Chapter 1

Introduction
1. INTRODUCTION

Crop plants growing under field conditions are directly or indirectly exposed to various biotic and abiotic stresses, which adversely affect the plants growth and yield. Among these, abiotic stresses are reported to hit our crops badly which cause a major decline in productivity and reduce average yields for major crop plants by more than 50%. With gradual increase in world population, there is an urgent demand for food. To maintain our crop yield which is threatened by abiotic stresses, several attempts have been made to adopt novel means to develop a crop with improved yield through biotechnological approaches. Transgenic plants have been developed with improved tolerance to abiotic stress with the help of genetic engineering tools which require a candidate gene to be identified, isolated and finally transformed into a suitable genotype. Various candidate genes have been isolated and characterized successfully so far, but the information available in literature related to their signaling cascades is very limited.

Amongst various stresses, salinity is one of the major factor which has been reported to affect crop yield worldwide. About 20% of irrigated agricultural land and 2% of the dry land is adversely affected by salinity (Flowers and Yeo, 1995). Salinity affects plant growth and development at physiological, biochemical and molecular level (Hasegawa et al., 2000a). High concentrations of salt cause both ionic stress and osmotic stress in the plants which in turn, leads to secondary stresses such as oxidative stress and nutritional disorders (Hasegawa et al., 2000; Zhu, 2001; Chinnusamy et al., 2006). Most of the crop plants are glycophytes and thus fail to withstand a prolonged exposure to even mild salinity (Zhu, 2001). A realistic solution to this problem might be provided by breeding or engineering the crops for improved tolerance to salt. More recently, transfer of single genes has resulted in production of transgenic plants with
improved tolerance to salinity (Zhang and Blumwald 2001, Singla-Pareek et al., 2003; Singh et al., 2010).

Keeping in view the complexity of signal transduction mechanisms operative in plants in response to abiotic stresses, single gene transfers may not be as efficacious as engineering a signaling pathway per se (Kawasaki et al., 2001). However, over expression of multiple genes may improve stress tolerance in plants more effectively. Initial studies for crop improvement focused only on the transfer of a single gene at a given time, but presently multigene transfer to crop plants is a great concern to researchers of different disciplines (Yang et al., 2009).

Plants are reported to use mainly three strategies to maintain low cytosolic sodium concentration: sodium exclusion, compartmentation or secretion. One mechanism responsible for transporting sodium out of the cell is through plasma membrane-bound Na⁺/H⁺ antiporter, as evidenced by the characterization of SOS1 (Salt Overly Sensitive), a putative plasma membrane Na⁺/H⁺ antiporter from Arabidopsis thaliana (Shi et al., 2000). The efficient compartmentation of sodium is likewise accomplished through operation of vacuolar Na⁺/H⁺ antiporter that move potentially harmful ions from cytosol into large, internally acidic, tonoplast-bound vacuoles. In yeast, simultaneous expression of SOS1, SOS2 and SOS3 increased the salt tolerance of transformed yeast cells much more than the individual expression of one or two SOS proteins (Quintero et al., 2002) suggesting that SOS1 requires SOS2/SOS3 complex for its complete activity. Genetic analysis of A. thaliana has shown that SOS3, SOS2 and SOS1 are involved in a common pathway of salt tolerance (Zhu et al., 1998). The SOS pathway has recently been shown to be conserved in rice as well (Martinez et al., 2007). Out of these, SOS2 is responsible for maintenance of intracellular Na⁺ and K⁺ homeostasis in A. thaliana. The kinase activity of SOS2 protein is responsible for providing salt tolerance to the plant (Liu et al., 2000). Abiotic stresses induce Ca²⁺ signals (Knight et al., 1998; Sanders et al., 2000) that are perceived by SOS3. The SOS3 then interacts physically with SOS2 a serine threonine protein kinase (Guo et al., 2001). The activated SOS2 kinase
phosphorylates the SOS1, a Na\(^+\)/H\(^+\) antiporter which is responsible for pumping Na\(^+\) out of the cytoplasm (Shi et al., 2000). In recent studies, the SOS3-SOS2 complex also has been found to regulate the transcript level of SOS1 and other genes (Chinnusamy et al., 2006). In order to define which proteins might be forming parts of nodes for stress-related signaling, SOS2 is reported to be a good candidate (Verslues et al., 2007). However, in addition to the well-established role of SOS2 in regulating ion transports (Qiu et al., 2002; Quintero et al., 2002; Cheng et al., 2004), SOS2 protein has been reported to have interaction with the H\(_2\)O\(_2\) signaling protein nucleoside diphosphate kinase 2 (NDPK2) and catalases (CATs) activity in Arabidopsis (Verslues et al., 2007). Furthermore, regulation of vacuolar H\(^+\)-ATPase activity is also a key function of SOS2 which helps in coordinating changes in ion transport during salt stress and in promoting salt tolerance.

Variation in salinity tolerance is reported more frequently in dicots as compared to monocots. It is interesting to note that within Brassica species, considerable variation has been reported in salinity tolerance wherein the amphidiploid species have been found to be more tolerant than the diploid species (Kumar et al., 2009). In recent years, contrasting genotypes have served as a useful tool in several genome-wide transcriptome analysis aimed towards understanding the physiological as well as molecular mechanisms of salinity tolerance in rice and Brassica (Kawasaki et al., 2001, Karan et al., 2009, Kumari et al., 2009, Kumar et al., 2009). A comparative study among rice varieties reveals that Pokkali has been a preferred salt tolerant variety which is explored in conjunction with various salt sensitive varieties. Similarly, among the different improved genotypes raised at IRRI, IR64 (released in the year 1985) is one of the most widely grown IR varieties and well adapted in more than 13 countries including India. IR64 is well suited for irrigated and rain fed lowland areas with moderate to high levels of tolerance towards various toxicities (such as salt, alkali, iron and boron) as well as deficiencies (such as phosphorus and zinc) for wetland adverse soils.
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Though SOS pathway has been dissected out in detail in Arabidopsis and component genes involved into it are well characterized, the characterization of the important genes of this pathway from crop plants such as rice and Brassica is yet to be attempted. Work carried out during M.Phil dissertation of the candidate has resulted in isolation of partial clones for various SOS genes from Brassica species. In the present study, main emphasis has been towards characterization of SOS2 serine threonine protein kinase, an important component of SOS pathway, which is yet to be explored in other crop plants with respect to salinity stress.

For this purpose, following objectives have been set forth in the present study:

1. Isolation of members of SOS2 gene family from crop plants such as rice and Brassica sp.
2. Characterization of expression patterns of SOS pathway candidates in rice and Brassica with respect to various abiotic stresses such as salinity, drought, heat and ABA.
3. Isolation of SOS2 promoter from crop plants to comment on its fine regulation.
4. Developing transgenic Brassica and rice plants with over-expression as well as RNAi constructs and thus carry out studies related to their behaviour under salinity stress employing physiological, biochemical and molecular tools.
5. Finally, to comment upon the role of SOS2 as “candidate gene” for raising transgenic crop plants with improved tolerance towards salinity stress.