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DNA-binding One Zinc Finger (DOF) transcription factors in *Vigna* species: A review

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Legumes that belong to the family *Fabaceae*, are considered as one of the largest groups in edible plants. Among these, the genus *Vigna* stands out as a powerful representative of Legumes. It consists of more than 200 highly nutritional and economically important species for food security worldwide. The most commonly domesticated legume crops are mung bean, adzuki bean, urd bean, rice bean, and cowpea. However, these crops are vulnerable to several biotic and abiotic stresses. The DNA-binding one zinc finger (DOF) is an important transcriptional factor, specific to plants that play a vital role in plant-linked cellular functions such as plant growth, differentiation, seed development and germination as well as their response to biotic and abiotic stresses. DOF family is characterized by a presence of highly conserved DNA binding one zinc finger domain of 50-52 amino acids (DOF domain). These transcription factors are widely distributed in all plant species. This review mainly focuses on identifying and characterizing of factors in three *Vigna* species (*Vigna radiata*, *Vigna angularis*, and *Vigna unguiculata*).

Keywords: V*igna* species, Transcription factors, DOF transcription factor, biotic stress, abiotic stresses, characterization.

INTRODUCTION

Beans or pulses are considered the source of human and animal diets having high nutritional value. Beans are the edible legume plants that belong to family *Leguminosae*, also known as *Fabaceae* (Shavanov, 2021). Legumes are potential contributors to human food as a rich source of nutrients. These nutrients are either macronutrients like carbohydrates, proteins, and fats or micronutrients like minerals and vitamins. Cereals that belong to family Poaceae include oats, wheat, rice, maize, sorghum, barley, etc. Cereal-based diet has sufficient source of carbohydrates but not enough sources of proteins. About 800 million people are affected by malnutrition so beans are the primary source of proteins in the human diet as a food crop to meet this nutrition demand (Broughton *et al*., 2003). Beans belong to the *Vigna* genus that has flowering plants in the *Fabaceae* family. The genus *Vigna* consists of approximately 200 species having pantropical distribution, native to tropical regions worldwide (Fery *et al*., 2002). Several species of *Vigna* genus are considered as potential food crops for millions of people in developing countries. Many *Vigna* species like mungbean (*V. radiata*), cowpea (*V. unguiculata*), mat bean (*V. aconitifolia*), urd bean (*V. mungo*), rice bean (*V. umbellate*), bambara groundnuts (*V. subterranea*) and adzuki bean (*V. angularis)* are key food staples as standard diet. These *Vigna* species exhibit considerable economic importance in recent decades (Fery et al., 2002; Somta *et al*., 2009; Tomooka *et al*., 2006; Tomooka *et al*., 2011).

Vigna species are capable to grow under extreme environmental conditions such as nutrient-poor soil, high temperature, salinity, and low rainfall. The products of beans are leaves as well as tips of tender shoots consumed during the seedling stage and immature seeds and immature pods consumed during the fruiting stage. Dry seeds of beans are easy to transport and store. Some *Vigna* species are used as ground cover, forage for farm animals and green manure crops and many *Vigna* species produce edible products. Beans can be used as bean sprouts, bean paste, or as a whole bean, flour from its seeds. The overall annual production of various edible *Vigna* species approaches about 20 million hectares worldwide but most of these productions come from the developing countries (Fery *et al*., 2002). Among all *Vigna* species, *Vigna* radiata alone contributes significantly to producing a protein-based diet for societies of developing and under-developing countries (Somta *et al*., 2009). Globally, the area covered by pulse crops is approximately 73.2 million hectares followed by 61.72 million tons of productivity and

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the average production of these crops is 843 kg/ha worldwide (Pandiyan *et al*., 2011). In 2014, production and yield of total pulse crops has been increased up to 77 million tonnes and 929 kilograms per hectare respectively stated by FAOSTAT.

In Pakistan, majorly produced summer and winter pulse crops are mungbean, black gram, red gram and chickpea, lentils, field pea respectively (Ullah *et al*., 2020). These crops are grown in the spring season (March-June) and a rainy season (July-October). Most of the *Vigna* crops are foremost, short duration, warm seasonal legume crops being used as a supplement of cereal-based crops in Pakistan. These are widely grown crops all over Pakistan especially in Punjab and other provinces of Pakistan that include Sindh, Baluchistan, and Khyber Pakhtunkhwa (Haqqani *et al*., 2000). In Punjab, *Vigna* plants are cultivated in different districts including Bhakkar, Layyah, Narowal, T.T Singh, Kasoor, Nankana Sahib, Sheikhupura, Chakwal, Jehlam, Sialkot, Mianwali, Faisalabad, Muzaffargarh, Dera Ghazi Khan, and Dera Ismail Khan. Mungbean is grown in the Federal region in Islamabad, Rawalpindi, and Attock. In Sindh, it is cultivated in Sajawal, Thatta, and Larkana (Iqbal and Mukhtar, 2014). In Pakistan, 20.40 million hectares area is under cultivation out of the total geological area of about 79.61 million hectares. The area under productivity has increased from 100,000 hectares to above 200,000 hectares hence increased in production from 50,000 (fifty thousands) metric tonnes to 100,000 (1 lac) metric tonnes since 2000 (Weinberger, 2005).

Vigna species are important pulse crops that supplement the cereal-based diet for poor societies specifically in Asian countries. These crops are widely distributed throughout South Asia, Southeast Asia, West Indies, tropical Africa, Subtropical Africa, Australia, North and South America. Among Southeast and South Asia, Pakistan, Nepal, India, Burma, Sri Lanka, Thailand, Bangladesh, Indonesia, Philippines, Malaysia, Taiwan, Bhutan, and Myanmar are countries contributing to production lead to enhancement of overall production of *Vigna* plants worldwide (Karthikeyan *et al*., 2014; Lakhanpaul *et al*., 2000; Singh, 1988; Chadha *et al*., 2010).

***Stresses in Vigna species*:** Species of *Vigna* genus are susceptible to many environmental as well as biological stresses. These stresses are the reason in reduced production of *Vigna* plants that ultimately led to decline in yield.

Vigna plants are susceptible to many biotic and abiotic factors hence decreasing the overall production and causing great economic losses. Abiotic factors include drought, waterlogging, temperature, salinity, and many other stresses (Arora *et al*., 2002; Srivalli *et al*., 2003). These stresses affect cellular homeostasis, disrupt metabolism, and interrupt the biochemical and physiological processes of Vigna plants. Vigna plants must tolerate this stressful environment to survive. Unable to survive this stressful environment leads to disturbance in the functionality of Vigna plant systems. In drought stress, the physiological state linked with development, growth, and economic yield of *Vigna* species is affected. Water loss basically affects the turgor pressure of the *Vigna* plant hence loss in turgidity cause a decrease in cell growth. Mungbean is more susceptible to drought stress than other pulses because it requires more water for its cultivation. So its productivity is more affected during drought stress periods like summer and spring (Singh *et al*., 2011).

Waterlogging stress mainly disrupts the early stages of development in the *Vigna* plants. The *Vigna* plants play an important role in atmospheric nitrogen fixation to fulfill its own nitrogen demand and many other surrounding plants. Waterlogging or flooding reduces the ability of nodule activity and nitrogen fixation. Winds coupled with heavy rainfall caused extensive yield loss by damaging mature crops (Tomooka *et al*., 2006). *Vigna* species production is greatly affected by a change in temperature and photoperiod. Elevated temperature stress causes negative impact on flower retention as well as pod formation. High temperature leads to rise in flower shedding and a decline in seed production due to a lack of reproduction and pollen grain's inviability (Kumari and Verma, 1983; Rainey and Griffiths, 2005). Salinity stress cause a decline in shoot and root length, no branches and root hairs, seed germination, and seedling that leads to a decrease in *Vigna* production (Promila and Kumar, 2000; Saha *et al*., 2010). Salt stress causes distinct symptoms like a decrease in the content of pigment chlorophyll A, B, and carotenoids and enhanced necrosis and chlorosis (Sehrawat *et al*., 2015). Other abiotic stresses include ultraviolet and ionizing radiations, metal pollutants, insecticide residue, prolonged rainy conditions, and many other mechanical factors like wind, water pressure (HanumanthaRao *et al*., 2016). These abiotic stresses make crops more vulnerable to biotic stresses.

Biotic stress plays an important role in agriculture as it immensely damages agricultural products that lead to malnutrition in different regions of the world (Moustafa-Fraag *et al*., 2020). Biotic stress includes different organisms like bacteria, fungi, viruses, pests, nematodes, weeds, and parasites. These organisms are of great economic importance because of their impact on *Vigna* plants especially in Asia (Taylor *et al*., 1996; Raguchander *et al*., 2005; Singh *et al*., 2000). These biotic stresses cause severe losses in production and ultimately in its yield. About 47% of emerging infectious diseases have been caused by plant viruses (Yadava *et al*., 2010). Among all the infectious diseases, YMV is the group of pathogens severely affecting *Vigna* crop production. Major viral diseases of *Vigna* species are mungbean yellow mosaic virus (MYMV), urdbean yellow mosaic virus, cucumber mosaic virus (CMV), alfalfa mosaic virus (AMV), mosaic mottle virus (BCMV), mungbean leaf curl virus (MLCV) and leaf crinkle virus (ULCV) (Singh *et al*., 2018).

The most important fungal diseases of *Vigna* crops are powdery mildew caused by *Podosphaera fusca* and *C. truncatum*, Cercospora leaf spot (CLS) caused by *Cercospora*

canescens and anthracnose disease caused by *Collectotrichum acutatum*, *C. gloeosporioides*, and *C. truncatum.* Dry root rot is now considered as the emerging disease caused by *Macrophomina phaseolina*. Other fungal diseases are Fusarium wilt, web blight, and Alternaria leaf spot caused by *Fusarium solani*, *Rhizoctonia solani*, and *Alternaria alternata* respectively (Ryley and Tatnell, 2011). The main bacterial diseases of *Vigna* plants are bacterial leaf spot, halo blight as well as tan spot caused by *Xanthomonas campestris*, *Pseudomonas syringae*, and *Curtobacterium flaccumfaciens* respectively (Pratap *et al*., 2020).

Insect-pests may directly affect the *Vigna* plants for feeding purposes or indirectly for being a vector for many pathogens. Insect-pests adversely affect *Vigna* production by attacking the crop from sowing to cultivation period and till the end step of storage. For *Vigna* pnats, most important insect-pests are thrips, whitefly, aphids, stem fly, pod bugs, bruchids, and pod borer complex (Swaminathan *et al*., 2012). Stem fly is one of the chief insect-pest that takes an adverse toll on *Vigna* yield. *Vigna* species are mainly infested by *Ophiomyia phaseoli* specie of stem fly. Other common species are *Ophiomyia centrosematis* and *Melanagromyza sojae* (Talekar, 1990). Thrips are also economically important insect-pests that damage *Vigna* crops. They affect plants in both the seedling stage as well as flowering stages. The *thrips tabaci* and *Thrips palmi* are pests of the seedling stage while *Caliothrips indicus* is the pest of the flowering stage. The *Maruca vitrata* specie of spotted pod borer present in the tropical and subtropical regions causes immense damages to *Vigna* plants (Zahid *et al*., 2008). The identification of the genes linked to these stresses can play an important part to deal with these stresses. Regulatory genes are important for controlling genes expression linked to these stresses. Among the regulatory genes, stress-responsive transcriptional factor genes can control the plant functions under stress conditions (Shao *et al*., 2015; Wang *et al*., 2016). Regulation of the expression of genes is compulsory for many developmental processes occurring in plants under normal and stress conditions.

***Transcription Factors*:** Transcription factors (TFs) are the particular sequence-binding proteins that control the expression of many genes necessary in plant growth. These factors control the expression of many genes at transcription level through binding with the particular complementary sequences on the genome. Transcription factors can either act as activators or repressors. They can activate the particular genes that lead to up-regulation of the gene expression or repress or halt the gene activity causing down-regulation of the gene expression. These factors bind to the particular sequences within the promoter region of the DNA and then control the activity of RNA polymerase, an enzyme necessary for transcription process. The TFs can either directly or indirectly interact with the basal transcriptional apparatus to control the RNA polymerase activity by altering its properties. In case of indirect interaction, transcription factors can control polymerase activity through co-repressors and co-activators (Yang *et al*., 2010; Gupta *et al*., 2015).

Transcriptional factors attach to promoter region of DNA where they can up-regulate or down-regulate the gene expression present next to the promoter region. A transcription factor consists of three domains named as DNA-binding domain (DBD), activation domain (AD) or trans-activating (TAD), an oligomerization domain and signal-sensing domain (SSD) (Liu *et al*, 1999). The DBD binds to the response elements also known as promoters or enhancers present in the DNA. This domain is more significant because of its binding to the DNA. The activation domain has the specific site for the attachment of many proteins such as co-regulators (co-repressors or co-activators). The signal-sensing domain can sense the external environment signals and transmit them to the rest of the transcription complex. The oligomerization domain can find out the interaction between the proteins. Transcription factors can either make homodimers, heterdimers or both homo and heterodimers at the same time. This domain can eventually alter the function of the rest of the domains depending on its dimerization capability (Kono *et al*., 2012; Gupta *et al*., 2015). The cis-regulatory site in the DNA binds to the particular amino acid (AA) sequences present in the DNA-binding site of the transcription factor that determines the affinity, specificity and selectivity of the transcription factors for the DNA. This amino acid arrangement can affect the interaction between the TF and the DNA (Kono *et al*., 2012).

DNA-binding one zinc finger (Dof) transcription factor: Among all these transcription factors, DNA-binding with one zinc factor (DOF) is a particular plant-linked transcription factor present in all plants. The first Dof transcription factor was identified in maize plant (Yanagisawa and Izui, 1993). Dof factors are the specific proteins with 200-400 amino acids sequence. Dof factor has a dof DNA-binding domain responsible for the transcription process's repression and activation. The members of the Dof family don't have conserved sequences of amino acids, and even individual proteins are very deviating in sequences from each other. Some transcription factors have single and multiple DNA-binding domains, but Dof family have only a single DNA-binding domain (Yanigasawa, 2002).

Dof transcription factors involved in many biological processes distinctive to plants such as morphogenesis (Guo *et al*., 2009; Gardiner *et al*., 2010), accumulation and regulation of seed storage proteins that are specific to endosperm (Vicente-Carbosa *et al*., 1997; Mena *et al*., 1998; Yamamoto *et al*., 2006; Dong *et al*., 2007; Kushwaha *et al*., 2008), secondary metabolism (Yang *et al*., 2012), signaling of plant pigments such as phytochrome (blue green pigment) and cytochrome (Iwamoto *et al*., 2009), glucosinolates biosynthesis and metabolism of phenyl propanoid, seed germination (Papi *et al*., 2000; Papi *et al*., 2002; Gualberti *et al*., 2002; Rueda-Romero *et al*., 2012), nitrogen assimilation

(Yanagisawa *et al*., 2004), root storage development (Yang *et al*., 2011), nitrogen use efficiency (Kumar *et al*., 2006), carbon fixation (Yanagisawa, 2000; Tanaka *et al*., 2009), expression of genes related to cell wall (Wei *et al*., 2010). There are several other functions reported that are regulated by Dof transcription factors that including regulation of the genes involved in defensive activities (Yanagisawa, 2000) and cell cycle, expression of glutamine synthetase (Rueda-lopez *et al*., 2008), regulation of stomatal functioning (Negi *et al*., 2013) and oil fraction in seeds (Wang *et al*., 2007), photoperiodism (Imaizumi *et al*., 2005; Iwamoto *et al*., 2009; Li *et al*., 2008; Fornara *et al*., 2009), regulation of development of vascular tissues (Guo *et al*., 2009; Gardiner *et al*., 2010). In recent years, role of Dof family have been reported in plants including abscission control (Kim *et al*., 2010; Wei *et al*., 2010), circadian clock influenced by light (Yang *et al*., 2010; Yang *et al*., 2011).

Phytohormones are important plant hormones such as axins, cytokinins, gibberllinis that control the plant growth by regulating their gene expression. These hormones are involved in signaling pathways that are somehow complex and the regulation of these pathways is attained by attachment and coordination of many Dof factors at the cis-acting sites of promoter region of genes linked to these phytohormones (Yanagisawa, 1997). Studies indicated that Dof transcription factors have regulated the expression of auxins (Baumann et al., 1999) and gibberellins (Washio, 2003) responsive genes in many plants. Gibberellins are diterpenoid plant hormones responsible for plants' growth and developmental processes, including seed germination, elongation of the stem, leaf expansion, and flower and fruit maturity (Hooley, 1994). In rice, wheat, and barley, the alpha-amylase promoters play important role in GA induction. The analysis of these promoters has been identified conserved cis regulatory sites, within these promoters. These cis-regulatoryy elements have been known as GA-responsive elements (GARE). These elements can be either tripartite or bipartitie having three sequence motifs. These motifs involve the CCTTTT (pyrimidine box), the TATCCAC box, and the TAACAAA box (Skriver *et al*., 1991; Gubler and Jacobsen, 1992; Rogers and Rogers, 1992). The pyrimidine box in GARE is the conserved motif present in Dof proteins, showing that Dof TFs are involved in controlling GA responses.

The rolB is an important gene that involves in the induction of auxins in plants. The NtBBF1 is a Dof TF that regulates the transcription of rolB gene responsible for auxin induction (Baumann *et al*., 1999). Auxin-responsive genes are regulated by Dof transcription factors and these genes then control the expression of oxidase enzymes involved in plant growth. Ascorbate oxidase is a particular enzyme involved in meristem growth of roots, cell division and growth in higher plants (Kisu et al., 1998). The endosperm cells are the specific storage cells that store the sulphur and nitrogen during the development of seeds in cereals. These elements are being stored in the forms of complex molecules of proteins such as prolamines. The genes encoding prolamines are coordinately expressed in the developing endosperm where they are under spatial and temporal transcription control, involving cis-acting motifs in their promoters and trans-acting transcription factors. A PBF (Prolamin box)-DOF associated with regulation of endosperm-specific seed storage proteins has been studied in maize, barley, wheat, rice and finger millet (Noguero *et al*., 2013). The first PBF has been cloned from maize and identified as a transcriptional activator of zein gene, a seed storage protein of maize (Vicente-Carbajosa *et al*., 1997).

A cDNA encoding a DNA-binding protein of the Dof class of transcription factor has been isolated from a barley endosperm library (Mena *et al*., 1998). Transient expression experiments in developing barley endosperms demonstrated that BPBF trans-activates transcription from the P-box element of a native Hor2 (hordein) promoter. Similarly, rice PBF (RPBF), isolated from rice cDNA expressed sequence tag clones, is known to play important role as an activator for seed storage protein genes and involved in quantitative regulation by combinatorial interactions with RISBZ1 in determining endosperm-specific expression (Yamamoto *et al*.,2006). A transient expression experiment also demonstrated that the wheat prolamin-box binding factor (WPBF), isolated from wheat endosperm, could trans-activate transcription of native alpha-gliadin promoter by binding to the intact PB (Dong *et al*., 2007).

Dof proteins are important for metabolism of many elements specific to plants. They are linked to metabolism of organic acids and control many genes expression like PEPC gene (Yanagisawa, 2000). Dof1 in maize is responsible for activation of PEPC gene that leads to increase in transcription (Yanagisawa, 2000). They are also necessary for production of carbon skeleton (2-oxogluterate). SRF1 is a Dof factor that controls carbohydrates' metabolism in plant roots (Tanaka *et al*., 2009). Plants have to ability to detect the slight changes in the quality and quantity of light by means of photoreceptors. These photoreceptors include cytochtomes as well as phytochromes. When these receptors sense any light change, they start a signaling pathway leading to photomorphogenesis changes during developmental stages. Irrespective of these receptors, the Dof transcription factors are also involved in plant responses to light. In Arabidopsis, OBP3 is an important protein that acts as a component of signaling pathways of photoreceptors such as phytochrome B (PhyB) and cytochrome 1 (Cyr1). The OBP3 acts as a positive regulator of phytochrome B and negative regulator for cytochrome 1 (Ward *et al*., 2005).

The COG1 is another Dof transcription factor present in Arabidopsis. This Dof protein negatively regulated the signaling pathways of the phytoreceptors, phytochrome A as well as Phytochrome B (Park *et al*., 2003). Many Dof transcription factors such as CDF1, CDF2 as well as CDF3

and CDF5 have been reported in Arabidopsis, involved in photoperiodism in flowering process. The mutation in CDF factors led to insensitivity of photoperiodism that cause either premature or late flowering in plants (Imaizumi *et al*., 2005; Fornara *et al*., 2009). The Dof genes in rice, OsDof12 and Rdd1, are important phyto-regulated genes whose expression has been changed in response to light. The OsDof12 factor regulates the flowering during long day conditions but inhibits the flowering during short-day or dark period (Li *et al*., 2009).

The Rdd1 gene regulates grain size in rice whose expression can be cg=hanged in response to prolonged light and dark conditions (Iwamoto *et al*., 2009). The genes of Dof transcription factor also show circadian rhythms and control flowering duration in plants. In an experiment, two full-length complemantry DNA, JcDof1 and JcDof3, were isolated from seedlings of Jatropha curcas by one-hybrid library of yeast. This experiment was conducted by exploring plants during continuous light, long day, and short day light conditions in interaction with the F-box proteins for regulating the flowering (Yang *et al*., 2011). Recently, a full-length Dof1 associated with circadian cycle, has been reported in finger millet (Kumar *et al*., 2014).

Dof transcription factors also play a vital role in morphological pattern of plants. Recently, these morphological-related Dof factors have been reported in Arabidopsis. Studiesrevealed that the role of AtDof5.6, a Dof transcription factor, in regulating genes involved in cambium formation and development of vascular tissues (xylem and phloem) (Guo *et al*., 2009). The three Dof proteins, Dof2.1, Dof4.6 and Dof5.3, have been expressed in leaves of Arabidopsis plants' dring preprocambial stage of development. This study suggested that these factors are specific to preprocambial developmental stage of plants (Gardiner *et al*., 2010). The Dof transcription factor, AtDod2.4 and AtDof5.8, has been reported that undergo promoter activation in procambial cells of roots and leaf, embryo, veins of leaves in primordial seedlings and cotyledons of embryo under development (Konishi and Yanagisawa 2007).

AtDof5.1 is a Dof factor that regulates the expression of genes responsible for the axial pattern in leaves (Kim *et al*., 2010). In other studies, it was demonstrated that AtDof4.2 controls the gene expression particular for the branching of shots and formation of seed coat in Arabidopsis. It was further studied that over-expression of AtDof4.2 led to enhanced branching pattern in phenotypes but mutation in this factor led to change in the T-M-D motif or AtDof4.2 that led to decreased branching pattern in transgenic plants. The seeds of plants with over-expression of AtDof4.2 showed a collapse-like appearance in the epidermal cells of the over-expressed seed coat. The mutated plants did not show any changes in the branching pattern of seed coat (Zou *et al*., 2013). SCAP1 is an important Dof transcription factor that has been identified in Arabidopsis plants. These factors are only expressed in mature guard cells. In mother guard cells, these factors are not expressive. These transcription factors regulate the expression of genes involved in morphogenesis, functioning of stomata (opening and closing of stomata). They also regulate the expression of other transcription factors such as MYB60 and enzymes like pectin methyl esterases (Negi *et al*., 2013). These studies indicated that SCAP1 plays an important part in the maturation of stomata guard cells of stomata. This transcription factor control the differentiation of the guard cells final stages after maturation and ultimately affects the morophological changes in plants. Another Dof transcription factor, StDof1, was reported in potato that controls the expression of genes responsible for the guard cells activity and development (Plesch *et al*., 2001).

The genes that are specific to guard cells have binding sites for the Dof transcription factor in their promoter region. These binding sites were reported in Arabidopsis (Galbiati *et al*., 2008). Studies revealed that a particular number of binding sites for Dof factors could be present in the promoter region of guard cell genes. This was demonstrated in GAL-GFP lines of Arabidopsis (Gardner *et al*., 2009). Dof binding sites within promoter also contribute to the expression of AtMYB60 in guard cells of Arabidopsis.

Studies indicated that Dof transcription factors are very important in regulation of germination processes in seeds (Noguero *et al*., 2013). The first germination related Dof factor, Dof affecting Germination, was identified in Arabidopsis. This DAG1 factor is predominantly involved in seed germination only in case of maternal control. These factors regulate the germination process in vascular systems of the mother plants but not of the embryo or any other development stage of plant (Papi *et al*., 2000 and 2002). DAG2 is another Dof transcription factor that is responsible for controlling the seed germination process. The germination characteristics of DAG2 transcription factor are different in mutant seeds. The mutation in DAG2 transcription factor is maternally inherited. Studies revealed that overexpression of DAG2 gene have same germination properties as in DAG2 mutated seeds. But in case of double mutation, double mutated (DAG2DAG1) plants have germination characteristics similar to DAG1 seeds. Results showed that DAG1 and DAG2 have opposite regulatory roles (Gualberti *et al*., 2002).

The Dof6 is another transcription factor responsible for seed germination in plants. This Dof factor was first reported in Arabidopsis. The expression level of this gene has been increased in case of dry seeds but decreased after ripening of fruits and during imbibition of seeds. The constitutive up-regulation of Dof6 gene led to sterility and abnormal growth in plants. Its over-expression also caused delay in germination and produced ABA hypersensitive (abscisic acid) phyenotypes with enhanced expression of stress-related ABA1 and ABA2 genes. Results indicated that Dof6

transcription factor unconstructively influences seeds' germination (Rueda-Romero *et al*., 2012).

***Domains of Dof transcription factor*:** D of gene has two domains referred as N-terminal domain (DNA-binding region) and C-terminal domain (transcriptional regulation region of dof gene). The N-terminal conserved domain covers the 50-52 amino acid residues region that contains CX2CX21CX2C sequence. This particular sequence binds zinc ions (Zn^{2+}) in a zinc-finger motif. This configuration is necessary for transcription process because change in this cysteine sequence will distort the zinc-finger motif resulting in halted DNA binding for transcription (Yanagisawa, 2001; Umemura *et al*., 2004). Studies showed that AAAG and its reversible sequence CTTT is necessary for the DNA binding of DOF proteins in all plants except the pumpkin protein (Yangisawa, 2002). So this conserved sequence is responsible for DNA binding irrespective of the outside sequence of this domain (Yangisawa, 1995; Kisu *et al*., 1998; Yangisawa and Schmidt, 1999).

The C-terminal domain has tryptophan amino acids at its base that are also required for significant DNA binding (Shimofurutani *et al*., 1998). The N- and C-terminal regions of the DOF domain are very distinguishable among many DOF proteins, but these domains are not differentiated among the proteins. Because of short recognition sequence of DOF transcriptional factors, they perform functions in contact with other TFs that enhance promoter specificity. So, these regions interact with other regulatory factors and control the gene expression. These interactions can cause adverse effects on DOF functions (Yangisawa *et al*., 2001). Furthermore, domains of DOF proteins can physically interact with itself, other DOFs and several other different transcriptional factors (Yanagisawa and Schmidt, 1999; Yanagisava, 2000). The Dof DNA-binding domain of Dof transcription factor interacts with several different proteins such as OBF and GAMYB and performs important functions that are unique to plants.

***Interaction of Dof domain with other transcription factors*:** An OBP1 (OBF binding protein 1 transcription factor) was the first discovered interacting Dof transcription factor with further other transcriptional factors. OBF is an octopine synthase (OCS) binding factor in plants. The OBP1 transcription factor coordinates with the OBF4 and OBF5 transcription factors that belong to the bZIP factors. The interaction between OBP1 and bZIP factors is governed by the OBP1 Dof domain (Zhang *et al*., 1995). The OBP1 factor helps in enhanced attachment of OBF5 to the OCS elements present within the promoter region of the targeted gene. The GST6 (glutathione S-transferase6) is an targeted Arabidopsis gene and OBP1-bZIP interacting complex binds with the OCS elements and OBP1 binding region present in this targeted gene with a difference of 13 bp (Chen *et al*., 1996).

Another interaction was reported between the Dof and bZIP transcription factors. PBF is a prolamine box binding factor that belongs to endosperm-specific Dof transcription factor in maize. This PBF Dof factors interacts with the bZIP factors. Endosperm proteins are the certain storage proteins in cereals whose gene expression is controlled by the cis elements present in its promoter region. These regulatory elements have a P-box of 7 bp having TGTAAAG sequence and GCN4-like motif (TGAGTCA/ TGACTCA) closet to P-box. The PBF factor interacts with the Opaque2 bZIP transcription factor and attaches with the P-box present in the targeted gene. In PBF-Opaque2 interaction, Opaque binds with the GCN4-like motif present in the targeted gene. P-box is compulsory for the binding of Opaque2 with the motif for transcription initiation (Vicente-Carbajosa *et al*., 1997). Activation of transcription by P-box interaction was also studied in rice in which RPBF (a rice PBF homolog) interacts with RISBZI bZIP transcription factor (Yamamoto *et al*., 2006). So the interaction of Dof domain of transcription factors with the bZIP transcription factors is necessary for the activation of transcription of many important genes in plants. The GAMYB is an R2R3- type MYB protein that is involved in the regulation of gibberellic acid (GA). Several Dof transcription factors interact with GAMYB proteins. This Dof-GAMYB protein interaction plays an important part in the GA-induced regulation of the genes that encode for the hydrolytic enzymes. These enzymes are important for germination of seeds in cereals. BPBF is a Dof homolog of maize PBF in barley and these Dof factors are necessary for the activation of the promoter present in the B-hordein gene (Mena *et al*., 1998; Mena *et al*., 2002) the BPBF Dof factor interacts with the GAMYB (HvGAMYB) in barley and this HvGAMYB protein requires BPBF Dof factor for gene activation. In case of Dof-bZIP interaction, N-terminal of Dof domain interacts with other proteins but in Dof-BPBF interaction, C-terminal of Dof domain in BPBF factor interacts with the HvGAMYB protein (Diaz *et al*., 2002). Another Dof factor in barley, SAD, interacts woth the HvGAMYB protein whose interaction enhances the transcription of genes specific to endosperm. These genes are important that encode B-hordein and a trypsin inhibitor (BTI-CMe) in barley (Isabel-LaMoneda *et al*., 2003; Diaz *et al*., 2005). A Dof factor (OsDOF3) in rice also interacts with GAMYB protein (Washio, 2003).

The DOF6 and AtDof3.3 are the Dof transcription factors that majorly affect the germination of non after-ripened seeds in Arabidopsis. These factors interact with the TCP14 transcription factor, which belong to TCP (TEOSINTE BRANCHED1, CYCLOIDEA, and PCF domain). TCP14 is a bHLH-type (basic helix loop helix) transcription factor that controls the germination of seeds (Rueda-Romero *et al*., 2012). AtDof4.7 is an Arabidopsis Dof transcription factor that controls the process of absicission (premature shedding of various parts of plant) by regulating the expreesion of certain genes that encode for enzymes involved in hydrolysis of cell wall. These transcription factors interact with other

Zinc Finger Protein 2 TFs that are involved in abscession for enhancing transcriptional activity (Wei *et al*., 2010).

Dof transcription factors can also interact with HMG proteins that belong to the HMGB (High Mobility Group-box) family. These are the chromosomal proteins involved in DNA-dependant processes like DNA-repair, recombination, replication and transcription. These proteins have a single DNA-binding domain that nonspecifically binds to DNA. This DNA domain can interacts with the Dof domain of Dof factors (Yanagisawa, 1997; Krohn *et al*., 2002). These proteins enhance the binding efficiency of Dof transcription factors to the DNA. About five HMGB proteins were found to interact with Dof2 in maize and increase their binding with DNA. Studies revealed that CK2 (protein kinase) is an enzyme that halts the interaction of Dof1 and HMGB1 by phosphorylation. So, phosphorylation is necessary for their interaction to perform different plant activities (Krohn *et al*., 2002).

Dof transcription factors play an important part in plants to provide tolerant against biotic stresses. In recent years, Dof role has been identified in barley seeds where they interact with the cystatic gene (Martinez *et al*. 2005). Cystatins are a particular cluster of cystein proteinase inhibitors that were first identified in vertebrates, then in invertebrates and plant crops (Barrett, 1981). Different disease causing agents produces specific enzymes like cystein peptidases and proteinases. As a result, these enzymes help then to colonize and multiply within the plant cells. The cystatin proteins are helpful in inhibition of these degrading enzymes by restricting their proliferation and growth in host cells (Barrett, 1981). In an experiment conducted in vitro, two Dof factors (BPBF and SAD) interact with the oligonucletides. Ologonucleotides are the specific sequences that binding sites for Dof TF, derived from promoter of cystatin gene. OBP1 is another Dof factor from Arabidopsis that interacts with OBF4 OCS elements to provide resistance in plants against biotic stresses (Singh *et al*. 2002; Zhang *et al*. 1995).

Kishwaha *et al*. (2010) performed a study in which collectively twenty eight putative Dof genes have been recognized in sorghum. In sorghum, these genes have been identified by the in silico characterization from the entire genome shotgun sequence. These identified SbDof gene are widely distributed on nine chromosomes out of ten chromosomes of sorghum plant. These Dof genes don't have introns in their gene structure and this was achieved by canonical intron/exon structure tool. Phylogenetic analysis of these twenty eight SbDof proteins presented their evolutionary relationship with other plants. This analysis resulted in 4 subgroups consisting of 6 clusters. The comparative phylogenetic studies showed the relationship of these 28 genes with other 30 genes of rice and 36 genes of Arabidopsis. This comparative study generated six main groups similar to wheat genes. Motif analysis indicated the existence of 50 to 52 conserved amino acids domains of Dof transcription factor that are equally distributed all over the 28 Dof genes within sorghum crops. The *in silico* OCS analysis of these SbDof genes recommended its variety of functions that are linked with hormone and light responsiveness, particular expression of endosperm, stress responsiveness and particular expression of meristematic cells.

There is a great variety of Dof genes that are distributed among different plants. The characterization of these genes is very limited in case of beans specifically soybean. In this study a total of 78 putative GmDof factors have been identified in the whole-genome sequence from soybean. The characterization of these Dof genes have indicated that these genes are uniformly distributed within and all around the 19 chromosomes out twenty chromosomes. About 97.4% or 38 pairs have kept the duplicate paralogous genes on the duplicated portion of the soybean genome. These segmental duplications in soybean have contributed to the development of Dof genes specific to soybean plant.

The phylogenetic analysis of the proteins of these Dof genes have categorized into nine discrete subgroups. Among these subgroups, the gene structure and motif composition are conserved. The relative phylogenetic analysis of these proteins identified four main groups that resemble to those present in rice and Arabidopsis. The characterization of these genes showed particular pattern of expression depending on RNA sequence data analysis. The expression pattern of some of the duplicated genes has proven to be partially unnecessary but some of them are functionally diverse. Comprehensive expression profile analysis provided information about the functional divergence among the family members of Dof gene family specific to soybean. Cis-regulatory element analysis has indicated various functions of these genes in plants (Guo and Qiu, 2013).

This study was conducted by Jin *et al*. (2014) for the genome-wide analysis of gene family of Dof transcription factors in castor bean genome. In this analysis, a total of 21 RcDof genes were recorded in the genome of castor bean. These genes were then classified into 4 classes and 7 subgroups. This classification was based on their gene structure similarity and conserved regions of Dof factors. The size of Dof family in castor bean is comparatively smaller than those of 30 genes in rice and 36 genes in Arabidopsis. The fewer numbers of introns in the castor bean has indicated that they are much responsive to different environmental stresses. The global expression profile analysis of these genes has indicated that these genes are expressed in many tissues giving an indication of their involvement in many physiological functions. Cis-elements analysis also given its functional diversity associated with many activities like hormone, light and stress responses. Studies indicated these genes are highly expressed in leaves linked with light responses and seedling morphogenesis (Park *et al*., 2003; Ward *et al*., 2005). In castor bean, four genes (RcDof6, RcDof12, RcDof18, and RcDof20) are particularly expressed in leaves. The expression profile of

these 21 gene indicated only 19 out of these 21 genes respond to abscisic acid and gibberellic acid signals. This research has provided enough information about the molecular characterization of genes of RcDof family and their functions associated with maturity and development of castor bean plants.

This study was performed by Malavia *et al.* (2014) for the genome-wide analysis of genes of Dof family in pigeonpea. The analysis indicated 38 putative genes of Dof family by using BLAST search comparing with other conserved domains of amino acids. the in silicio characterization have given information about the gene structure, location on chromosomes, conserved protein motifs, phylogenetic relationship, duplication of genes, and divergence in their functions. The gene structure analysis showed that most of the genes are intronless and have no intron regions in their structure. The number of introns is less in CcDof genes ranging from 0 to 4. The gene structure analysis gave data about the intron number indicating only 17 out of 38 genes are intronless. A total of 17 genes from 36 in rice, 35 from 78 genes in pigeonpea, and 21 from 43 genes in Arabidopsis have been identified in recent studies (Lijavetzky *et al*., 2003; Guo and Qiu, 2013). The CcDof 6, CcDof14 have 2 introns and CcDof18, and CcDof25 genes have 3 and 4 introns respectively.

These putative genes are located on 8 chromosomes. Chromosome Visualization Tool (CViT) identified the location of these genes. The results revealed that most CcDof genes are distributed on chromosome 11 rather than on 2, 5, and 9. A total of 9 and 11 genes were located on chromosome 1 and 11 respectively. Only one gene was found on chromosome number 3. The phylogenetic analysis has given the evolutionary relationship of these 38 CcDof genes. These genes were categorized into three main clusters and 2 subclasses. The phylogenetic analysis was conducted by using MEGA 5.2.2 software. A total of 25 conserved domains of amino acid motifs were identified in this analysis among 38 putative genes. After that Cis-regulatory element analysis was performed for the identification of 13 elements in 11 CcDof genes involved in light response and other activities. These elements are associated with biotic, abiotic stress responses, light response and photoperiodic opening and closing of flowers.

In gene duplication analysis, 14 paralogous gene pairs were identified present either on the same chromosome or different chromosomes. Five paralogous gene pairs were scattered throughtout the genome, resulting in segmental duplication and 9 pairs co-located on chromosome, resulting in tandem duplication. These tandem or segmental duplications on the chromosomes contributed to the wide expansion of genes of Dof family in pegionpea. The comparative studies of these 38 putative CcDof genes with 36, 30, 78 genes of Arabidopsis, rice and soybean based on phylogenetic analysis have given seven major clusters. Many ortholog and paralog groups were constructed based on these phylogenetic studies. The characterization of these putative genes has provided enough information in peigonpea for further research purposes.

The current research was planned by Wu *et al*. (2015) to identify the St Dof genes in potato. For that purpose, a in silico genome-wide analysis of the potato genome was conducted to find the location, structure of these genes on chromosomes, phylogenetic relationship and protein motifs of these gene products. The analysis indicated the presence of 35 Dof genes in potato genome that encode for about 43 proteins. These genes are distributed on 10 chromosomes excluding chromosome 7 as well as 12. The transcriptional products of these genes are ST DOF proteins and these proteins have a particular length of 165-503 amino acids. The isoelectiric point for these proteins is 4.72-10.04. The phylogenetic analysis of ST proteins led to 4 classes. *In silico* analysis of St Dof genes indicated their role in biotic and abiotic stresses as well as their response to different hormones. An expression profile of these genes was generated based on Illumina RNA-seq transcriptome data. These profiles provide a clue for improving genetic traits in potato under different stress conditions.

In this study, various Dof genes were identified throughout the genome and the expression profiles of these genes at different stages of development and their responses to abiotic stresses. Data was retrieved using BLAST tool, considering Arabidopsis Dof genes as reference. Gene structure analysis showed that No. of introns varying from 0 to 4 and exons. The genome wide analysis of these genes revealed a total of 35 StDof genes randomly found on chromosome No. 10 from total of 12 chromosomes. There was no StDof gene, found on chromosome 7 and 12. A total of 9 genes were found on chromosome 2. A number of 5 and 6 genes were indicted on chromosome No. 3 and 6 respectively. Only two genes were found on chromosome No. 1, 4, 5, and 8 and only one gene was present on chromosome No. 9. Cis-regulatory elements analysis was performed for identifying the cis elements present in the promoter region of StDof genes by using PGSC database and *in silico* analysis was conducted using PLACE database. Stress-responsive cis elements like MYCCONSENSUSAT and CURECORECR, and hormonal responsive elements like WRKY71OS and ARR1AT were revealed in this analysis. The phylogenetic analysis of StDof proteins categorized them into 4 subgroups (StDof1-4) and also showed their evolutionary relationship with other genes. The expression analysis of these genes indicated their role in different plant functions. Various StDof genes showed higher expression in reaction to ABA, salinity and drought stress in potato crop (Venkatesh and Park, 2015).

This study was conducted by Wen *et al*. (2016) for the genome wide analysis of cucumber plant. The analysis identified 36 CcDof genes (CsDof1-CsDof36) distributed throughout the genome. The predicted proteins of these genes consist of 150-503 amino acids with isoelectric point of 4.8-

9.6. These genes were located on 6 chromosomes out of 7 chromosomes. Eleven out of thirty-six genes were mapped on chromosome No. 6. A total of 10, 8, and 5 genes were located on chromosomes 1, 3, and 5. Only 1 gene was found on chromosome No. 2 and 4 and no gene was found on chromosome 7. Duplication analysis resulted in identification of 2 pairs of tandem duplications and 6 pairs of segmental duplications that led to widespread of cucumber genome. The phylogenetic analysis of CsDof factors resulted in 4 groups and 8 subgroups. About 15 conserved motifs were identified based on this analysis. The expression profile of CsDof genes indicated that these genes are overexpressed in response to biotic factors specifically against downy mildew and watermelon mosaic virus disease. The results showed that these genes can be responsible for conferring resistance to cucumber plant against biotic stresses. All these genes were identified based on structural and functional characterization. The studies suggested that these defense-related genes are potential approaches to combat biotic stresses.

This study was conducted by Dong *et al*. (2016) for the identification of various genes within the genome of *Musa acuminata* (banana). A total of 74 MaDof (MaDof1-MaDof73) genes were mapped in this analysis. These genes are widely distributed on chromosome 1-11. The size of resulted proteins range from 119 to 466 aa. The average size of these proteins is 284 amino acids. The structural analysis of these genes indicated the No. of exons and inrons ranging from 0-4 using GSDS software. About 28 MaDof genes have no intron in their structure. A total of 1, 2, 3 and, 4 introns were found in 21, 12, 9 and, 4 MaDof genes. The studies about gene duplications showed a total of 17 gene pair duplications. These duplications resulted from 16 segmental and 1 tandem duplications lead to expansion of Dof family of MaDof genes. The phylogenetic analysis of MaDof proteins resulted in 4 subgroups (StDof1-4). About 20 conserved motifs were identified in these MaDof proteins. The expression analysis showed increased regulation of these genes in response to salt and drought stresses, other abiotic stresses, and their involvement in other developmental responses.

This research carried out by Wu *et a*l. (2016), genes of Dof family were identified in pepper (*Capsicum annuum*) genome. The data mining of Dof genes led to identification of 33 CaDof genes lacking redundancy. The BLAST searches resulted in the detection of coding sequences of these genes ranging from 441bp to 1512bp. The size of resulted proteins ranges from 46aa to 503aa with average size of 317aa. These genes' isoelectric point (PI value) of these genes can be 4.15 to 9.69. These genes are located on different chromosomes (0-11 chromosomes except No. 7 chromosome). The results of this analysis indicated that twenty-nine out of thirty-three chromosomes found on 11 chromosomes except No. 7 chromosome. Chromosome no 2 consists of most of the genes of CaDof family (eight members). On the other hand, only 1 CaDof gene located on chromosome No. 8, 9, 10, and 11. After that intron-exon structure of CaDof genes was analyzed. A range of 0-2 introns were found in these CaDof genes. A total of 15 CaDof genes were intronless having no intron, 12 genes have only 1 intron, and 6 of them have 2 introns in their structure.

The phylogenetic analysis of the CaDof proteins classified them into four main groups. CaDof genes within the same group have same gene structure and other characteristics. Group 1 has 10 genes and group 2 and 3 have 8 genes and in the end group 4 has minimum of 7 genes. The analysis showed 25 conserved motifs present within CaDof proteins. Based on conserved motifs, these gene families are clustered into classes. Class 1 had only one similar conserved motif based on functions. Class 2 contained conserved motif 6 and 10. Class 3 showed a particular motif known as motif 13. Class 4 has 10 conserved motifs (motifs 2, 4, 5, 6, 9, 11, 13, 16, and 17. The phylogenetic relationship within the pepper and with other plants such as *Solanum lycopersicum*, *Arabidopsis thaliana*, *Oryza sativa*, *Vitis vinifera and Sorghum bicolor* was also analyzed. The differential expression analysis explained the roles of these CaDof genes in different tissues and developmental stages under abiotic stresses such as heat stress and salinity stress. The results indicated that several genes including CaDof 1, 7, 9, 11, 14, 20, and 24 were up-regulated in response to heat stress. Several other genes that includes CaDof 10, 13, 15 16, 24, and 26 were highly expressed under salt stress. The results showed that these genes play a particular part in providing tolerance to plants against abiotic stresses.

A genome-wide analysis of foxtail millet (Seteria italica) was conducted to identify the Dof genes known as SiDof. The analysis indicated 35 genes of SiDof family (SiDof1-SiDof35) located throughout the genome. The sequences of these genes were identified by using BLAST database then these SiDof genes were verified by SMART database. ProtParam tool (ExPASy) was used for the identification of size and isoelectric point of predicted SiDof proteins. The size ranges from 168aa (shortest protein) to 62aa (longest protein) with the average size of 328 amino acids. The isoelectric point of these proteins varies from 4.81 to 10.06. A chromosomal localization analysis was conducted for identifying the location of SiDof genes on chromosomes. These SiDof genes were distributed on 9 chromosomes. A total of 9 from 35 SiDof genes were located on chromosome No. 9. Only 1 gene was present on chromosome 4 and 6 in contrast to chromosome No.9. Four SiDof genes were found on chromosome 1 and 8. In contrast, 2, 3, 5, and 6 SiDof genes were found on chromosome 7, 2, 5, and 3 respectively.

The structure of SiDof genes were analyzed using GSDS 2.0 indicating number of introns and exons within gene structure. The analysis showed that 20 SiDof genes have no intron and 15 SiDof genes have only one intron in their structyre. Conserved domain analysis was conducted for the identification of conserved motifs using MEME software.

Fifteen conserved motifs were detected present on SiDof genes. A conserved motif of 55 amino acids was present in all genes. One group of SiDof 1, 23, 24, and 25 had motifs 2, 3 as well as 4. Another group of SiDof 15, 32, and 35 contained motifs 5, 8 along with motif 12. Phylogenetic analysis was conducted for revealing the evolutionary relationship of SiDof proteins of foxtail millet and of other plants such as Arabidopsis thaliana, sorghum, and rice. These proteins were then categorized into six main groups. The differential expression analysis of these genes indicated their role in different plant tissues. The resulted showed that SiDof 7 and 15 were up-regulated in response to water deficit stress. These findings are helpful for developing tolerance in foxtail millet and other plants (Zhang *et al*., 2017).

The current investigation was carried out by Kang *et al*. (2016) for the genome-wide analysis of pepper. The data was searched using the BLAST tool that gave 33 Dof genes known as CaDof (CaDof1-CaDof33) in the pepper genome. The size of coding sequence ranges from 525bp to 1512bp. The size of predicted CaDof proteins ranges from 173aa to 503aa. The isoelectric point of these proteins varies from 3.61 to 9.79. The structural analysis showed the number of exons and introns within CaDof genes. The results indicated that 22 from the 33 Dof genes were intronless. Four and seven CaDof genes had two and one introns respectively. The exon-intron structure in pepper was similar to those in rice, tomato, and Arabidopsis. The localization analysis of CaDof genes revealed that these genes were unevenly located on chromosomes. About 26 CaDof genes were found on eight chromosomes from total of twelve chromosomes. Chromosomes 7, 8, 9, and showed no presence of genes. One CaDof gene was mapped on chromosomes 1, 4, and 11, two genes were mapped on chromosome 5, three genes were on chromosome 3 as well as 10, six were on chromosome 6 and nine CaDof genes were on chromosome 2 respectively.

The analysis of gene duplication resulted in expansion of gene family of CaDof factors in pepper based on seven pairs of segmental duplication and one pair of tandem duplication within the genome of pepper. The phylogenetic analysis revealed the dividion of these CaDof genes into four main groups. These were then further divided into seven subgroups. A total of twenty-five conserved motifs were identified based on phylogenetic studies in 33 CsDof genes using MEME tool. The size of conserved motifs could be from eight to one hundred and thirteen amino acids. Motif 1 known as Dof domain was found on all 33 CaDof genes. The expression analysis of CaDof genes indicated their role in plant physiology under stress conditions. Studies showed that these genes were highly regulated and overexpressed in response to tobacco mosaic virus (TMV), PepMoV and *Phytophthora capsici*. Results showed that pepper plants were resistant to these biotic stresses and these genes are valuable for developing several other resistant plants.

The present research was carried out by Wang *et al*. (2016) for the detection of genes from Dof family in moso bamboo plant (*Phyllostachys heterocycla*). A total of 26 Dof genes (PhDog1-PhDof22) were identified based on genome wide analysis in moso bamboo plant. BLASTP tool was used to identify these genes' sequences by giving query from Arabidopsis and rice Dof genes. After that sequences were verified using InterProScan and SMART tool for confirming the Dof domains. Further studies showed the predicted proteins of PhDof genes size that 197aa to 542aa. Gene structure analysis indicated the number of exons and introns present in PhDof genes using GSDS tool. A number of 0-4 exons were found in PhDof genes. Among these genes, 1 gene had 4 exons. Six, nine and ten genes had 3, 1, and 2 exons respectively.

The phylogenetic studies showed the evolutionary relation of these genes within the moso bamboo plant and with other plants. These genes were classified into 4 classes consisting of seven clusters (subgroup A, B1, C1, C2 as well as D1, 2, and 3. Twenty-five conserved motifs were identified in these PhDof genes based on evolutionary studies. Motif 1 was found in all genes considering it as Dof domain. Subgroup A, B1 as well as D3 showed no motifs while D1 subgroup showed maximum number of motifs that included 2-8, 10, 19, 11, 16, 25, 14 and 20-23. Subgroup C1 showed particular motifs 9 as well as 17. Subgroup C2 showed 21, 12, 24, and 18 and subgroup D2 showed motif 13 respectively. The expression analysis of these genes showed their expression in flowers and shoots under various conditions. PhDof4 and 5 were over expressed during flowering under drought stress. These results are helpful for increasing moso bamboo and many other plants efficiency under stress conditions.

The genome wide analysis of Populus (*Populus trichocarpa*) genome revealed 41 PtrDof genes (PtrDof1-PtrDof41). The size of encoded proteins was 159aa to 1485aa. The average size of these proteins was 684 amino acids. These genes were then classified into 4 major subgroups from A to D. Subgroups A and D were considered as the largest groups containing 12 PtrDof genes in each subgroup. Subgroup B and C had 8 and 9 genes respectively. Structural analysis of these genes was conducted using GSDS tool showing number of introns and exons found within the gene sequences. The No. of exons varied with minimum 1 to maximum 4. Nineteen genes out of 41 had 1 exon, nineteen genes had 2 exons. Only three genes showed presence of 3 exons. Motif 1 was present in all PtrDof genes, considered the Dof domain of PtrDof genes. Further, subgroup A showed 1 or 2 genes and subgroup C showed 0 introns. Additionally, 15 conserved motifs were identified in these 41 genes using MEME tool.

All the genes were found on chromosomes 18 from total of 19 chromosomes in populous plant. Chromosome 4 and 11 showed 4 genes out of 41 genes. In addition, only one gene was found on chromosome 9, 13, 16, 17 and 19. Two or three genes were found on the remaining chromosomes. Genome

wide duplication analysis revealed the expansion of DOf gene family in populous plant. These expansions were due to several segmental and tandem duplications. Cis-regulatory analysis indicated that several phytohormones such as ABA, ethylene, and salicylic acid activated under stress conditions. The expression analysis showed over-expression of some genes that were PtrDof14, 16, 27, 39, 25, 37, and 28. The results showed that these genes were highly expressed in leaves as well as in shoots during osmotic and ABA stresses. So these genes are important for providing resistance in populous and other plants under various abiotic stresses (Wang *et al*., 2017).

The identification and characterization of Dof genes have been done in many plant species. There is not study reported in case of coffee plants. In this study, a genome wide analysis of *Coffea canephora* genome has been conducted resulting in identification of 24 genes (CcDof1-CcDof24) of Dof family. These genes were identified from a *Coffea canephora* database known as Coffee Genome Hub. The analysis showed the size of resulted proteins ranging from 167-513aa. The isoelectric point for these proteins could be 4.24-10.13. The distribution and localization of these 24 CcDof genes on eleven chromosomes was uneven and random. These genes were not found on chromosome No. 3, 5 as well as 9. The greatest number of CcDof genes (6) was found on chromosome 2, which is also a largest chromosome in coffee plant having size of 55Mb.

The structural analysis indicated the number of exons and introns and their organization within 24 CcDof genes. In case of coffee plant, the number of introns was in a range of 0-5. The CcDof19 gene contained the maximum number of introns which were five. The phylogenetic analysis of these genes allowed them to categorize into 6 major groups (1-6). These groups were phylogenetically identical having the same functions, structure, and origin, each group comprised of CcDof genes ranging from 2 to 8. In these 24 CcDof genes, 24 conserved motifs were identified. Motif 1 was considered as the Dof domain found in all the CcDof proteins. The studies indicated that several genes such as CcDof1 and 2, 10 to12 as well as 15 were expressed in tissues of roots and leaves. They were involved in several biological functions, growth and development. They also involved in protecting plants against biotic and abiotic stresses. These genes are important for further studies in coffee plants (Gracia *et al*., 2018).

The Dof genes are ubiquitous to plants and involved in several biological and physiological functions as well as ripening processes. In this study, Dof genes have been identified in eggplant (*Solanum melongena*) using Eggplant Genome Database. The genome wide analysis of eggplant genome has been conducted resulting in identification of 29 SmeDof genes (SmeDof1-SmeDof29). These SmeDof members have been categorized into four main groups as well as 9 subgroups. These groups were homologous to each other having similar structure and origin. The structural analysis resulted in the detection of exon and intron numbers in eggplant genes. The organization of exons and introns were important for identifying the evolutionary relationship between these genes. Eggplant genes had 0 to 2 intons in their structure. The 24 from 29 genes were intronless. Three genes (SmeDof15, 17, 29) comprised of only one intron and two genes (SmeDof2 and 7) comprised only one intron and two genes (SmeDof2 and 7) comprised 2 introns. Fourteen motifs have been identified in these SmeDof genes using MEME tool (Wei *et al*., 2018).

In the current studies performed by Azam *et al*. (2018), a total of 26 Dof genes (AcoDof1-26) have been identified in pineapple (*Ananas comosus*) based on genome wide analysis using HMMER database. The number of amino acids fro resulted proteins ranged from 137 to 479 and the isoelectric point was 16.3 to 51.5. The structural analysis showed the number of introns and exons (1-5) present within the genes. Three genes from 26 total genes had five exons. One gene comprised of three exons. Eighteen and four genes consisted of two and 1 exon respectively. Four genes AcoDof3, 8, 13 and 25 were inronless having just exons in their structure. Based on phylogenetic tree, these genes were classified into 5 main groups. group 1, 2, and 4 had 4, 3, and 5 AcoDof genes respectively. Group 3 and 5 had 7 and 5 AcoDof genes respectively. For diversification of AcoDof genes, MEME program was used for the identification of putative motifs. The results showed ten conserved motifs in all 26 genes collectively. Motifs 1, 2 as well as 3 were found in all AcoDof genes representing the Dof domain.

These 26 genes were randomly distributed on sixteen chromosomes. Eleven genes were located on individual chromosomes. In contrast, rest of the genes was located as group of 2 or 3 genes on the same chromosome. Three Dof genes (AcoDof24-26) were located on scaffold regions (scaffold_502, 1002 and 2504). These regions were not assigned to chromosomes yet. The expression analysis of these genes indicated their expression in leaf and flower tissues. The AcoDof1, 12, 26 genes were highly expressed in roots. The AcoDof9, 22, 19 and AcoDof23, 25 genes were expressed in fruits and ovule respectively. The AcoDof 11, 17 and 20 genes were expressed on petals. The expression analysis also showed role of genes in development of female gametophyte. The studies indicated that several genes were up-regulated under abiotic stresses. The AcoDof2, 8 and 12 genes were highly expressed in different tissues under salt stress. The AcoDof3, 9, 11 and 25 genes were up-regulated in cold stress providing tolerance to plant against cold stress. The AcoDof genes (2, 3, 13, 15, and 20) were highly regulated under heat stress. Several genes (8, 11, 12, 20, 22, and 26) were up-regulated under water scarcity.

Khaksar *et al*. (2019) performed a genome-wide analysis that detected twenty-five putative Dof (AcoDof1-25) genes within the pineapple genome using the pineapple genome using pineapple genome database. The size of proteins varied from

136aa to 497aa. These genes were classified into seven groups and nine subgroups in phylogenetic analysis. The expression analysis of these genes indicated that twenty genes (AcDof3, 4, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, and 23) showed high expression during internal browning of pineapple. Studies indicated these genes' role in the pineapple's internal browning. Genome-wide analysis was also conducted in durian resulting in the identification of 24 Dof genes (DzDof1-DzDof25). Fifteen genes out of 24 DzDof genes were also expressed in fruit pulp. These genes led to high expression of other genes responsible for auxin biosynthesis during fruit ripening.

Liu *et al*. (2020) conducted a research in which a total of 108 Dof genes were detected across the genome of wheat (*Triticum aestivum*) known as TaDof (TaDof1-TaDof108). These genes' sequences were identified using queries from Arabidopsis, rice, and maize. These genes were distributed on twenty-one chromosomes. These genes were clustered into seven subgroups based on structural similarity. The structural analysis showed the number of exons and introns in members of all groups. Most of the genes had a range of 0-2 introns. TaDof4.1 genes comprised of seven introns. There was no intron found in subgroup 1 and 7. Among the thirteen genes of subgroup 2, eleven members contained 7 introns and two genes had no intron. Twenty conserved motifs were identified in TaDof genes. Motif 1 was present in all genes known as Dof domain. Motif 10 and motifs 4 as well as 9 were found in subgroups 2 and 3 respectively. The expression analysis of TaDof genes showed the expression of several genes in tissues under different stress and growth conditions. Various TaDof genes were up-regulated under salt as well as drought stress. In other studies, 96 Dof genes were identified in wheat located on 1-7 chromosomes. These genes were clustered into 5 subfamilies based on evolutionary relationship. The expression profile of these genes were highly expressed under heavy metal, PEG as well as heat stress.

In-silico genome wide analysis of cotton species was conducted by Chatta *et al*. (2020) to identify the Dof genes. In this study, the cotton species were either diploid (*Gossypium raimondii* & *Gossypium arboretum*) or allotetraploid (*Gossypium barbadense* & *Gossypium hirsutum*). In this analysis, 58 Dof genes were found in *G. arboretum* (GaDof01.1-GaDof13.4), 55 Dof genes in *G. raimondii* (GrDof01.1-GrDof13.1), 89 genes in *G. hirsutum* (GhDof1.1-GhDog13.1), and the maximum number of Dof genes (110) were recognized in *G. barbadense* (GbDof). In case of *G. arboretum*, all 58 genes were located on thirteen chromosomes. Only one GaDof (GaDof12.1) was found on chromosome 12. Rest of the chromosomes contained more than one gene. The size and PI value for GaDof proteins was ranged from 167 to 515aa and 4.82-9.66 respectively. In case of *G. raimondii*, all 55 genes were located on 12 from 13 chromosomes. The size and PI value for these proteins was 155-515aa and 4.81-10.57 respectively. In *G. hirsutum*, 89 GhDof genes were located on 12 chromosomes. The average size of GhDof proteins was 292aa with a range of 162-515aa and the PI value was 4.86-9.60. In *G. barbadense*, all 110 genes were located on eleven chromosomes.

The phylogenetic analysis of Dof genes from all these species was conducted resulting in generation of an evolutionary tree. This evolutionary tree indicated the evolautionary relationship and their origin. Based on similarity, GaDof genes were organized into five groups in *G. arboretum*, GrDof genes were clustered into four groups in *G. raimondii*, GhDof genes were classifies into seven groups in *G. hirsutum*. The structural analysis showed the distribution of exons and introns in GaDof, GrDof, GhDof, and GbDof genes. Expression analysis indicated the expression of these Dof genes in particular cotton species. Twenty-one GhDof genes were expressed in *G. hirsutum* during growth and developmental stages. These genes also played an important role in response to biotic and abiotic stresses. In current study carried out a total of 33 Dof genes were identified using BLAST tool in *Eragrotis tef*. The evolutionary relationship between Dofs resulted in categorization of 10 groups. These genes' expression and promoter analysis indicated that they were involved in photosynthesis and biotic and abiotic stresses.

This recent studies was conducted by Zhou *et al*. (2020) for genome wide analysis of watermelon (*Cittrulus lanatus*) in order to identify genes involved in several developmental processes. So as a result of this analysis, 36 Dof genes were collectively recognized as ClDof genes (ClDof1-ClDof36). The length of sequence for ClDof genes ranged from 492-1575 bp and the size of predicted ClDof proteins ranged from 163-424 amino acids. The PI value for these proteins was 5 to 9.95. For evolutionary studies, a phylogenetic tree was constructed from ClDof proteins of watermelon and other plants like Arabidopsis, cucumber, and rice. These studies indicated the evolutionary relationship of watermelon plants with each other and with other plants. The resulting evolutionary tree showed that these genes 36 were organized into 9 major groups (A, B1 and B2, C1, C3, C2.1 and C2.2, D1 and D2. All Dofs were present in all the groups with exception of C3. Only one gene was found in C3 group. Two genes were found in group C2.2. Three genes were found in members of each A, B2, and C1 groups. Four, five, seven, and eight genes were found in D2, C2.1, B1, D1 groups.

The Dof domains were identified using MEME program. A total of 10 conserved domains were detected in 36 ClDof genes. Conserved motif 1 was considered to be present in all the ClDof genes except ClDof4, known as Dof domain. Motifs three, four, six, seven as well as ten were found in ClDofs of D1 group. Motif two was found in ClDofs of B1 group. Motif nine was present in all ClDofs of B1 group with exception of ClDof20. Motif eight was found in all members of C1, C2.1, and C2.2 groups. The structural analysis of ClDof proteins showed the number on exons and introns.

Twenty from thirty-six ClDof genes were lack of introns. One intron was found in eleven genes (ClDof5, 10, 15, 21, 23, 24, 27, 28, 32, 33, and 34). Two introns were present in 5 ClDof genes (ClDof4, 11, 13, 20, and 36). All the 36 ClDof genes were found on ten chromosomes from total of twelve chromosomes. Two genes, ClDof1 and ClDof2, were located on 0 chromosomes. In contrast, chromosome 2, 4, 7, and 8 were comprised of 2 ClDof genes. Chromosomes 1, 3, 5, and 6 contained 10, 5, 3 and 3 ClDof genes. One and 4 ClDof genes were found on chromosome 9 as well as 10. In cis-element analysis, five elements were identified; linked to biotic and abiotic stress conditions and nine elements were linked to hormonal responses. The expression pattern of these genes showed their up-regulation in several tissues under stress conditions.

Conclusion: This study provided the review on characterization of DNA binding one zinc finger (Dof) transcription factors in *Vigna* species. These Dof factors are plant-specific and involved in several plant activities. These factors also played an important part in response to biotic and abiotic stresses. The gene structure, conserved motifs, and homologous genes of Dof factors were extensively reviewed in different *Vigna* species. In future it is of our interest that how these factors perform their part to facilitate plant defense against environmental stresses to increase crop yield.

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