REVIEW OF LITERATURE
Homeodomain Class of Transcription Factors

Introduction

Transcription factors are proteins that bind to DNA and regulate gene expression by controlling the process of transcription. They bind at the enhancer sites or to other proteins present in the promoter regions and thus their interaction regulates gene expression. Till date, many transcription factors have been identified in both animal and plant kingdom. Some transcription factor families characterised in plants till date include MYB, WRKY, NAC, b-ZIP, MADS, AP2, GRAS, b-HLH and zinc finger family (Qu et al., 2006). They have been ascribed roles in controlling plant growth and development by regulating processes like seed germination, photomorphogenesis, hormone signalling, defence responses and stress responses.

Amongst the different types of transcription factors are a group of homeodomain proteins that have been shown to be present in plants, animals and fungi (Bharathan et al., 1997). They regulate gene expression of their target genes both positively and/or negatively by binding to their cis-regulatory regions (Gehring et al., 1990). The genes encoding for these proteins are known as homeobox genes, which have been named so because they all harbour a 180 bp sequence called homeobox that encodes for a 60 amino acid long DNA binding homeodomain. Apart from this typical homeodomain is also present in some proteins another atypical homeodomain that is variable in length, being 65 amino acid long, having three amino acids extra inserted between helix 1 and 2 (Burglin et al., 1994; Burglin et al., 1995; Bertolino et al., 1995; Chan et al., 1998).

An interesting observation was made in early 1980’s where it was found that a mutant Drosophila melanogaster had legs on its head, i.e. at position where antennae were supposed to be. Later, the Antp gene, responsible for this homeotic transformation was cloned and it was found to have this 180 bp homeobox sequence (McGinnis 1984; Scott et al., 1984). Thus, homeobox genes were identified as factors important in maintaining homeosis. Till date, homeobox genes have been identified and studied from various other organisms as well. In human beings a total of 300 homeobox loci with 235 functional genes have been reported.
Among different plant species, there are 110 homeodomain genes in *Arabidopsis thaliana*, 117 genes in *Oryza sativa*, 149 in *Populus trichocarpa*, 45 in *Selaginella moellendorfii* and 66 in *Physcomitrella patens*. Among algae, green alga *Chlamydomonas reinhardtii* has a total of five homeobox genes and red alga *Cyanodioschyzon merolae* has six genes as summarised in a recent paper by Mukherjee et al. (2009). Present review focuses on different aspects of plant homeobox genes like their structure, classification, evolution and functional roles.

**Discovery of homeodomain proteins**

The very first report in plants came way back in 1991 by Vollbrecht et al. where they isolated maize *knotted1* gene through transposon tagging and it was seen that dominant gain of function mutation in this gene affected the leaf development, leading to the formation of finger-like outgrowths or knots on its leaf blades. On further analysis it was found that the gene encodes for a homeodomain containing protein designated as *Kn1*, and it acts as a transcription factor. (Freeling et al., 1985; Vollbrecht et al., 1991; Hake et al., 1992). Thus, *kn1* was used to isolate more members of homebox gene family from maize that have been classified as knox genes, i.e. knotted-like homeobox genes (Kerstetter et al., 1994). Since then many other homeobox genes have been isolated and characterised from other plant species like *Arabidopsis*, rice, carrot, soybean, apple, tomato and barley.

**Basic structure of homeodomain proteins**

The homeobox is a 180 nucleotide long consensus sequence which encodes for a 60 amino acid long homeodomain that binds to DNA in a sequence specific manner. Following is the consensus sixty amino acid sequence known as the homeobox domain.

\[
\text{RRRKRTA-}
\begin{array}{l}
\text{YTRYQLLE-LEKEFL-}
\end{array}
\begin{array}{l}
\text{NRYLRRRRIELAHSL-NLTERHIWFON-RRMK-}
\end{array}
\begin{array}{l}
\text{WKKEN}
\end{array}
\]

The homeodomain forms an alpha-helical structure composed of three helices where α-helix 3 binds to the major groove of DNA in sequence-specific manner (Gehring et al., 1994). As mentioned in later part of review, a class of homeodomain
proteins contain plant-specific leucine zipper motif present adjacent to the homeodomain. This leucine zipper is a short peptide that consists of four to six heptad repeat of leucine residues designated \((abcdefg)_n\) with a characteristic pattern of hydrophobic and hydrophilic residues (Landschulz et al., 1988; McLachlan et al., 1975). They all have \(\alpha\) helical domain that forms a coiled coil structure while binding to DNA. The d-position is always occupied by a leucine and a position by \(\beta\) branched residues (Hurst et al., 1994). The residues a, d are hydrophobic, present on same side of one helix and they interact with other hydrophobic residues of second helix and thus stabilise the dimer. Dimerisation is also affected by other residues present at e and g positions which form complimentary charge interactions (Szilak et al., 1997). Thus, leucine zipper present downstream of the homeodomain (HD) is necessary for dimerization of HD-ZIP proteins. The position of zipper and homeodomain is critical as the distance between them if modified affects DNA binding (Sessa et al., 1994).

**Figure 1:** Three dimensional structure of *Drosophila* Homeodomain Engrailed protein. Homeodomain in form of helix-loop-helix binds to DNA major groove. (DNA double helix shown in brown, \(\alpha\)-helices are in grey. Amino acid residues important for base-specific contacts to DNA and main determinants of binding specificity are shown in red and remaining in yellow). (Adapted from Kissinger et al., 1990).
Classification of plant homeodomain proteins

Till date, various schemes of classification have been proposed for these group of transcription factors, wherein different homeobox genes have been classified according to their domain composition, i.e. position and nature of different amino acids present within the homeobox domain and also if any additional domains exist alongwith this signature motif, in these transcription factors. Chan et al. (1998) aligned different plant homeobox genes according to their composition and divided into five major families, i.e. HD-ZIP, GLABRA, KNOTTED, PHD and BELL. Another scheme of classification came up next in 1997 by Bharathan et al., where they proposed seven different families of homeobox transcription factors which were KNOX, BELL, HD-PHD finger, HAT1, HAT2, GL2 and ATHB8.

However, on detailed sequence analysis, it was found that in some of the above listed families there was present a leucine-zipper motif next to the C-terminus of homeodomain, therefore, these class of genes have been renamed as HD-ZIP gene family (Ruberti et al., 1991, Schena et al., 1992). They have been further divided into four subfamilies HD-ZIP I, HD-ZIP II, HD-ZIP III and HD-ZIP IV, according to their sequence composition and functional roles they play in the plant kingdom (Meijer et al., 1997, Aso et al., 1999, Sakakibara et al., 2001). Genome wide analysis of rice homeodomain genes was done and they were classified into ten sub-families based on their domain composition and phylogenetic relationships which included HD-ZIP I, II, III, IV, KNOX I, II, BLH, WOX, ZF-HD and PHD family (Jain et al., 2008).

Recently, a more comprehensive classification has been proposed for all plant homeobox genes by Mukherjee et al. (2009) where they have analysed ten complete genomes of different angiosperms, moss, Selaginella, unicellular green algae and red algae. They have proposed a new scheme of classification where they have divided all homeobox genes into 14 different classes with each having unique structural features. Various sub-classes of homeobox genes are described below with some of their characteristics (also shown diagrammatically in Figure 2)

**HD-ZIP Superclass** comprises of all the four previously characterised HD-ZIP I, II, III and IV families (Sessa et al., 1994). As mentioned, they all are identified by the
plant specific leucine zipper motif (HALZ-Homeodomain associated leucine zipper) adjacent to the C-terminus of homeodomain (HD). Apart from the leucine-zipper, class II members have a CPSCE motif in them (Chan et al., 1998) that has a role in redox regulation (Tron et al., 2002) and a ZIBEL domain for interaction with other target proteins (Mukherjee et al., 2009). The class III members have a START (Steroidogenic Acute Regulatory protein related lipid transfer) domain that has lipid binding capability (Ponting et al., 1999; Schrick et al., 2004) and MEKHLA domain with light, oxygen and redox sensing capability (Mukherjee et al., 2006) and an additional SAD (START associated conserved domain) with transcriptional activity (De Castecker et al., 2000). The genes belonging to class IV family also have START and SAD domains in them. HD ZIP III members have extra four residues insertion between helix 2 and helix 3 as seen by alignment of different HDZIP members. The HD-ZIP superclass is the largest among all other families and it makes up of around 40-50% of total homeodomain genes present within the plants. Strikingly, no HD-ZIP member has been reported to be present in green algae or red algae (Mukherjee et al., 2009). Recently, 63 HD-ZIP members have been identified from *Populus trichocarpa* and classified into different subfamilies based on their amino acid composition (Hu et al., 2011).

**PLINC ZINC Finger Class** has plant specific Zinc Finger motifs or better known now as a PLINC domain upstream of the homeodomain and it is different from zinc finger class of proteins found in animals (Burglin et al., 1994). Other characteristic structural features of this class of proteins are presence of one extra amino acid between helix 1 and helix 2 and presence of a methionine at F49 which is otherwise a conserved homeodomain residue (Mukherjee et al., 2009). Apart from this, it has also been seen that no members of this class has been reported in unicellular algae. It has been found that the zinc finger domain is involved in protein-protein interactions by mediating homodimer or heterodimer formation and also regulate DNA binding activity of the adjacent homeodomain present (Windhovel et al., 2001).

**WOX Class** has a WUS box motif at C-terminus of the homeodomain. The motif has been found to regulate shoot stem cells and floral patterning (Haecker et al., 2004; Ikeda et al., 2009). In some of *Arabidopsis* WOX proteins, an additional EAR motif has also been reported which acts as a transcriptional repressor (Graaff et al., 2009; Paponov et al., 2009). This class has been subdivided into different families based
on presence or absence of various amino acid residues. Interestingly, WOX genes have been reported in all plant species, moss, *Selaginella* and unicellular green algae *Ostreococcus* sp. but none of them is present in *Chlamydomonas reinhardtii* (Mukherjee et al., 2009). As far as the structural features are concerned, the WOX members also have extra two residues between helix 1 and helix 2 and four to five extra residues between helix 2 and helix 3 (Mukherjee et al., 2009). Recently, a comprehensive genome wide analysis has been done for all the WOX family members in rice, sorghum, maize, poplar and *Arabidopsis* and then grouped into different subgroups according to their homeodomain amino acid composition. The presence of conserved WUS (Wushel) box and EAR (the ERF-associated amphiphillic repression) like motif was observed in all the subgroups (Zhang et al., 2010).

**TALE Superclass** is a combination of two major and extensively studied homeodomain proteins, namely KNOX and BEL proteins. Both the groups have an extra three residue insertion between their helix 1 and helix 2 and they both are the most primitive members amongst the whole class of homeodomain genes (Bertolino et al., 1995; Burglin et al., 1997).

Members of KNOX class have been divided into two families, KNOX I and KNOX II (Kerstetter et al., 1994), and then these families have been further divided into subclasses namely STM, KNAT2/6, KNAT1, KNAT7 and KNAT3/4/5 (Mukherjee et al., 2009). As far as the protein composition is concerned, KNOX family members have a characteristic KNOX domain which is further divided into two blocks, KNOX A and KNOX B, with a variable length region in between (Burglin et al., 1997), and an ELF motif (Vollbrecht et al., 1991) alongwith the homeodomain present at the C-terminus. Recently, Mukherjee et al. (2009) reported that there is a 30 amino acid insertion in KNOX B of KNOX II family and also there was presence of a conserved 20 amino acid KNOX II C motif in all KNOX II members. The primary role of KNOX domain is in binding with other homeodomain proteins like BEL family members and thus regulate developmental processes in plants.

The BEL class members have been characterised to have a SKY domain and a BELL domain in addition to the homeodomain present in them (Bellaoui et al., 2001; Becker et al., 2002). However, recently, another new domain within this class
of proteins has been found, it was named as ZIBEL domain. It was present at both the ends of BEL proteins in all subclasses except ATH1 subclass members which also lack the SKY domain (Mukherjee et al., 2009). Various new subclasses have also been identified and added to this class of homeodomain proteins. Moreover, it can be said that all these domains identified within them are important as they all play a role by mediating interaction with other KNOX members and regulate plant development.

**DDT Class** has been recently added to the family of homeodomain proteins, although DDT domain (after the better characterized DNA-binding homeobox containing proteins and the different transcription and chromatin remodelling factors in which it is found) was discovered long back and was present in a group of homeodomain proteins next to the homeodomain (Doerks et al., 2001). Detailed alignment analysis revealed presence of more motifs present throughout the length and have been named as D-TOX A to G respectively. Primary role of this domain present within the homeodomain proteins and other transcription factors is DNA binding. Different proteins have been reported containing this domain in all organisms except none have been found in red algae (Mukherjee et al., 2009).

**PHD Class** has been one of the oldest amongst all other homeodomain proteins which has a PHD domain present upstream of the homeodomain (Plesch et al., 1997; Schindler et al., 1993). In recent analysis by Mukherjee et al. (2009), they have divided the family into two subclasses based on their amino acid composition and have even identified an extra 90 amino acid long motif present adjacent to PHD domain that they named it as PEX-PHD, which is plant specific.

**NDX Class** is a small class that is expressed in nodules and also amongst the oldest class found long back in some plant species (Jorgensen et al., 1999). Further detailed analysis has revealed that they have an insertion of 6 amino acids between helix 2 and helix 3. They all harbour an atypical type of homeodomain and alongwith it are present two NDX (Nodulin homebox genes) domains namely NDX-A and NDX–B. Moreover, it was found that no NDX gene is present in unicellular green algae or red algae but members have been found in other plant species (Mukherjee et al., 2009).
**LD Class** is another class with a very few members present in different plant species like *Arabidopsis* and maize (Van Nocke et al., 2000). No members have been reported in either green algae or red algae. They all harbour a homeodomain and additional LD domain that has been recently found to be present in more than a single copy, thus named as LD1 to LD5, respectively. Detailed structural analysis also revealed certain unusual substitutions within the homeodomain and even within LD3 a region, which was renamed as LUMINI domain characteristic of this family (Mukherjee et al., 2009).

**PINTOX Class** is one of the newly characterised classes of proteins that have an additional PINTOX domain and an acidic Acid Pint domain apart from the characteristic homeodomain present downstream. Moreover, members have been found in all plant genomes and algae too. Another feature found after analysing in detail is the substitution of N15D residue of the homeodomain. No specific function has been assigned to this newly identified domain (Mukherjee et al., 2009).

**SWADEE Class** has a 130-140 amino acid long SWADEE domain present at C-terminus of the homeodomain. Another important feature found in these newly identified class of proteins is an insertion of ten amino acids between helix 2 and helix 3 (Mukherjee et al., 2009). The function of SWADEE domain is not known yet but since there are many cysteine and histidine residues within so it is speculated to have a role in binding. However, in a recent study two *Arabidopsis* members SWADEE HOMEODOMAIN HOMOLOG 1(SHH1) and SWADEE HOMEODOMAIN HOMOLOG 2 (SHH2) have been identified. They analysed that in shh1 mutant both DNA methylation levels and siRNA levels were decreased. Moreover, it was also shown to be co-immunoprecipitated with NRPD1 (Nuclear RNA Polymerase D1), i.e. a RNA Polymerase IV subunit. Thus, it was concluded that SHH1 plays a role in RdDM (RNA-directed DNA methylation) pathway although the exact role of homeodomain and SWADEE domain still needs to be examined (Law et al., 2011).
Evolution of plant homeodomain proteins

Genome duplications have occurred for different plant species and have been accompanied by gene duplication and loss as well, thus accounting for variable number of homebox genes in different species, their number being more in more complex and highly developed ones (Adams et al., 2005; Rensing et al., 2008). In a recent report by Mukherjee et al. (2009), an extensive search and comparison of different homeobox genes was done. They analysed different genomes of different phyla including angiosperms, Tracheophyta (Selaginella sp.), Bryophyta (moss), Chlorophyta (unicellular green algae) and Rhodophyta (red algae). They have identified some new classes and subclasses of homeobox genes in addition to

Figure 2: Different classes of plant homeodomain proteins with their domain compositions. All classes have the characteristic homeodomain (in black) and along with are present co-domains characteristic of each class (Adapted from Mukherjee et al., 2009).
earlier known ones, all having some characteristic domains and motifs in them. Further, it has been found that of all the classes, TALE class is the oldest one with representative members present in all different phyla analysed. However, PLINC zinc finger, WOX, HD-ZIP, DDT, NDX, LD and SAWADEE class members are absent in either Rhodophyta or Chlorophyta or both of them but have their representative members in flowering plants and moss suggesting that they originated early during evolution before separation of moss and vascular plants. However, PINTOX genes have been found to be present in green plants and green algae thus may have originated before divergence of Chlorophyta and Streptophyta.

There are many reports on the evolution of HD-ZIP class suggesting that there is a difference in rate of evolution between its different subclasses as well. It has been found that genes belonging to HD-ZIP I subclass have evolved at a faster rate than others (Sakakibara et al., 2001). HD-ZIP class members are present in flowering plants, moss and Selaginella but not in green algae or red algae with an exception of one HD-ZIP III gene in alga Chara coralline (Floyd et al., 2006) suggesting that a parental gene was present in the last common ancestor of Charales and land plants, before separation of moss, Tracheophyta and vascular plants, which then gave rise to newer subclasses.

Thus, a model has been proposed by this group depicting evolutionary history of plant homeobox gene classes and co-domains. On the basis of their analysis, it has been concluded that all the 14 classes were present during the evolution of land plants before separation of moss and vascular plants. However, with the increase in complexity of organisms the number of homeobox genes has increased with acquisition of new domains and structural modifications. So there has been proliferation of homeodomain gene paralogs within each class in different flowering plants.

**Structural features and binding properties**

It has been well established that the HD-ZIP class of homeodomain proteins bind to DNA as dimers where both the monomers bind to a specific DNA sequence (Palena et al., 1999) Various in vitro studies like by chromatin-immuno precipitation and DNA footprinting assays have been done to characterise the binding site sequences. HD
ZIP I and HD ZIP II members show high specificity for sequence CAAT (A/T) ATTG or CAAT(C/G) ATTG, also named as binding site 1 and binding site 2, respectively, and HD-ZIP III binds to sequence GTAAT (G/C) ATTAC (Sessa et al., 1994, Sessa et al., 1997). Later, a group of researchers studied that the nature of amino acids which compose the leucine zipper of these homeodomain proteins had a great effect on the dimerisation capability of these proteins. They found that in sunflower hahb-1 and hahb-10, when conserved leucine and threonine present at a1 and d1 position if exchanged or modified, then the dimer formation was hampered (Gonzalez et al., 1997).

It has been deduced that not only the sequence composition of binding site but homeodomain and leucine zipper composition affects the binding between DNA and the protein. It was seen that some conserved cysteine residues are present among the variable residues within the dimerisation motif. Further, it was seen for Hahb-10, a HD-ZIP II member, that these cysteines affected DNA binding of the transcription factors by regulating the redox conditions (Tron et al., 2002). Park et al. (2007) isolated zinc finger homeodomain proteins from soybean that could bind to stress inducible GmCAM-4 promoter. Later, by binding assays, same group has shown that the homeodomain binds to ATTA sequence and an AG present within 30 nucleotide A/T rich sequence within the GmCAM-4 promoter.

Similarly, homodimerisation and heterodimerisation between KNOX-KNOX and KNOX-BELL family members, respectively, has been demonstrated in different plant species (Bellaoui et al., 2001; Muller et al., 2001; Smith et al., 2002; Smith et al., 2003). Studies have revealed that MEINOX domain is a conserved domain present in both plants and animals and it is responsible for KNOX protein interactions (Burglin et al., 1998). Other homeodomain family members like WUSCHEL (WUS), WUSCHEL-related homeobox (WOX) and PRESSED FLOWER (PRS) have also been speculated to form similar homo-dimers and hetero-dimers which is important in their regulation and for their functioning (Haecker et al., 2004; Nagasaki et al., 2005)
Role of homeodomain proteins in plants

Different approaches have been followed to know more functional roles of homeodomain proteins in plants. Over-expression, silencing, and/or mutational studies have been done and it has helped in finding out the role of these transcription factors in regulating plant growth and development. Various roles are discussed in following pages, citing importance of these transcription factors in regulating different aspects of plant development.

Role in photomorphogenesis

Light regulates plant growth and development right from early stage of seed germination to its flowering. There is a marked difference between the phenotype of a light-grown seedling and a dark-grown seedling. It is well-known that light promotes differentiation of etioplasts into chloroplasts together with promoting expression of different development related genes and thus is necessary for leaf development. It also affects other aspects of plant development, including phototropism, stomatal movement, stem growth and floral initiation. Various receptors like phytochromes, cryptochromes and phototropins are known that perceive light of different spectral regions and regulate diverse plant responses (Quail et al., 1991; Ahmad and Cashmore 1993; Khurana et al., 2009).

ARABIDOPSIS THALIANA HOMEobox 1 (ATH1) gene has been found to be light inducible as its mRNA levels increased on transferring the seedlings from dark to light. Moreover, it was observed that in dark-grown (constitutively photomorphogenic) cop and (de-etiolated) det mutant seedlings, the expression of ATH1 mRNA was higher, thus emphasising that it may act downstream of COP1 in the signal transduction pathway (Quaedvlieg et al., 1995).

When a plant senses shade of another plant, there is a change in ratio of far-red light to red light perceived by it through the photoreceptor phytochromes and a response known as shade avoidance syndrome (SAS) is induced which is characterised by enhanced elongation, decreased branching, increased apical dominance and an early flowering (Smith et al., 1995). It is known that of all the phytochromes present, PhyB has major role to play in this shade avoidance phenomenon. It regulates the expression of PAR (Phytochrome Rapidly Regulated)
genes like $ATHB4$, $HAT2$, $HFR1$ and $PAR1$ (Roig et al., 2006). Over-expression of
$PAR$ genes affected hypocotyl elongation and root development as reported for
$HAT1$ and $HAT2$ (Ciarbelli et al., 2008; Sawa et al., 2002). Another example is
$AtHB2/HAT4$, a HD-ZIP II member, which was shown to be up-regulated by far-red
light (Carabelli et al., 1993). Over-expression of the gene resulted in phenotypes
such as longer hypocotyls and plants with lesser leaf expansion (Schena et al.,
1993), which was later shown to be controlled by auxin (Steindler et al., 1999).
Further, it was reported that $AtHB2$ mediates light responses by negatively
regulating itself via a negative auto-regulatory loop (Ohgishi et al., 2001) and it
induces shade avoidance response as well (Morelli et al., 2000). Kunihiro et al.
(2011) have proposed that ARABIDOPSIS THALIANA HOMEOBOX PROTEIN 2
($ATHB2$) has a diurnal expression pattern where its transcript is induced in night and
it is dependent on $\beta$-helix-loop-helix transcription factors PHYTOCHROME
INTERACTING FACTOR 4 ($PIF4$) and $PIF5$ in short days. Moreover, they supported
it by immunoprecipitation assay that $PIF5$ binds to G-box regions present upstream
of $ATHB2$. Similar reports have been published for $ATHB4$, another HD-ZIP II
protein, that forms a negative regulatory loop and integrates shade avoidance
response and hormone BR response (Sorin et al., 2009). $AtHB16$, a HD-ZIP I
member, which is expressed mainly in leaves, has also been shown to regulate blue
light responses like hypocotyl growth inhibition and is thus proposed to act
downstream of the blue light receptors $CRY1$ and/or $CRY2$ (Wang et al., 2003).
Other HD-ZIP members, like $AtHB52$, $AtHB5$ and $AtHB6$ have also been found to be
up-regulated in dark conditions (Henriksson et al., 2005), thus implicating their role
in photomorphogenesis and plant development.

Similar reports have also been published from other plant species. For
example $Hahb-10$ from sunflower when overexpressed in Arabidopsis gave
transgenics which had leaves with altered shape, form and colour. They had a short
life cycle and an early flowering was seen in the transgenics as compared to the
wild type plants. The transcript levels of light inducible genes were also lower in
$Hahb$ over-expressors, thus giving a hint that the gene may have a role in controlling
light responses (Rueda et al., 2005). $Hahb4$, a HD-ZIPI member from sunflower,
was also reported to be up-regulated in darkness, transcript remained constant
throughout night in photosynthetic tissues and then declined during the day
(Manavella et al., 2008). In potato, StBEL5, a member of BELL1 family, plays a role in tuber formation and its growth. There was a reduction in GA levels in the transgenics over-expressing this gene (Xu et al., 1998; Rosin et al., 2003; Kloosterman et al., 2007). Later, it was observed that the promoter of StBEL5 was light-inducible both by red light and blue light (Chatterjee et al., 2007). Recently, it has been reported in maize that HD-ZIPI member, grassy tiller (gt), regulates lateral branching. It was shown that in response to shade there was an increase in gt level and it acts downstream of teosinte branched 1 (tb1) and thus promotes bud dormancy and mediates shade avoidance response in maize (Whipple et al., 2011).

**Role in abiotic and biotic stress**

It has been well-established that plant hormone ABA is produced under stress conditions like water deficit, salt stress and cold (Lang et al., 1994). It helps the plant to overcome the stress by reducing its rate of transpiration. Among the different homeodomain proteins, Arabidopsis HD-ZIP members AtHB7 (Soderman et al., 1996) and AtHB12 (Lee et al., 1998) are induced by ABA or water deficit. AtHB6 has also been shown previously to be induced under water deficit conditions and osmotic stress which was dependent on ABI1 and ABI2 (Sodermann et al., 1999). The transgenic Arabidopsis plants over-expressing AtHB6 showed reduced inhibition of germination on ABA and reduced stomatal closure, thereby implying that this gene may act as a negative regulator during water stress (Himmelbach et al., 2002). Lechner et al. (2011) have recently shown that MATH-BTB group of proteins bind to ATHB6 and promote its proteasomal-dependent degradation via cullin3-ubiquitin ligase.

Similarly, AtHB7 overexpressing plants also had decreased stem growth, increased branching of inflorescence meristem and made them hypersensitive to ABA (Hjellstrom et al., 2003; Olsson et al., 2004). However, it was seen that ATHB5 over-expression transgenics showed enhanced sensitivity to ABA as observed by seed germination and plant growth of the transgenics (Johannesson et al., 2003). In a recent report, authors have compared the length of inflorescence of AtHB12 overexpressor and athb12 T-DNA insertional mutant and it was seen that decrease in the expression of GA20ox1 (gibberellin 20-oxidase1) by overexpression on AtHB12 resulted in shorter inflorescence as compared to that in mutant. It was thus
concluded that there was cross-talk between plant hormones via this homeodomain gene wherein ABA induced the levels of AtHB12, and which in turn affected GA biosynthesis thereby regulating the growth of the inflorescence (Son et al., 2010).

HOS9 is another nuclear-localised homeodomain transcription factor from Arabidopsis that has been reported to control cold tolerance. It was observed that in hos-9 (high expression of osmotically responsive genes) mutant there was an increased freezing stress tolerance. They concluded that it controlled the expression of genes involved in cold tolerance independent of CBF (C repeat-dehydration responsive element-binding factor) pathway (Zhu et al., 2004). Zinc finger homeodomain gene, ZFHD1, from Arabidopsis was cloned and found to be upregulated in drought, salt and ABA (Tran et al., 2006). Transgenics produced by its overexpression were also found to be tolerant to drought stress. Further, it was also shown that a 14 bp sequence which they named as ZFHDRS (zinc finger homeodomain recognition sequence) of this protein binds to promoter region of ERD1 (early responsive to dehydration stress1) gene (Tran et al., 2006). Zinc finger transcription factors are induced during floral development, pathogenesis, stress-like dehydration, salinity and ABA and recently AtHB29 was shown to interact with heavy metal induced protein HIPP26 during stress response in Arabidopsis (Tan et al., 2006; Tran et al., 2007; Park et al., 2007; Barth et al., 2009).

Members of homeodomain class from other plant species have also been studied and their role in abiotic stress analysed. HD-ZIP genes isolated from resurrection plant Craterostigma plantagineum, named as CPHB1 and CPHB2, were found to be induced by dehydration and out of the two CPHB2 levels increased by ABA treatment (Frank et al., 1998). Similarly, in sunflower, Hahb-4 was transcriptionally regulated by ABA (Gago et al., 2002) and when expressed under the control of 35S promoter in Arabidopsis displayed a retarded growth phenotype and plants were water-stress tolerant (Dezar et al., 2005). Transgenics produced by Hahb-4 over-expression under the control of its own promoter were also water stress tolerant (Manavella et al., 2006). Cotton HD-ZIP factor, GhHB1, was also reported to have an enhanced expression under salt stress conditions and in response to ABA (Ni et al., 2008). NaHD20 from Nicotiana attenuate is another HD-ZIP member for which transcript levels increased in response to water stress and caused ABA accumulation in the plant. Further, it was observed that NaHD20 also
controlled BA (benzylacetone) emission from plants and affected the flowering time in both NaHD20 over-expressors or NaHD20 silenced plants (Re et al., 2010). Rice OsBHID gene is a member of BELL family and when functionally analysed by its overexpression in tobacco imparted reduced tolerance to salinity and oxidative stress in the transgenic tobacco plants (Luo et al., 2005). HD-ZIPI from *Medicago truncatula* named as HB1, was salt stress inducible (Gruber et al., 2009) and it was responsible for regulating root architecture and lateral root emergence under stress conditions, by repressing transcription of an auxin regulated factor LBD1 (Ariel et al., 2010).

Apart from abiotic stress, homeodomain proteins have been proposed to regulate pathogen defence-related gene expression (Korfhage et al., 1994). In tomato, HD-ZIP gene H52, was shown to regulate programmed cell-death (Mayda et al., 1999). *Arabidopsis* homeodomain protein OVEREXPRESSOR OF CATIONIC PEROXIDASE 3 (*ocp3*) was shown to regulate defence response to necrotrophic pathogens and may act through jasmonic acid pathway (Coego et al., 2005). Rice OsBIHD1, was when over-expressed in tobacco, resulted in an elevated level of expression of defence-related gene *PR-1* and it was also able to confer an enhanced disease resistance against virus and fungus (Luo et al., 2005). In sunflower, Hahb4 expression was studied in response to wounding and biotic stress. It is well known that levels of phytohormones like jasmonic acid (JA) and ethylene (ET) increase in the cell upon wounding or insect attack and comparatively levels of salicylic acid (SA) decrease. So, in *Arabidopsis* plants ectopically expressing sunflower Hahb4, the transcript levels of Hahb4 increased several folds upon biotic stress. This transcription factor was also seen to be responsible for an increase in JA levels in the transgenics and accompanied by an up-regulation of defence-related genes in response to biotic stress (Manavella et al., 2008). In another study, homeobox gene from *Nicotiana benthamiana*, *NbHB1*, was found to positively regulate pathogen induced cell death and it was speculated that it does so possibly by induction of jasmonic acid pathway (Yoon et al., 2009). NaHD20, another HD-ZIPI member from *Nicotiana attenuata*, that is induced by water stress and ABA, was also reported to be affected by wounding. The transcript levels increased after insect elicitation but jasmonic acid accumulation was not similar as observed in plants over-expressing Hahb4 (Re et al., 2010).
Role in plant development

There are several reports published about homeodomain proteins where they have been shown to regulate different aspects of plant growth and development like embryo development, meristem development and vascular patterning. Below are discussed some of the examples citing their importance in controlling plant development.

Formation of a proper vasculature is very important as it helps the plant by transporting all the essential nutrients and also provides it a physical support determining the architecture. Various studies have revealed that HD-ZIP III members like REVOLUTA/INTERFASCICULARFIBERLESS1 (REV/IFL1), PHABULOSA (PHB)/ATHB14, PHAVOLUTA (PHV)/ATH14, AtHB8 and AtHB15/CORONA (CNA)/ INCURVATA4 (ICU4) play a role in vascular development. The dominant mutations in both PHABULOSA (phb) and PHAVOLUTA (phv) resulted in the transformation of abaxial to adaxial fate in leaves formed and it also resulted in organs developing with radial symmetry, thus implicating their role in determining leaf polarity ( McConnell et al., 2001). Similarly analysing these phb, phv and rev mutants further helped in unravelling their additional roles in embryo patterning, meristem initiation, organ polarity and regulating meristem development (Prigge et al., 2005; Baima et al., 1995, 2001; Kang et al., 2002, Green et al., 2005; Ochando et al., 2008). KANADI (KAN) is another group of transcription factors that acts as a negative player which controls organ polarity and embryo patterning in an antagonistic manner as compared to the HD-ZIP III members and acts by regulating the auxin flow through the plant (Kerstetter et al., 2001; Emery et al.,2003; Izhaki et al., 2007; Illegems et al., 2010). Kim et al. (2008) also reported a small group of proteins called ZIP proteins that also negatively regulate the HD ZIP III activity in meristem development. They showed ZPR3 interacted with phabulosa and even other HD ZIP III members via its ZIP motif and, thus, a feedback loop functions in the development of SAM in *Arabidopsis*. AP2 domain containing transcription factors DORONROSCHEN (DRN) and DRN-LIKE (DRNL) have been shown to interact with HD-ZIP III member PHAVOLUTA (PHV) and regulate embryo patterning in *Arabidopsis* (Chandler et al., 2007).
The HD-ZIP I member, *ATHB23*, is ubiquitously expressed in all vegetative organs (Henriksson et al., 2005), induced by hormone GA, and had a higher expression in shoot apical meristem (SAM) and adaxial region of the developing leaves. It was observed that levels of *ATHB23* were affected by the activators of leaf development mentioned before like KANADI (KAN), PHABULOSA (PHB) and SERRATE (SE). Moreover, its expression was altered in mutants related to adaxial-abaxial leaf polarity thus ascribing its role in leaf development (Kim et al., 2007). Over-expression studies with *ATHB-1* and other homeodomain members like *ATHB3*, *ATHB20* and *ATHB23* also revealed their role in leaf development and cotyledon expansion (Aoyama et al., 1995; Kim et al., 2007). In *ATHB13* over-expression plants, the cotyledon and leaf development was altered and this was found to be related to sucrose signalling. Thus, it was the first report where HD-ZIP member was shown to be a link between development and sugar signalling (Hanson et al., 2001). Recently, AtHB13 has been shown to be up-regulated under stress conditions, and when AtHB13 or its sunflower homolog HaHB1 was ectopically expressed in *Arabidopsis*, the transgenic plants were found to be abiotic stress tolerant (Cabello et al. 2012). Tomato homeobox gene *LeHB1*, a HD-ZIPI member, has been shown to bind to *LeACO1* (ACC oxidase) promoter, and in *LeHB1* silenced plants there was a reduction in ACO1 levels, causing delayed ripening. In contrast, the plants over-expressing *LeHB1*, the floral organ development were altered, thus implicating the role of gene in both flower and fruit development (Lin et al., 2008). HD-ZIP II member, JAIBA (JAB) from Arabidopsis, has been found to regulate floral meristem activity and also important for proper gynoecium and fruit development (Zuniga-Mayo et al., 2012).

Most of the genes belonging to HD-ZIPIV family play a role during differentiation and maintaining epidermal cell fate. Amongst them, *OCL4* of maize has been functionally characterised in detail. Phenotypic analysis of its insertional mutants and maize *OCL4*-RNAi plants helped researchers to define its role in regulating trichome differentiation and anther development in maize (Vernoud et al., 2009). An epidermis specific member, *OCL1* from maize, has been functionally analysed (Ingram et al., 1999, 2000) and its over-expression caused a delay in flowering time and other phenotypic aberrations like narrow and shorter leaves with no starch accumulation in bundle sheath cells, tassels with shorter lateral branches
and aborted flowers (Depege-Fargeix et al., 2010). GLABRA2/ATHB10 are other HD-ZIP IV members found to regulate epidermal cell fate, trichome development, root development as seen in gl2 mutants which had defects in trichome and root hair development, and also controls oil accumulation in seeds (Rerie et al., 1994; Di Cristina et al., 1996; Masucci et al., 1996; Ohashi et al., 2002; Abe et al., 2003; Shen et al., 2006). Cotton GaHOX1, is the functional homolog of Arabidopsis GL2 as it was able to complement the glabrous mutant gl2-2, and its expression was higher at early developmental stages in cotton fiber cells. GaHOX2 was also observed to have an integument-specific expression (Guan et al., 2008). Recently, GhHD-1, another HD-ZIP IV member from cotton has been shown to have a high expression in fibre cells and was found to regulate epidermal cell differentiation, thus important for trichome and fibre cell development (Walford et al., 2012). ANL2 (ANTHOCYANINLESS2) regulates anthocyanin accumulation in epidermal cells (Kubo et al., 1999). Members from this class are also involved in cuticle development as reported in maize and tomato (Isaacson et al., 2009; Javelle et al., 2010). It has been shown that in Arabidopsis 35S:AtCFL1 overexpression plants and rice cfl1 mutants, CFL1 interacts with HDG1, a HD-ZIP IV member, and plays an important role in regulating cuticle development by affecting downstream genes (Wu et al., 2011).

Homeodomain BELL1 family member, AtBEL1, regulates ovule development via Agamous (AG) the homeotic gene (Robinson-Beers et al., 1992; Reiser et al., 1995; Western et al., 1999). BEL1 has been said to be integument identity factor that interacts with other ovule identity factors like Seedstick (STK) and Shatterproof (SHP). BEL1 interacts with AG-SEP3 dimer and thereby controls ovule development. It was also shown that a proper balance between all the members, i.e. carpel identity factor and ovule integument factor, is required for correct ovule formation (Brambilla et al., 2007). BEL1 has also been shown to act with SPL/NZZ to regulate ovule development by controlling chalaza development (Balasubramanian and Schneitz, 2002) and recently the role of cytokinin was analysed where both these transcription factors were shown to be important for chalaza development by regulating the levels of PIN1 (Bencivenga et al., 2012). MDH1 from apple belonging to the BEL1 family of homeodomain proteins, when overexpressed in Arabidopsis, imparts different phenotypes like reduced fruit size

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and shape, reduced leaf, shortened petioles, leaf curling and reduced plant height, probably by affecting cell differentiation. Another important observation recorded was reduced male and female sterility, thereby implicating its role in controlling ovule development and plant fertility (Dong et al., 2000). This family has also been shown to be involved in other developmental aspects like maintaining leaf shape in \textit{Arabidopsis} (Kumar et al., 2007) and seed shattering in rice (Konishi et al., 2006). Barley \textit{JuBEL1} when over-expressed in tobacco led to male sterility and other phenotypic developmental abnormalities like bushy growth, ectopic outgrowths on floral organs, thus implicating its role in development (Muller et al., 2001). Wheat \textit{BLH} (BELL-LIKE HOMEODOMAIN) genes have been recently isolated and observed to express in basal boundary region of ovary and thus speculated to play a role in development of pistils and transformed stamens (Mizumoto et al., 2011). Over-expression of rice \textit{OsBIHD1} in tobacco resulted in transgenics with morphological abnormalities like abnormal roots, and reduced fertility and, as discussed before, it is speculated to have a role in disease resistance (Luo et al., 2005).

WOX family members have been speculated to have a role in controlling cell division and cell differentiation and in early embryo pattern formation in \textit{Arabidopsis}. For example, WOX3 has a role in lateral organ development (Matsumoto et al., 2001), WOX2, WOX8 and WOX9 have apical and basal daughter cell specific expression and have been implicated in early embryo patterning, WOX6 regulates ovule development (Park et al., 2005), WOX5 is involved in stem cell maintainence (Gonzali et al., 2005) and WOX1, WOX2 and WOX9 in meristem growth and development (Wu et al., 2005; Zhang et al., 2011). Another member of homeobox gene belonging to WOX family, i.e. WOX 11, was found to be involved in crown root growth in rice, where it has been shown to act by coordinating auxin and cytokinin signals and regulate cell division during root development (Zhao et al., 2009). Similarly, WOX family members were characterised from \textit{Vitis vinifera} and their expression levels were compared at different stages of embryo development, and out of the total 12 members studied, VvWOX2 and VvWOX9 could be tagged as early expression markers of somatic embryogenesis (Gambino et al., 2011).

By analysing \textit{Arabidopsis obe1} and \textit{obe2} mutants, researchers have concluded that these group of homeodomain proteins, i.e. OBERON1 (OBE1) and
OBERON2 (OBE2), are nuclear localised transcriptional regulators that function mainly in maintaining shoot apical meristem, root apical meristem by regulating the expression of meristem genes like WUS and PLT1 (Saiga et al., 2008). Both of them also promote vascular development in the embryo by regulating auxin mediated development wherein they act downstream of auxin accumulation (Thomas et al., 2009).

Class I KNOX genes are important regulators of SAM development as seen by knox mutants in rice and maize which lacked proper SAM (Long et al., 1996; Vollbrecht et al., 2000). It has been well-documented that they have a shoot apical meristem specific expression and play a role in its maintainence and development (Hake et al., 2004), which in contrast to class II KNOX members that have diverse expression patterns (Zhong et al., 2008). HBK1 was cloned from the conifer Picea abies and it was characterised as KNOX 1 family member, was also shown to play a role in meristem development as studied by its overexpression in Arabidopsis (Larsson et al., 1998). Populus KNOX gene, ARBORKNOX1, had SAM and cambium specific expression and its over-expression affected vascular development (Groover et al., 2006).

Arabidopsis SHOOTMERISTEMLESS (STM), a class I KNOX member, is expressed at early stages of embryogenesis in shoot apical meristem (Long et al., 1996) and has been shown to hetero-dimerize with BELLRINGER (BLR)/PENNYWISE (PNY), a BELL family member (interact through MEINOX domain), and thus maintain shoot apical meristem and also influence inflorescence development (Bellaoui et al., 2001; Byrne et al., 2003; Roeder et al., 2003; Smith et al., 2003). Other BELL family members from Arabidopsis like ARABIDOPSIS THALIANA HOMEBOX1 (ATH1) and POUND-FOOLISH (PNF) have also been shown to interact with STM and regulate SAM development and influence inflorescence architecture (Rutjens et al., 2009). VAAMANA (VAN), a BELL family member, was previously characterised and found to influence inflorescence growth and meristem function in Arabidopsis (Smith et al., 2003). Later, another group demonstrated its interaction with class I KNOX genes STM, BP and KNAT6. It was also shown that nuclear localisation of this protein was dependent on its interaction with these KNOX proteins (Bhatt et al., 2004).
Similarly, other KNOX members from *Arabidopsis*, i.e. *BREVIPEDICELLUS* (BP)/KNAT1, KNAT2 and KNAT6, have been reported to have a SAM specific expression at different stages of embryogenesis and they help in maintaining shoot apical meristem activity throughout the plant life cycle (Dockx et al., 1995; Byrne et al., 2002; Belles et al., 2006). Apart from influencing the floral development, Shi et al., recently showed that BP regulated floral organ abscission. It was observed that BP acted as a negative regulator thereby limiting the size of abscission zone (AZ) and, in contrast, KNAT2 and KNAT6 positively regulated abscission. BP when ectopically expressed caused formation of lobed leaves (Chuck et al., 1996). In contrast, *bp* mutant had downward pointing pedicels and defective and compact inflorescence architecture, and it was shown to act in conjugation with ERECTA and determine inflorescence architecture (Douglas et al., 2002; Venglat et al., 2002).

ARABIDOPSIS THALIANA HOMEBOX1 (*ATH1*), as mentioned before, acts as a regulator of photomorphogenesis. In addition, it has been observed that it plays a role in establishing basal boundaries of shoot organs (Gomez et al., 2008), and acts as an activator of floral repressor *FLC* (Proveniers et al., 2007). In a recent study by Li et al. (2011), they have shown that *ATH1* interacts with *KNAT2* and regulates pedicel development as *ath1* mutant also displayed downward-pointing pedicels like *bp* mutant. It has been postulated that *BP* and *PNY* interact with KNAT members *KNAT2* and *KNAT6* and downregulate them, which is important for pedicel development and establishment of a correct inflorescence (Ragini et al., 2008). BOP1/2, added as new members, have been shown to induce *ATH1* and *KNAT6* and function downstream of BP-PNY and thus regulate plant inflorescence architecture (Khan et al., 2012). As postulated previously that KNOX-BELL dimerization affected localisation of fusion proteins, similarly, STM protein is localised to nucleus as determined by its interaction with *ATH1*, *PNY* and *BLH3* (Cole et al., 2006; Rutjens et al., 2009). Likewise, it was speculated that ATH1-KNAT2 dimer may influence its localisation and it then interacts with downstream factors to promote inflorescence development in *Arabidopsis*. Thus, one can conclude that *ATH1-KNAT2, KNAT6* and *BP* act as important regulators in *Arabidopsis* pedicel development. Other functional roles that class I KNOX genes play in plant development include compound leaf development.
In rice loss of function knox mutants for OSH1 and OSH15 were isolated and the analysis confirmed their role in SAM formation and maintenance in rice. OSH1 not only positively regulates expression of other KNOX genes but is also positively auto-regulated, which is thought to be important for SAM development in rice (Tsuda et al., 2011)

**Other roles of homeodomain proteins**

Homeodomain transcription factors have also been found to control the levels of plant hormones, which are well characterised in terms of their role in regulating plant development. The homeodomain genes have been studied to affect both synthesis and/or signalling pathways of plant hormones. In tobacco, over-expression of KNOX member, NTH15, resulted in repression of GA-20 oxidase (involved in GA biosynthesis) and accompanied with an increase in cytokinin levels in transgenics which therefore also had an abnormal leaf morphology (Tamoki et al., 1997; Tanaka-Ueguchi et al., 1998). When over-expressed under the control of inducible promoter, similar decrease in GA levels [because of repression of GA 20-oxidase (Ntc12) activity] were observed in shoot apical meristem of transgenic tobacco plants (Sakamoto et al., 2001). In maize, KN1, another knotted class member up-regulates the level of catabolic enzyme GA2-ox1 (Bolduc et al., 2009). Over-expression of potato homeodomain gene POTH1 also resulted in decrease in gibberellin levels by causing a decrease in GA20-oxidase levels (Rosin et al., 2003). Later, Chen et al. (2004) reported that, in addition to POTH1, other homeodomain member, StBEL1, could also decrease GA levels by suppressing GA20-ox promoter and thus reducing its levels. They observed that the POTH1-StBEL1 heterodimer binding affinity (to the GA20-ox promoter) was higher than that of individual member, thereby repressing ga20-ox1. In rice plants over-expressing HD-ZIP member, Oshox4, had a semidwarf phenotype with higher levels of catabolic enzyme GA2ox3 but GA biosynthetic enzymes were unaffected, suggesting that Oshox4 repressed GA signalling. In addition, there was an up-regulation of YAB1 levels, which has been previously shown to control GA biosynthesis by feedback regulation (Dai et al., 2007, 2008). Recently, Wen et al. (2011) have reported a PHD-family member from rice to be involved in GA signalling. They found that in plants over-expressing HOX1a the levels of GA biosynthetic genes were decreased but the expression of GA catabolic genes was more. More importantly, they have
shown that HOX1a could bind to early flowering1 (EL1) promoter, which has been shown to be a negative regulator of GA signalling (Dai et al., 2010). In rice plants over-expressing HOX1a, downregulation of EL1 levels was observed based on which they concluded that HOX1a acts as a positive regulator in GA signalling. Cytokinin (CK) levels have also been shown to be modulated by KNOX-I family members. There was an increase in levels of cytokinin caused by an increased level of CK biosynthetic enzyme adenosine phosphate isopentyltransferase (IPT) as studied in Arabidopsis and rice (Jasinski et al., 2005; Yanai et al., 2005; Sakamoto et al., 2006).

WUSCHEL (WUS) and CLAVATA (CLV) are two well-studied homeodomain transcription factors in Arabidopsis which have been shown to act through a feedback loop where WUS positively regulates the CLV3 expression and, in turn, CLV3 localised above the organizing centre (OC) represses WUS, i.e. present in the OC. CLV2 and CLV1 have also been shown to be a part of this loop (Ogawa et al., 2008; Muller et al., 2008) wherein they all regulate WUS activity and thus are responsible in maintaining the size of stem cell population of shoot meristem (Clark et al., 1995,1997; Schoof et al., 2000; Brand et al., 2000). A new family of proteins named as FANTASTIC FOUR (FAF) has been recently identified that affect CLV3-WUS loop activity. Out of the four FAF members discovered, FAF2 and FAF4 were expressed in the center of shoot apex. It was seen that in these FAF over-expression lines, there was a strong decline in WUS expression and also the size of meristem was reduced. In addition, it was observed that FAF2 and FAF4 were under negative control of CLV3 and thus they also indirectly regulate the size of shoot meristem in Arabidopsis through the feedback loop (Wahl et al., 2011).

Conclusions and perspectives

Till date a large number of homeodomain genes have been identified from a variety of plant species. All of them harbour the signature motif, i.e. homeodomain, but with time additional domains and motifs have been acquired by most of the classes, thereby adding to their functional diversity. They have been classified into various families and subfamilies according to their sequence compositions, presence or absence of specific amino acid residues. Different molecular and genetic approaches have been used for knowing their functional roles in plants in more
detail. Studies employing transgenics and mutants (both gain of function and loss of function) have also been carried out that have further helped in functional characterisation of these homeodomain proteins in plants. They have been shown to be involved in different aspects of plant life cycle ranging from morphogenesis and development to their participation in stress responses. However, there are certain aspects that still need to be studied in detail so as to know how these proteins induce the downstream target genes and bind to their cis-elements and regulate the signalling pathways under normal conditions and when induced by stress. More in vivo and in vitro studies still need to be done for knowing the protein-protein interactions and protein-DNA interaction between different homeodomain proteins and homeodomain targets, respectively, that would help us in characterising the regulatory networks of homeodomain proteins and elucidating their specific roles in regulating plant development.