2.1 WHEAT CROP

Cultivation of wheat, which along with rice and maize, is among the top three cereal crops, started around 10,000 years ago (Briggle, 1980; Briggle and Curtis, 1987; Curtis, 2002). The annual production of wheat in India stands at 80-90 million tonnes, thereby, making India the second largest producer of wheat in the world (Food and Agriculture Organisation of the United Nations, 2012). Wheat is a highly adaptable crop and is widely grown in temperate as well as tropical regions of the world. In India, it is basically a winter crop, grown in rabi season with temperatures between 10-15 °C at the time of sowing and increasing up to 25 °C during grain filling. The rainfall requirement of the crop is between 5-15 cm.

Most of the present day cultivated wheat is the hexaploid wheat (AABBDD genome) that originated by hybridization of cultivar emmer (AABB genome) with wild type Triticum tauschii, also known as Aegilops tauschii. In the hexaploid wheat, A genome is found to be related to A genome of primitive wheat cultivar, called einkorn (tertaploid), while B and D genomes are derived from T. tauschii (Feldman, 2001). Wheat has 23 species with ploidy levels 14, 28 and 42 chromosomes (Morris and Sears, 1967).

The plant of hexaploid wheat, Triticum aestivum, grows up to 2-4 feet and flower is a terminal spike comprising around 15-20 spikelets that are borne on an axis. Individual spikelets are sessile and solitary, consisting of 1-5 flowers each. Anatomical studies of wheat reveal that the grains generally have oval shape, although it varies from spherical to long, narrow and flattened shapes. Usual length of wheat seed is 5-9 mm and the weight is around 35 to 50 mg. The wheat grain contains three layers: germ (2-3%), bran (13-17%) and endosperm (80-85%) (Belderok et al., 2000). Bran is the outer layer of the grain and plays the role of a protector. Bran is made up of water-insoluble fibre complex, protein bound molecules such as xylose and arabinose, and also contains, cellulose and pentosans (Cornell, 2003). The endosperm is enclosed by a fused pericarp and seed coat, hence called caryopsis. Simmonds and O’Brien (1981) studied the course of endosperm development in detail and divided in four phases, where phase I corresponds to 1-5 days post anthesis (DPA) that is characterized by triploid endosperm division, which leads to formation of multinucleate cytoplasmic matrix around central cell. In phase II i.e. 6-13 DPA, starch and protein body
formation and gluten proteins deposition in cell is observed. In phase III (14-24 DPA), endosperm continues to divide and the grain is released. Amino acids synthesis and sucrose synthase activity is also observed. The phase IV is marked by dehydration process (25-38 DPA), putting a stop to the seed vascular flow. Phase II is the most important stage of endosperm development, since it determines the commercial value of wheat due to gluten protein quality and quantity. The outer layer of endosperm is the aleurone which is rich in proteins and enzyme necessary for germination. The inner layer of endosperm is primarily composed of starch that is needed as food reserve for the growth of the seedling. Starch amount vary from 60 to 70% of the total dry weight of the wheat grain. Wheat consists of starch granules which are large lenticular and small spherical shaped. The large granules are synthesized up to 15 DPA, whereas, the spherical granules appear after 10-30 days of pollination. In addition to carbohydrates, endosperm also contains fat (1-5%) and protein (13%). Wheat germ is a rich source of several amino acids and vitamin E (Belderok et al., 2000).

The key characteristic feature of wheat flour is dough formation, which is affected by storage proteins, known as ‘gluten proteins’. Wheat flour is used for production of number of end products, such as cookies, noodles, pasta and the leavened and unleavened breads, etc. Wheat is an important part of nutrition as it provides substantial proteins and calories. Wheat also fulfils our nutritional needs as wheat product intake helps to combat iron and zinc deficiency (Henderson et al., 2007). It is also one of the most important livestock feed in the form of hay, pasture and silage.

2.2 Seed storage proteins

Functionality of wheat flour for different applications is governed by starch and proteins present in it. The quantity and quality of gluten proteins, which form visco-elastic mass on addition of water to flour, governs the bread making quality of wheat. Wheat gluten was first isolated by Beccari in 1745. Osborne (1924) classified storage proteins on the basis of extraction and solubility in different solvents. Albumins are soluble in water, globulins in dilute saline, and gliadins and glutenins in alcohol/water mixtures. The elasticity or strength characteristic of wheat dough is imparted by glutenins, whereas, gliadins provide viscosity to flour. Wheat gluten proteins are rich in proline residues and, hence, are also known as prolamins.
2.2.1 Glutenins

Glutenins are polymers comprising of aggregated proteins linked by interchain disulphide bonds, soluble in alcohol/water mixture (Wieser et al., 2006). Glutenins consist of high molecular weight (HMW-GS) and low molecular weight (LMW-GS) subunits ranging between 30-120 kDa (Kreis et al., 1985). HMW-GS range from 80-120 kDa. Hexaploid wheat genomes (A, B and D) encode two HMW-GS, mapping both at locus Glu-1 on the long arm of the group I homoeologous chromosome (Payne et al., 1987). The two genes at each locus have been classified as x- and y-types according to their molecular weights (Payne et al., 1983). Therefore, six HMW-GS are expected; however, genes encoding x-type and y-type HMW-GS at locus Glu-A1 and Glu-B1, respectively, are not always expressed, hence, 3 to 5 HMW-GS are observed (Forde et al., 1985, Werner et al., 1994). The genes encoding HMW-GS are highly conserved and comprise of three domains (Shewry et al., 1992; Wan et al., 2002). The central domain is 420-700 amino acids long comprising of repetitive peptides which are in glutamine, glycine and proline rich, whereas, the flanked domains are composed of non-repetitive amino acids. The C-terminus domain comprising of 42 a.a. residues is conserved in size, whereas, the N-terminus varies between 80-90 a.a. residues in length. The N- and C-termini structure is mainly α-helix, whereas the central domain has a β-spiral structure (Veraverbeke and Delcour, 2002). The repetitive central domain, which forms a β-reverse turn, results in elasticity, as the loop elongates and compress when the stress is applied (Belton et al., 1999).

Varying length of central domain is the main reason for difference in molecular weights of x-type (80-90 kDa) and y-type (65-75 kDa). Other difference between the subtypes is that x-type repeats are nonapeptide, hexapeptide and tri-repeats, whereas, these repeats are absent in central domain of y-type (Shewry et al., 1992). Further, position and number of cysteine residues in the x- and y-type GS are also different (Shewry and Tatham, 1997). In HMW-GS, the cysteine residues form intermolecular disulfide bonds that lead to large polymeric aggregates of glutenins in the dough. This cysteine cross-linking and the natural elasticity of repetitive domain impart elasticity to glutenin.

LMW-GS are more heterogeneous than the HMW-GS. These proteins are grouped as B- (40-50 kDa), C- (30-40 kDa) and D-subunits (Payne et al., 1979). The D-subunit
constitutes a minor fraction and has lower molecular weight than the B-subunit (Tao and Kasarda, 1989). B-type and C-type LMW-GS are encoded at Glu-3 and Gli-1 loci, respectively, on short arms of chromosome group I. B- and C-type LMW-GS are similar to α- and β-type monomeric gliadins and are sulphur-rich. They also have a similar two domain structure, where the N-terminal domain comprises of repetitive a.a. residues like glutamine and proline, forming β-reverse turns. The C-terminus domain forms α-helix structure and comprises of non-repetitive sequences (Gianibelli et al., 2001). Hence, B- and C-type LMW-GS have asymmetrical structure. D-type LMW-GS are sulphur poor glutenin subunits, similar to ω-gliadins (Shewry et al., 1986). They consist of repetitive amino acid sequence of proline, glutamine and phenylalanine forming overlapping β-reverse structure which is rod shaped (Shewry et al., 1986). Masci et al., (1999) reported that a single unpaired cysteine residue in C- and D-type LMW-GS which are involved in chain termination to stop glutenin polymerization, which weakens the dough strength. In contrast, the LMW-GS with more than two cysteine residues, found in majority of B-type LMW-GS, can increase dough strength by forming aggregates through intermolecular disulfide bonds with HMW-GS (D’Ovidio and Masci, 2004).

2.2.2 Gliadins

Gliadins are soluble in aqueous alcohol mixture and their molecular weights range from 30-80 kDa. They are rich in proline and glutamine (MacRitchie et al., 1990). These are monomeric gluten proteins and are of three types: α-, γ-, and ω-types (Shewry et al., 1986). Sequence of these protein types is encoded on short arms of group I and group 6 chromosomes. α- and γ-type gliadins are ‘sulphur rich’ prolams, as they consist of cysteine residues which form disulphide bonds within polypeptide chain or with other polypeptides. On the contrary, the ω-type gliadins are sulphur poor due to lack of cysteine residues. α-gliadins have five distinct domains and one of the domains is flanked by two stretches of polyglutamine domains. The conserved cysteine residues present in polyglutamine domains form intermolecular disulphide bonds (Kasarda et al., 1984; Muller and Weiser, 1997; Anderson et al., 1997). In γ-gliadins, a 20 amino acids long signal peptide is present, followed by a non-repetitive N-terminal domain and a highly repetitive domain. The C-terminal domain is a non-repetitive region with two conserved cys residues (Cassidy et al., 1998; Anderson et al., 2001). γ-gliadins contain uneven distribution of cysteine residues due to which the free –SH groups form intermolecular disulfide bonds. These bonds contribute to
gliadin to glutenin polymer formation. ω-gliadins have a relatively simple structure comprising of a putative signal peptide followed by non-repetitive N- and C-termini regions. These proteins lack cysteine residues and hence no disulphide bonds are observed (Kasarda et al., 1983; Hisa and Anderson, 2001).

Elasticity of glutenins is due to non-covalent interactions, mainly hydrogen bonds within or between glutenin chains. Viscosity is provided to dough by gliadins as it interferes and weakens these non-covalent interactions within glutenin chains (Ciaffi et al., 1996). Viscosity and elasticity of dough is determined, therefore, by maintaining the ratio between monomeric gliadins and polymeric glutenins (Popineau et al., 1994), which is important for the breadmaking and other end products.

2.2.3 Non-gluten proteins

Non-gluten proteins form 15-20% of total storage proteins and are soluble in dilute salt solutions. Two major proteins belonging to this class of proteins are the albumins (water soluble) and the globulins (salt soluble) (Osborne, 1907). These are basically low molecular weight proteins with size lower than 25 kDa, and they migrate ahead of gliadins. Deposition of albumins and globulins in the grains increases from anthesis to up to 20 days after anthesis (DAA), before stabilizing at maturity (Gupta et al., 1996; Stone and Nicolas 1996; Panozzo et al., 2001). Globulins and albumins are also polymeric in nature and are stabilized by inter-chain disulphide bonds (Gupta et al., 1996). This group consists of structural proteins like amylases, endogenous wheat enzyme inhibitors i.e. protease inhibitor and xylanase inhibitors. Genes encoding 64, 62 and 16 kDa albumins are localized to chromosome 4DL, 4BS and 3DS, respectively (Forsyth et al., 1992), whereas, globulins of 38 kDa and 39 kDa are encoded by genes present on chromosomes 2A, 2B, 2D, 3BS, 3DS, 4DS, 5DL, 6DL, 6DS, 7BS or 7DL (Fra-Mon et al., 1984; Neito-Taladriz et al., 1996).

Non-gluten protein family also contains enzymes which function in metabolic pathways and in protective mechanisms (Luckow and Mcvetty, 1991). One of the major factors affecting the protein content is temperature and, hence, the end product. The increase in temperature at grain filling stage is important for induction of various non-prolamins, as endogenous level of number of albumins increase or their expression decreases under heat stress. Two-dimensional electrophoresis (2-DE) showed lower levels of glucose-1-phosphate adenyl transferase and granule-bound glycogen starch synthase, which are involved in starch
synthesis and metabolism, under heat stress conditions (Majoul et al., 2004). Gupta et al. (1991) observed that some HMW albumins and globulins function as storage proteins also by forming disulfide bonds. These proteins are observed at early stages of development but are not detected after early seedling development stage, therefore, suggesting that they provide nutrition. Non-prolamins contain significant amounts of essential amino acids like aspartate, threonine, lysine and tryptophan, which are absent in prolamins. Thus, these proteins are highly important, since they increase the nutritional value of wheat grains (Weiss et al., 1997; Takizawa et al., 2001). The non-prolamin proteins also affect the rheological parameters of wheat dough since addition of certain enzymes, like amylases, result in change in the dough properties (Dogan, 2002). Another type of non-gluten protein is lipid bound i.e. amphiphil (Marion et al., 1994), which are implicated in synthesis of hydrophobic membranes such as cutin and suberin, and is involved in fatty acid transport (Douleiz et al., 2000). Several of these proteins are also known to cause baker’s asthma, which is a major occupational disease. It is an IgE-mediated type I allergic response due to inhalation of cereal flour (Baur et al., 1998), and it is primarily due to salt soluble proteins i.e. albumins/globulins (Weiss et al., 1997).

2.2.4 Synthesis, assembly and deposition of seed storage proteins

Secretory proteins are synthesized with a peptide signal, which is cleaved and protein is translocated into the lumen of ER, where the folding is completed (Shewry, 1999; Shewry et al., 2002). Various lumen proteins, like BiP (binding protein), further assist these peptides to attain their final conformation from intermediate nascent polypeptide by preventing formation of incorrect transient polypeptides. Another class of proteins, peptidyl prolyl cis-trans isomerases (PPIases) catalyze cis to trans isomerisation of peptide bonds preceding proline (Fischer et al., 1984). The PPIases accelerate the process of protein folding in ER lumen. Protein-disulphide isomerases (PDI), which promote correct disulphide bond formation in proteins (Freedman, 1989), are also involved in synthesis of proteins in ER. The storage proteins are deposited as small discrete protein bodies in the mature seed (Shewry et al., 1995). The protein bodies can originate from vacuole or ER (Rubin et al., 1992; Li et al., 1993). Some proteins follow a pathway, wherein they are transported via golgi apparatus to the vacuole before forming protein bodies, or they are retained in ER and form protein bodies (Levanony et al., 1992; Shewry et al., 1995). Wheat storage proteins synthesis is transcriptionally regulated (Bartels and Thompson 1986). The up- and down-regulatory
motifs, known as ‘endosperm motifs’ and ‘GCN4 motifs’, respectively, form ‘prolamin box’ in the promoter regions of several genes encoding storage proteins (Muller and Knudsen, 1993; Hammond-Kosack et al., 1993).

Since the wheat prolamins have high content of prolyl residues (10-30%), it is, therefore, likely that PPIases may be playing a vital role in the catalysis of folding process of proteins during deposition. Variations in these isomerases may affect the folding process, thereby, leading to variation in wheat flour and dough quality. Hence, the role of immunophilins in protein folding needs to be studied in wheat.

2.3 Peptidyl prolyl cis-trans isomerases (PPIase)

In cellular machinery, protein folding is very accurate and stability of the intermediates and their conversion to matured protein is of utmost importance. Among all the peptide bonds in the protein, rotation of prolyl imide bond is the slowest and a rate limiting step in protein folding (Brandts et al., 1975; Schmid and Baldwin, 1978; Lang et al., 1987). Peptide bonds not preceding proline are almost always in trans folded state, which is a stable conformer, but 6.5-10% of the peptide bonds preceding proline residue remain as cis-isomer. Rate limiting nature of prolyl imide bond isomerization is due to partial double bond character of C-N bond in proline, which causes restrictions for the amide bond torsion. This also influences the relative free energies of cis and trans isomeric states (ΔG). The difference in free energy between cis and trans states determines cis and trans population ratio at thermal equilibrium. The catalysis of cis to trans isomer in proteins is governed by slow rotation by PPIases, and for their slow rotation during catalysis, PPIase are also called Rotamases (Fischer et al., 1984). Fischer (1989) isolated porcine cyclophilin (Cyp), which showed homology to bovine Cyp and was inhibited by immunosuppressant drug cyclosporine A (CsA) (a cyclic undecapeptide) (Takahashi et al., 1989). FK506-binding proteins (FKBP), another class of proteins, which possess PPIase activity bind to another set of immunosuppressant drugs-FK506 and rapamycin. FK506 and rapamycin are structurally not similar to CsA and are macrocyclic lactones produced in fungi Streptomyces tsukubaensis (Harding et al., 1989). FKBP and Cyclophilins have distinct PPIase domains with unrelated a.a. sequence (Harrison and Stein, 1990). Cyp-CsA complex inhibits Ca$^{2+}$-dependent phosphatase activity of calcineurin, resulting in inhibition of translocation of nuclear factor required for the activation of T-cells (NT-AT) from cytosolic compartment to nucleus, thus, preventing transcription of gene encoding cytokine like IL-2 transcription factor (Scrieber, 1992;
McCaffrey et al., 1993). FKBP-FK506 complex binds to protein kinase (TOR-target of rapamycin), which further regulate p70S6 kinase activity that governs cell signal transduction and, therefore, inhibits IL-2 (Abraham and Wienderrecht, 1996). Due to formation of complex with immunosuppressant drugs, these PPIases are also known as immunophilins. Third class of PPIase gene family having similar isomerisation activity in protein folding, as displayed by Cyps and FKBPs, is parvulins (Rahfeld et al., 1994). Parvulins show no sequence similarity to Cyps and FKBPs, and do not bind to either CsA or FK506. However, these proteins bind to Juglone (5-hydroxy-1,4-naphthoquinone) which results in inhibition of their isomerase activity (Hennig et al., 1998).

2.3.1 FK506-binding proteins (FKBPs) in lower organisms

Peptidyl prolyl cis-trans isomerases are ubiquitous proteins and are present in bacteria, yeast, insects, plants and mammals. Their functions and structure have been conserved during evolution (Galat, 2000). The number of FKBP genes vary in different organisms, as four FKBP genes have been reported in yeast (Dolinski and Heitman, 1997), eight in Caenorhabditis elegans (He et al., 2004), 15 in human (Rulten et al., 2006), 23 in Arabidopsis (He et al., 2004) and 29 FKBPs in rice (Ahn et al., 2010). In lower organisms, a unique family of ribosome-associated PPIases, known as Trigger factor, are found which help in binding of polypeptide chains with ribosomes (Stoller et al., 1995). Guanidine-denatured bovine carbonic anhydrase II showed refolding in presence of wild type trigger factor, thereby, suggesting a role for this protein in folding process (Kramer et al., 2004; Liu et al., 2005). Bacterial trigger factor (EcFKBP48) was observed to bind to 50S ribosomal subunit for folding of proteins. Though this PPIase does not show affinity for FK506 or rapamycin, its activity is attributed to the presence of a FKBP domain. Archael microorganisms viz, hyperthermophiles, thermophiles, halophiles and methanogens live under extreme environment conditions. Along with PPIases, other chaperones like GroE, DnaK, DNaJ, GrpE, HSP70 and HSP60 are also present in archael genomes, which help in assisting the protein folding in bacterial cytosolic compartments (Langer et al., 1992). In E. coli, mutation in Dnak leads to accumulation of cytosolic proteins, suggesting its role in proper protein folding. Homologues of DnaK protein have been reported in hyperthermophilic bacteria Aquifex aeolicus (Deckert et al., 1998) and Thermotoga maritima, implying similar functionality (Nelson et al., 1999). Archael cyclophilin, HcCyp19, isolated from Halobacterium cutirubrum showed half maximal inhibitory concentration (IC$_{50}$) value of 15
nM in presence of 2.9 M KCl, whereas archael FKBP, MbtFKBP29, purified from *Methanobacterium thermoautotrophicum*, showed FK506-insensitive PPIase activity. Another FKBP from *Archaeoglobus fulgidus* (AfFKBP29) also showed weak PPIase activity (Nagashima *et al.*, 1999). Other bacterial immunophilins viz., SlpA and SlyD, which show homology to 12 kDa FKBP isoform, have been reported in *E. coli* (Bouvier and Stragir, 1991; Hottenrott *et al.*, 1997). A third FKBP isoform in *E. coli*, known as FkpA (Saul *et al.*, 2004), contains a FKBP-like PPIase domain which is conserved at C-terminus. FkpA orthologues are also reported in a number of pathogenic bacteria viz. *Trypanosoma cruzi* (TcMIP) (Pereira *et al.*, 2002), *Legionella pneumophila* (LpMIP) (Fischer *et al.*, 1992) and *Coxiella burnetti* (CbMIP) (Mo *et al.*, 1998). FKBP5s of Legionella and Chlamydia show similarity to mammalian FKBP25 (Bangsborg *et al.*, 1991; Lundemose *et al.*, 1992). Since, FKBP25 abrogate the defence systems of several hosts, this protein was termed as macrophage infectivity potentiatier (mip) (Cianciotto *et al.*, 1990).

### 2.3.2 Mammalian FKBP5s (mFKBP)

The minimal representative of FKBP5s in mammals is FKBP12, a 12kDa protein, which is a single domain immunophilin possessing FK506-inhibitable PPIase activity (He *et al.*, 2004; Barik, 2006). FKBP12 regulates activity of channels involved in calcium release, like the inositol (1,4,5-triphosphate) receptor and ryanodine receptor (RyR). The aforementioned regulation occurs when unbound or free FKBP12 binds to calcineurin and TOR/FRAP, which leads to T-cell activation. FKBP12 binds to cellular ryanodine receptor, and stabilizes and modulate this channel by increasing the ryanodine receptor number. It directly modifies calcium ions released in sarcoplasmic reticulum (Brillantes *et al.*, 1994; Breiman and Camus, 2002). The signal transduction is inhibited in the presence of FK506 or rapamycin, as FKBP12-FK506 complex blocks the phosphatase activity of calcineurin. Mutants of mice, impaired in FKBP12, showed severe cardiac defects (Shou *et al.*, 1998). Altered RyR function in FKBP12 mutant animals lead to cell cycle arrest, thus, implying that this immunophilin plays a crucial role in cell functioning (Breiman and Camus, 2002; Romano *et al.*, 2005).

Multidomain FKBP5s, such as FKBP51 and FKBP52, constitute an important component of progesterone receptor complex in rabbit (Nakao *et al.*, 1985; Sanchez *et al.*, 1990). These proteins also form a part of glucocorticoid receptor (GR) complex, along with other interacting proteins such as HSP90 and HSP70 (Pratt and Toft, 1997; Reynold *et al.*, 1992).
FKBP51 mediates receptor translocation to nucleus by linking the GR through TPR-bound HSP90 to the dynein motor that interacts with FKBP52 PPIase domain (Davies and Sanchez, 2005).

A human FKBP, hFKBP38, is involved in the regulation of apoptosis. The hFKBP38 interacts with an anti-apoptotic protein Bcl-2, and directs it to mitochondria, thereby, protecting the cells from apoptosis (Shirane and Nakayama, 2002; Kang et al., 2005). Reduced level of Bcl-2 protein was observed in FKB38 knockout mice produced using small interfering RNA technique, suggesting the role of FKB38 in stabilizing Bcl-2 and, thus, protecting the anti-apoptotic proteins from degradation pathways. NMR and X-ray crystallographic structure of Bcl-2 showed the presence of a flexible loop which binds to FKB38 and inhibits apoptosis (Petros et al., 2001; Kang et al., 2005).

2.3.3 FKBPs in plants

Immunophilins (FKBPs and cyclophilins) are involved in protein-protein interactions, plant growth regulation, chaperone mechanism, signaling pathways and stress regulation mechanisms (Chitti et al., 1999; Romano et al., 2005). These diverse functions could be explained better by understanding the domain structure of immunophilins. The structures of cyclophilins and FKBPs are distinct and are not related to each other. X-ray crystallography studies revealed that Cyclophilin A contains eight anti-parallel β-strands forming a right handed β-barrel, with two α-helices at either side (Ke, 1992). It has a unique closed β-barrel structure. Cyclosporine A (CsA) and proline-containing peptides bind to the outer hydrophobic surface, which contains seven aromatic residues and other similar residues. A loop is formed between Lys-118 and His-126. The four β-strands form the core of CsA-binding site (Kallen et al., 1991). Structure of FKB12 domain consists of six anti-parallel β-strands, which are wrapped around one short α-helix. The six anti-parallel β-sheets form a concave surface opposite the helix, and the hydrophobic side chains are projected towards the core of protein, forming a binding site for FK506 and Pro-peptide substrate (Griffith et al., 1995; Fanghanel and Fischer, 2004).

The FKBPs are named according to their molecular weights, from smallest FKB12 to largest FKB135, and are classified considering domain structure and subcellular localization. FKBPs and cyclophilins have been reported in all subcellular compartments of cell, and are present in cytoplasm, mitochondria, chloroplast and nucleus of number of plant
species (Galat et al., 2000). Luan et al. (1996) purified the first FKBP in plants from *Vicia faba* by employing the FK506 matrix for affinity chromatography. Rice has so far been reported to contain 29 FKBP s and 27 cyclophilin genes, highest number of immunophilins members in comparison to any other species (Gollan and Bhave, 2010a). Ahn et al. (2010) also identified 29 putative FKBP s in rice, which were classified as single domain (SD) and multiple domain (MD). In addition to FKBP12-like domain, additional domains such as localization signals, tetratricopeptide repeats (TPR), coiled-coil domain (CCD), WW domains (Tryptophan), calmodulin-binding sites, DNA- and RNA-binding sites have been putatively identified in high molecular weight FKBP s (Kurek et al., 1999; Aviezer-Hagai et al., 2007). These domains play a crucial role in interactions and assembly of complexes (Burkhard et al., 2000). Fourteen FKBP s in rice are multi-domain, with most of them being conserved for the a.a. residues essential for PPIase activity (Ahn et al., 2010). Some of the FKBP s in rice contain double arginine residues at N-termini and a hydrophobic region, which is necessary for chloroplast localization by twin arginine translocase pathway (Tat) (Brink et al., 1998).

Like Arabidopsis and Chlamydomonas, putative rice FKBP s, OsFKBP13, -16-1, -16-2, -16-3, -16-4, -17-1, -17-2, -18, -19 and -20-2 also contain peptidase cleavage site ‘Ala-Xaa-Ala’ at termini of hydrophobic region for thylakoid lumen target (Ahn et al., 2010). Putative OsFKBP15-1 and OsFKBP15-2 are 81% and 76% similar to AtFKBP15-1 and AtFKBP15-2, respectively. Rice lacks orthologue of AtFKBP15-3 (He et al., 2004). OsFKBP15-1 and OsFKBP15-2 contain ER-retention signal DSEL and NSEL. In comparison, rice has one putative OsFKBP20-1 localized to nuclear region, as compared to two orthologues in Arabidopsis.

The VfFKBP15 in *Vicia faba* contains a hydrophobic amino acid stretch, an ER-translocation signal peptide at N-terminus and an ER-retention signal, SSEL at C-terminus (Jin et al., 1991; Nielsen et al., 1992). VfFKBP15 was the first plant FKBP to show PPIase activity, which is specifically inhibited in presence of FK506 and rapamycin, with inhibition constants (Ki) of 30 nM and 0.9 nM, respectively. VfFKBP15 is homologous to yeast and mammalian FKBP13, having 55% and 53% identity, respectively (Luan et al., 1996). Using synthetic substrates containing variant amino acid preceding proline, it was observed that VfFKBP15 showed high affinity towards bulky hydrophobic residue (Luan et al., 1996). Another FKBP, VfFKBP25, showed nuclear localization and was reported to interact with casein kinaseII and nucleolin (Jin and Burakoff, 1993). FKBP12 in yeast is sensitive to inhibitors, FK506 and rapamycin, and after binding to FK506, inhibits calcineurin (CaN)
activity, which is essential for the survival of yeast under high salt conditions (Nakamura et al., 1993). Interaction between VfFKBP12 and calcineurin is drastically affected by reduction of disulphide bond, formed by conserved cysteine residues at position 26 and 80 of plant FKBP12, as demonstrated by a decline in CaN amount bound to VfFKBP12 in presence of reducing agent dithiothreitol (DTT). This was further validated by site directed mutagenesis, since replacement of cysteine residue with serine lead to significant decline in CaN content in mutant strain as compared to wild-type. It is, thus, evident that disulfide bonds are imperative for maintaining structure of VfFKBP12 and its interaction with other protein partners (Xu et al., 1998). Yeast two-hybrid assay revealed that unlike hFKBP12, VfFKBP12 did not restore the sensitivity of yeast FKB12 mutant to rapamycin or FK506, suggesting that plant FKB12-ligand complexes do not efficiently interact with yeast drug target (Koltin et al., 1991). Although VfFKBP12 is highly homologous to yeast and mammalian FKB12, but in plant FKB12, the critical amino acid residues Asp\(^{37}\) and Arg\(^{42}\) required for stabilizing the 40’s loop and the CaN binding are replaced by other distinct amino acid residues, leading to low affinity towards CaN (Yang et al., 1993; Kissinger et al., 1995). These findings validate plant FKBP’s inability to restore the drug sensitivity in yeast mutant.

Of the 23 isoforms of FKBP reported in Arabidopsis, AtFKBP42 (TWD1), AtFKBP53, AtFKBP61 (ROF1), AtFKBP65 (ROF2), AtFKBP70, AtFKBP71 (PAS1) and AtTIG are high molecular weight with the rest being low molecular weight (He et al., 2004). High molecular weight AtFKBPs are similar to mammalian orthologues and have number of FK506-binding domains at N-termini, and TPR domain at their C-termini. In Arabidopsis, AtFKBP70 and AtFKBP53 are different in comparison to other family members by virtue of presence of single FK506-binding domain (Harrar et al., 2001). Two active immunophilins, AtCyp20-2 and AtFKBP13, were reported in thylakoid lumen of Arabidopsis, but majority of the PPIase activity was contributed by the latter (Shapiguzov et al., 2006). This activity showed severe decline upon reduction of two redox active disulfide bonds, present in AtFKBP13, in the presence of thiol reducing agent DTT. It was observed that C-terminal disulfide linkage is an important structural feature of AtFKBP13 and governs substrate-binding (Gopalan et al., 2004). These studies indicated that AtFKBP13 is redox-activated, which also interacts with Rieske protein only in an oxidised state (Hall et al., 2010). Rieske protein is a subunit of cytochrome b\(_{6}\)f complex and a redox protein regulated by light intensity in the lumen. The interaction between FKBP13 and Rieske protein is not important
for electron transfer as FKBP13 is dispensable for plant growth (Ingelsson et al., 2009) but it is likely that this protein may be involved in other non-essential photosynthetic processes.

Two cyclophilins, TLP40 (Fulgosi et al., 1998) and TLP20 (Edvardsson et al., 2003) (Thylakoid Lumen Protein of 40 kDa and 20 kDa, respectively), isolated from thylakoid lumen of spinach chloroplast showed PPIase activity. TLP20 is inhibited by low concentrations (nM) of CsA in comparison to µM concentrations for TLP40. TLP40 is an active cyclophilin in spinach and regulates activity of photosystemII-specific protein phosphatase. On the contrary, its homologues Arabidopsis (AtCYP38) and maize (MzTLP40), also thylakoid bound immunophilins, showed no PPIase activity (Fulgosi et al., 1998; Vener et al., 1999).

CsA- and rapamycin-sensitive PPIase activity was also reported in pea. This activity was mainly localized in mitochondria, as PPIase activity of mitochondrial protein extract was inhibited 90% and 16% by CsA and rapamycin, respectively (Breiman et al., 1992). CsA-inhibitable activity was primarily associated with mitochondrial matrix, whereas, rapamycin-inhibitable PPIase activity was observed in mitochondrial membrane. This was validated employing immunodetection method by using yeast rapamycin-binding protein (RBP) antibody, which detected a 25 kDa protein in both matrix and membrane fractions. When membrane fraction was subjected to sodium carbonate treatment, it led to loss of PPIase activity and also no cross reactive 25 kDa protein was observed in this protein fraction, indicating that putative 25 kDa RBP is associated with pea mitochondrial membrane (Derocher and Vierling, 1994).

The first FKBP from wheat (wFKBP73) was cloned by Blecher et al. (1996). wFKBP73 is identical with mammalian FKBP, mFKBP52, and shows similar domain structure, possessing two to three FK506-binding domains at N-termini, CaM-binding domain and a TPR domain at C terminus. CaM-binding site contain five positively charged residues and hydrophic residues (Reddy et al., 1998; Kurek et al., 2002a). wFKBP77, the heat- induced isoform is 84% identical to wFKBP73, and is expressed in young meristematic tissue. The two isoforms differ in their molecular weight, which was attributed to the difference in amino acids (559 in wFKBP73 and 568 in wFKBP77) and the post-translational modification, as wFKBP77 possesses more putative phosphorylation sites (Blecher et al., 1996; Kurek et al., 1999). FKBP5s play a significant role in plant development. It was observed that full length wFKBP73, when over expressed in rice, produced normal fertile
plants but truncated wFKBP73, having only FKBP domain, produced male sterile transgenic rice (Kurek et al., 1998). The wFKBP73 and wFKBP77 are involved in assembly of functional GR complex with Hsp90 and p23 (Owens-Grillo et al. 1996; Pratt and Toft 1997), suggesting that binding through TPR domain is a conserved feature of plants and mammals FKBPs (Reddy et al., 1998, Pratt et al., 2004). The HSP90/HSP70 complexes and the plant FKBPs could be used to target the trafficking of signaling proteins in the plants.

A thylakoid localized FKBP (wFKPB16-1), linked to chloroplast biogenesis, was also cloned and characterised from wheat (Gollan and Bhave, 2010b). TaFKBP16-1 protein consists of a conserved signal peptide (valine-glutamic acid-alanine) for chloroplast localization (Mori and Cline, 1998). Compared to mature plants, the expression of TaFKBP16-1 was higher at the younger stage (14-day old). Further, this gene was not expressed in roots, suggesting its role in photosynthetic activity (Gollan and Bhave, 2010b). Similar to AtFKBP13, a 13 kDa FKBP in wheat (TaFKBP13) was proposed to play a role in assembly of cytochrome b₆f complex by checkin accumulation of the Rieske (an iron-sulphur protein) in membrane and is translocated to thylakoid membrane by pH gradient-dependent pathway (Gollan et al., 2011; Gupta et al., 2002). TaFKBP13 also consists of four conserved cysteine residues forming disulfide bonds (Gopalan et al., 2004). Presequence of AtFKBP13 mature protein is enriched in basic and hydroxylated residues, which are hydrophilic in nature, similar to chloroplast envelope signal. Twin arginine motif is also observed at N-terminus of mature protein, which helps in transport to periplasmic or extracellular compartment, and where peptides are folded prior to export (Berks et al., 2003). To validate the pH gradient-dependent import of AtFKBP13, nigercin, an inhibitor of ΔpH formation across thylakoid membrane was used. There was accumulation of the precursor protein, suggesting that it is completely inhibiting the import and formation of the 13 kDa mature protein (Keegstra and Cline, 1999).

Sec pathway, common for all chloroplast proteins, is involved in import of proteins across the double-membrane envelope by ATP-driven translocase (Hulford et al., 1994; Karnauchov et al., 1994). Sodium azide, an inhibitor of sec pathway (Mori and Ito, 2001), when employed to confirm the import, showed no affect on AtFKBP13, indicating that thylakoid translocation was not dependent upon ATP, thus, confirming that it did not depend on sec pathway (Gupta et al., 2002). Yeast two-hybrid assays, employing TaRieske encoding region from chloroplast as bait, revealed that TaFKBP13 interacts with stromal chloroplast
protein (Gollan et al., 2011). Yeast two-hybrid analysis also demonstrated that TaFKBP16-1 interacts with PsaL subunit of PSI, and TaFKBP16-3 with APO2 and Thf1 (thylakoid formation1), implying a role for these protein in thylakoid membrane formation (Zhang et al., 2009; Gollan et al., 2011). In higher plants, majority of the FKBPMainly function as chaperones (Gu et al., 2009) in assembly and formation of multiprotein photosynthetic complexes. AtCyp38 was reported to mediate regeneration of photosystemII (Sirpio et al., 2008), and AtFKBP16-2 was implicated in stabilization of NAD(P)H dehydrogenase (NDH) complex in the thylakoid membrane (Peng et al., 2009). The FKBP gene family has also been studied in two important horticulture fruits- Prunus persica (peach) (Zhang et al., 2014) and Fragaria ananassa (strawberry) (Leng et al., 2014). Employing FKBP domain as query, 21 and 23 putative FKBPss were identified in peach and strawberry, respectively. Seventeen FKBPss were single domain and four were multidomain in strawberry, whereas, in peach nineteen FKBPss show single domain structure.

2.4 FKBP in stress response

Abiotic stress conditions in plants induce the expression of several FKBPss (Kurek et al., 1999; Dwivedi et al., 2003), which may be required to accelerate the folding and maturation of newly synthesized proteins that are upregulated during stress and play protective roles (Vierling, 1991; Bohnert et al., 1995). Meiri et al. (2009, 2010) reported that ROF1 (AtFKBP61) and ROF2 (AtFKBP65) in Arabidopsis functions antagonistically in acquiring long term temperature tolerance. ROF1 and HSP90 form a complex via TPR domain of ROF1, which then binds to heat shock transcription factor, HsfA2. This complex is translocated in nucleus, inducing ROF2 and other heat shock proteins (Meiri et al., 2009). ROF2 disrupts this complex in nucleus and binds to ROF1, thereby, down-regulating HSPs’ expression during recovery phase (Meiri et al., 2010). This was further validated since rof1 mutants in Arabidopsis poorly coped with increase in temperature even after prolonged recovery period. Detrimental effects on plant growth were observed in HsfA2 knockouts also (Meiri et al., 2010). Wheat and rice orthologs of ROF1/ROF2 share similar domain structure, possessing 2 to 3 FKBP12-like domains at N-termini, three TPR domains and a C- termini CaM-binding site (Kurek et al., 1999; Magiri et al., 2006). These orthologs also show similar antagonist mechanism in acquisition of thermotolerance, thereby, implying this to a conserved feature in higher plants. Even mammalian FKBP52 and FKBP51 were also reported to interact with GR through TPR region (Owens-Grillo et al., 1996). FKBP52 is
involved in translocation of ligand bound GR to nucleus, leading to elevation in expression of stress-responsive genes like FKBP51 (Davies and Sanchez, 2005; Jaaskelainen et al., 2011). Variable expression pattern was observed for ROF1 under different stress conditions, as expression was up-regulated on wounding and in response to salt stress (Vucich and Gasser, 1996). Environmental stresses, like methyl viologen, hydrogen peroxide and high light intensity, are reported to regulate the expression of Arabidopsis FKBP16-1 (AtFKBP16-1) transcriptionally and post-transcriptionally (Seok et al., 2014). Constitutive overexpression of AtFKBP16-1 in transgenic Arabidopsis plants resulted in enhanced tolerance under high light intensity conditions, and plants overexpressing AtFKBP16-1 demonstrated higher accumulation of PsaL protein and increased tolerance to drought stress. Blue native/2-D electrophoresis showed that the increase in AtFKBP16-1 affected the levels of PSI-LHCI (photosystem I-light harvesting complex I) and PSI-LHCI-LHCCI supercomplex (photosystem I-light harvesting complex I-light harvesting complex II). Protease protection assay provided evidence that AtFKBP16-1 enhanced the stability of PsaL. These studies, therefore, revealed that AtFKBP16-1, probably by regulating PsaL stability, facilitated the acclimation of plants under stress conditions (Seok et al., 2014).

Small ubiquitin-like modifiers (SUMO) are part of post-translational modification of covalent protein interactions (Hochstrasser, 2000). Yeast two-hybrid analysis revealed interaction of SUMO-conjugating enzymes (Sce) with a 20 kDa nuclear-localized FKBP during heat stress response in rice plants. Expression of OsFKBP20 mRNA was induced by heat stress, and over expression of this gene in yeast conferred thermotolerance (Nigam et al., 2008). The expression of several rice FKBPs viz., OsFKBP20-1b, OsFKBP58, OsFKBP16-1 and OsFKBP62a was also reported to be induced by salt stress (Ahn et al., 2010). The salt stress-induced expression of rice FKBPs showed differential kinetics. OsFKBP62a was up-regulated with in 1 h of stress, whereas, other genes showed enhanced expression after 24 h of stress. OsFKBP19, -20-1a, -20-1b, -16-3, -42a, -42b, -58 and -62a showed upregulation under dessication stress as well, indicating that FKBPs respond to abiotic stress conditions in rice plants and regulate the changes in the cell. VfFKBP15 in fava bean, and AtFKBP15-1 and AtFKBP15-3 in Arabidopsis exhibited enhanced expression by 4- to 5-fold in response to heat shock, as was also observed for yeast homologue yFKBP13 (Partaledis and Berlin, 1993; Luan et al., 1996). Similarly, ZmFKBP15-3 in maize also showed higher expression under high temperature stress, while expression of isoforms ZmFKBP15-2 and ZmFKBP15-1 was unaffected (Yu et al., 2012).
The expression of wFKBP77 and VfFKBP13 was also induced by heat shock in wheat and V. faba, respectively (Kurek et al., 1999; Luan et al., 1996). wFKBP73 and wFKBP77 may have different functions as both isoforms show different distribution, and are localized from cytoplasm to nucleoplasm upon heat shock at 37°C. The expression of wFKBP73 was observed constitutively in young tissues and reproductive organs, whereas, wFKBP77 was expressed only under heat stress in these tissues. wFKBP73 was observed to be associated with prevacoules and vacuole ontogeny at room temperature (25°C), whereas at 37°C, wFKBP77 got localized to nucleus, thus, suggesting the role of this protein in stress-related function (Dwivedi et al., 2003). Crystal structure of N-terminal region of wFKBP73, possessing three FK domains viz., wFK73_1, wFK73_2, wFK73_3 was resolved to 2.1 Å by the Single-wavelength Anomalous Diffraction method, and a second structure to 2.3 Å resolution by the molecular replacement method (Unger et al., 2010). wFK73_1 structure revealed the presence of 12 characteristic conserved residues necessary for FK506-binding which form a hydrophobic cavity for isomerase activity, whereas, wFK73_2 and wFK73_3 do not show conserved residues, thus, resulting in lack of PPIase activity. A conserved FK fold consists of 5-6 stranded anti-parallel β-sheets and α-helix sheet, but wFK73_2 domain has only one conserved amino acid Tyr at position 183 and wFK73_3 have five. This may be the reason for lack of PPIase activity, as narrow cavity was observed in wFK73_3 when compared to wFK73_1, whereas, no such cavity is formed in wFK73_2. wFK73_1 has conserved PPIase activity domain, whereas, wFK73_3 comprise of conserved acidic amino acid residues necessary for TPR domain interactions. wFK73_2 lacks both and, therefore, different structural features were observed for it. Structure sequence analysis led to the conclusion that wFK73_2 is the most divergent domain in wFKBP73.

In living cells, intracellular pH is maintained near neutrality so that it is compatible with other cellular functions (Hochachka and Somera, 2002). Intracellular acid stress was reported to induce expression of ROF2 (Bissoli et al., 2012). ROF2 over-expressing lines demonstrated improved tolerance to acid stress, as indicated by the presence of normal green, expanded cotyledons in germinating seeds, which was attributed to enhanced K⁺ uptake, resulting in depolarizing of the plasma membrane and indirect activation of the electrogenic H⁺-ATPase. Induction of chaperones, such as ROF2, under intracellular acid stress condition protects the cellular proteins against denaturation and also activates H⁺ extrusion that restores intracellular pH. The role of ROF1/ROF2 in acid stress tolerance was further validated by analysing ROF2/ROF1 double mutant T-DNA lines, which showed impaired germination as
compared to ROF2 overexresser lines (Bissoli et al., 2012). Differential expression of
FKBPs in different stress conditions viz., heat, cold, salt and drought has also been reported
in maize. Quantitative real-time PCR analysis revealed suppression of ZmFKBP12, -13, -15-
3, -16-1 and -57 under cold stress, whereas, the accumulation of transcripts corresponding to
ZmFKBP19, -42, -53a, -53b was enhanced. Heat stress also resulted in enhanced expression
of ZmFKBP16-2, -17-2, -42, -53a, -62a, -62b, -72, -16-4, -15-3 and -18 but with different
kinetics (Yu et al., 2012). These observations suggest that the aforementioned multidomain
FKBPs may be implicated in heat stress by regulating the target proteins, since, these proteins
possess Arg/Lys rich N-terminal domain, which is a site for protein-protein interactions
(Bedford et al., 2000; Boisvert et al., 2005). The FKBPs genes in maize are also responsive to
salt- (ZmFKBP20-1, -53a and -62a), and drought stress (ZmFKBP15-2, -19, -20-1, -20-2, -
42, -53a, -57, -62a) (Yu et al., 2012). These observations indicate that FKBPs in plants play
vital role under different abiotic stress conditions.

2.5 Role of FKBPs in plant development

FKBPs have been demonstrated to play crucial role in plant development also. FKBP12, the
smallest FKBP present in plants and localized in cytosol, interacts with FKBP 12 interacting
protein 37 (FIP37) through its FKBD (Faure et al., 1998; Vespa et al., 2004). FIP37 is a
DNA-binding protein, which is involved in embryo development, and mutation in this gene
results in embryo lethality. FKBP12 was also implicated in pollen tube development through
its interaction with histone-associated transcription factor (HAP5) in Picea wilsoni (Yu et al.,
2011).

A genetic screen to identify cytokinin hyper responsive mutants led to identification
of a Pasticcino1 (pas1) gene in Arabidopsis (Vittoriosi et al., 1998; Faure et al., 1998). The
pas1 mutant is disrupted in gene encoding FKBP72 which comprises of three FKBP12-like
domains along with calmodulin-binding and TPR domain. The mutants showed ectopic cell
division and defects in differentiation in all tissues except roots. Formation of callus like
structure was observed in mutant plants on application of cytokinin, suggesting that PAS1
downregulates cell proliferation but positively regulates the cell differentiation (Vittoriosi et
al., 1998; Harrar et al., 2003). Yeast two-hybrid studies revealed interaction of PAS1 with
other proteins like FKBP-associated NAC-like (FAN) transcription factor via its CaM-
binding domain. Overexpression of FAN partially compensated for the deformities in
development of leaf becaused of uncoordinated and ectopic cell division in the apical
meristem and leaf primordial (Faure et al., 1998; Harrar et al., 2003). This interaction was further validated by pull-down assays, wherein PAS:GFP was recovered from maltose binding protein:FAN column, and fluorescence resonance energy transfer (FRET) analysis, which demonstrated that PAS1 facilitates translocation of FAN to cell nucleus, thereby, regulating the cell proliferation (Smyczynski et al., 2006).

T-DNA insertion in a 42 kDa FKBP in Arabidopsis resulted in isolation of another mutant, twisted dwarf 1 (TWD1), which exhibited smaller plant size, reduced cell elongation and helical rotation of roots and shoots (Kamphausen et al., 2002; Geisler et al., 2003). Lin and Wang (2005) provided evidence that FKBP domain of TWD1 interacts with four ATP-binding cassette (ABC) transporters, PGP1 and PGP19, in the plasma membrane. Similar interactions were observed with ABCC multidrug resistance associated proteins, MRP1 and MRP2, in the tonoplast (Geisler et al., 2004). Both the interactions occur via TPR domain of TWD1. Recent studies by Wu et al. (2010) have shown that TWD1 is necessary for localization of ABC transporters, which are also required for polar auxin transport (PAT) in Arabidopsis roots. Auxin is a known central regulator of plant growth and development (Woodward and Baetel, 2005). Confocal microscopy of fluorescently tagged fusion proteins and transmission microscopy of TWD1 plants revealed mislocalization of members of ABC transporters in ER instead of plasma membrane. These studies suggest that the abnormal spread of auxin signalling into the elongation zone of plant roots is due to mislocalization of ABC transporters, thus, causing twisted roots, a characteristic TWD1 phenotype (Wu et al., 2010). NMR analysis revealed the presence of a C-terminal in-plane membrane (IPM) anchor (Bailly et al., 2013), which is implicated in regulating ABCB-mediated auxin transport. Contrary to twd1 loss-of-function alleles, TWD1 gain-of-function lines lacking a putative in-plane membrane anchor (HA-TWD1-Ct) showed increased stem length and leaf surface but reduced shoot branching in comparison to wild type. This increase in shoot length was correlated to reduced polar auxin transport, since it leads to enhanced auxin levels and, thereby, increased hypocotyl length. The importance of C-terminal membrane anchoring of TWD1 for regulation of ABCB1-mediated export of auxin, which leads to cell elongation, was demonstrated recently (Bailly et al., 2013).

AtFKBP42 is a 365 amino acid long protein with single FKBP domain, TPR motif, putative CaM-binding site and hydrophobic C-terminus. This protein does not show any PPIase activity (Geisler et al., 2003). Domain interface is composed of hydrophobic network
surrounded by hydrogen bonds and electrostatic bonds. FKBP42 crystal structure was determined at 2.85 Å resolution. The N-terminal part of this protein show FKBP folding as 5-stranded anti-parallel β-sheet wrapped around α-helix, whereas, C-terminus represents helical region. AtFKBP42 protein is unfolded at physiological temperature but helical conformation is stabilized by HSP90 peptide binding (Grazin et al., 2006). FKBP domain interacts with ABC transporters, AtPGP1 and AtPGP19, which are plasma-membrane localized, whereas, TPR domain is associated with vacuolar transporter AtMRP1 and AtMRP2 (Geisler et al., 2004; Lin and Wang, 2005).

2.6 FKBPs are involved in redox control of photosystem

Several biochemical and proteomic analysis studies have shown that majority of FKBPs in higher plants reside in thylakoid membrane and lumen, and have cleavable signal peptide for chloroplast localization (Romano et al., 2005). Yeast two-hybrid assays revealed that Rieske-FeS protein, a subunit of the cytochrome bf complex in photosynthetic electron transport chain, interacts with AtFKBP13 (Gupta et al., 2002). Rieske protein interacts with precursor of AtFKBP13 and the import in chloroplast thylakoid is pH-dependent (Keegstra and Cline, 1999). AtFKBP13 interaction results in down-regulation of the Rieske protein accumulation, since, suppression of AtFKBP13 expression leads to increase in level of Rieske protein. In contrast to earlier reports (Gupta et al., 2002), luminal mature TaFKBP13, instead of precursor FKB13, was demonstrated to be involved in the interaction with Rieske protein (Gollan et al., 2011).

TaFKBP13 physically interacts with pro-loop (globular domain containing ‘Gly-Pro-Ala-Pro’ motif) as a substrate, which may regulate Rieske cluster by catalysing transition state intermediates to bf associated complexes (Gollan et al., 2011). In chloroplast lumen, AtFKBP13 and AtCyp20-2 are the only known active immunophilins (Shapiguzev et al., 2006; Edvardsson et al., 2007). These are also redox-activated, a feature controlled by C-terminal disulfide bonds (Gopalan et al., 2004). X-ray crystallography revealed unique disulphide bonds at residues Cys-5, 17 and Cys-106,111 (Gopalan et al., 2004). These residues are absent in animal homologues. AtFKBP13 comprises of six β-strands and two α-helices, where β-strands forms an integral β sheet that constitute the core of protein. Crystal structure analysis also demonstrated that these conserved amino acids are involved in binding and maintaining the hydrophobic core for binding (Radzicka et al., 1992). The disulphide bonds forms secondary structure which are located on either side of β sheet, reflecting
intrinsic versatility and flexibility of the region. AtFKBP13 is activated by oxidation in chloroplast lumen. These structural features correlate with its redox regulation in the thylakoid lumen and drastic reduction in PPIase activity was observed due to mutation in these cysteine residues (Gopalan et al., 2004).

Another chloroplast luminal FKBP, AtFKBP20-2, also plays a vital role in Photosystem II (PSII), which catalyzes the first two photosynthetic reactions that convert sunlight to chemical energy. Knockouts of AtFKBP20-2 (generated by t-DNA insertions) showed reduction in PSII supercomplexes and an increase in unassembled PSII monomers and dimers, which was also associated with lower rate of PSII activity in mutant plants. These observations suggest a role for plant FKBP20-2 in normal plant growth by regulating PSII supercomplex assembly and stability (Lima et al., 2006).

A thylakoid localized FKBP, TaFKBP16-1, was also demonstrated to interact with Psa-L, a subunit of Photosystem I (PSI), suggesting this protein to be involved in PSI assembly during thylakoid biogenesis (Gollan et al., 2011). Another isoform, FKBP16-3, showed interaction with thylakoid formation-1 (Thf1) and PSI-2 (APO2) and was proposed to play an important role in maturation of photosynthetic membranes. Thf1 regulates the chloroplastic FtsH protease complex, which is crucial for membrane biogenesis, whereas, APO (accumulation of photosystem one 2) proteins are involved in early assembly of [4Fe-4S]-containing proteins i.e. PsaA, PsaB, ferridoxin thioredoxin reductase (FTR) and NDH (NADH dehydrogenase-like complex) through cluster-binding during translation (Amann et al., 2004). These studies suggest that TaFKBP16-1 and TaFKBP16-3 may be playing chaperonic roles in the assembly of photosynthetic and thylakoid membrane complexes.

The role of FKBPs has also been suggested in photosynthetic electron flow regulation. NADPH dehydrogenase-like complex (NDH) is also known to be involved in cyclic electron flow (CEF) in PSI (Peng et al., 2009). Suppression of a luminal protein, AtFKBP16-2, by using RNA interference (RNAi) revealed its role in NDH assembly and also for NDH-PSI supercomplex formation that directs CEF. FKBP16-2, along with FKBP13, in plants have a conserved pair of cysteine residues, which form disulfide bonds only under oxidising conditions, which stabilize the active site and allows substrate binding (Gopalan et al., 2004, 2006). These results imply that thylakoid redox signals governs the substrate-binding in both the proteins (Iwai et al., 2010).
2.7 FKBPs as molecular chaperones

Chaperones are proteins that recognize non-native proteins, prevent unwanted inter- and intramolecular interactions, and influence the partitioning between the productive and unproductive folding steps (Hendrick and Hartl, 1995; Beissinger and Buchner, 1998). Recent studies have revealed a number of interacting partners for immunophilins, suggesting their role as chaperones. The mammalian FKBp, mFKBP52, possesses chaperonic activity in vitro, as it was able to suppress the aggregation of chemically and heat denatured citrate synthase (CS). Since this activity was not disrupted in presence of FK506 or rapamycin, it was concluded that the chaperonic activity is independent of PPIase activity. mFKBP52 also affected the reactivation of CS by increasing the level of reactive intermediates (Bose et al., 1996). Chaperonic activity was also demonstrated for E. coli immunophilin, FkpA, present in periplasm and induced under heat stress (Ramm and Pluckthun, 2000). It was observed that FkpA improved the expression of antibody single chain Fv, which is involved in early folding of intermediates. This interaction prevents aggregation and possesses the ability to relicense the inactive proteins. Similar activity was also observed for a 23.8 kDa archael FKBp, isolated from Methanobacterium thermoautotrophicum, which suppressed the aggregation of unfolded rhodanase and also provided protection from thermal denaturation (Ideno et al., 2000). wFKBP73 also showed chaperonic activity which was independent of PPIase activity. Contrary to wFKBP73, which interacts with intermediates and slows the inactivation process, the mammalian orthologue, mFKBP52, does not affect the rate of inactivation (Kurek et al., 2002b). The crystal structure of FKBp51 was resolved to 2.7 Å in human, and to 2.8 Å in squirrel monkey (Sinars et al., 2003). hFKBP51 consists of two close FKBp domains. The FK1 domain of mFKBP51, a homologue of hFKBP51 showed PPIase activity with inhibition constants (K_i) being 10 nM and less than 5 nM for FK506 and rapamycin, respectively. FK2 domain mainly contributes towards functionality aspect, as it interacts with other proteins via TPR region (Yeh et al., 1995; Sinars et al., 2003). FKBp51, along with FKBp52, participate in interaction of GR with HSP90, HSP70 and HSP40. The PPIase activity was localized to domain I, whereas, chaperonic activity was attributed to domain III towards C terminus, which has TPR domains that are involved in protein-protein interactions (Sinars et al., 2003).

FKBPs constitute an important gene family in plants, as they not only catalysse folding of proteins but are also involved in regulatory system via chaperonic activity, participate in
redox control of photosystem, control the plant development and signalling, perform housekeeping, and provide protection against different environmental stresses. However, the role of these genes in deposition of storage proteins in wheat grain has not been investigated as yet. The present study was, therefore, initiated to identify and clone FKBP genes in wheat and characterizes their biochemical activity.