Journal of Environment & Biotechnology Research
VINANIE PUBLISHERS
www.vinanie.com/jebr

Role of abcisic acid in regulating the expression of EcMyb gene for drought stress tolerance in *Eleusine coracana*

Sarita Kumari, Shipra Sharma, Anil Kumar, Pushpa Lohani*

Department of Molecular Biology & Genetic Engineering, College of Basic Science and Humanities, GB Pant University of Agriculture & Technology, Pantnagar. INDIA

ORIGINAL RESEARCH ARTICLE

ABSTRACT

Abcisic acid (ABA) plays many important roles in plants, including seed germination , dormancy, stomata closure, and adaptation to water stress. ABA has been implicated in responses to environmental stresses such as drought and salinity. Drought is a major abiotic factor that limits agricultural crop productivity. The study was performed to investigate the effect of drought on expression of Ecmyb gene on drought tolerant (PRM 6107) and sensitive genotypes (PES 400) of *Eleusine coracana* in seedling stage and investigate whether it followed the ABA dependent pathway. Different experiments were conducted wherein drought was induced by treatment with PEG (polyethylene glycol) and ABA (abscisic acid) was applied in three different concentrations i.e. 25, 50 and 100 µM. Expression of EcMyb gene was observed only in tolerant variety PRM 6107 under drought stress. It could be thought that the expression of EcMyb transcription factor gene may be upregulating the expression of many drought responsive genes which is providing drought tolerance to PRM 6107. In response to ABA, EcMyb gene was expressed in both the genotypes. However, expression was strong and early i.e. after 6 h of the treatment in PRM 6107 than in PES 400. As drought is a complex phenomenon, therefore, to understand how ABA accumulates in response to drought in both tolerant and sensitive varieties, primers were designed for NCED (9-cis epoxy carotenoid dioxygenase) gene, an important gene in ABA synthesis pathway and co-expression of NCED and EcMyb gene was recorded. The co-expression of NCED gene and EcMyb gene was observed only in PRM 6107 in drought conditions since first day whereas in PES 400 a basal level expression of NCED gene was observed after 5th day. There was no expression of EcMyb gene. It was concluded that EcMyb gene expressed only in that genotype which can synthesise a certain level of ABA. Morphological and physiological parameters were also recorded in both the genotypes after drought and ABA treatment. Root length increased, whereas shoot length, relative water content (RWC) and chlorophyll content decreased with exposure of drought as well as ABA treatment. The study suggests that EcMyb gene expresses only when ABA accumulates in plant cell and may follow ABA dependent signalling pathway.

KEYWORDS
abcisic acid; drought; EcMyb gene; *Eleusine coracana*; NCED gene

1. INTRODUCTION

Global warming possesses a serious threat to contemporary agriculture. It is speculated that average global temperature will rise by 1 °C till 2050 and by 2-4 °C by 2100 (Nelson and Gerald, 2010). This increase in temperature may adversely affect agricultural production and productivity. Conditions that adversely affect the crop growth and yield is called as stress. Stress may be biotic or abiotic. Abiotic stress such as drought and salinity causes loss up to 50 % of the potential yield in major crop plants. On the other hand the world population is estimated to increase from 6.8 billion in 2011 to 9.3 billion by 2050 (United Nations, 2011). So it is a great challenge to feed the growing population with shrinking agricultural land. In such

Corresponding author: **P.Lohani**
Tel: +91-9411539749
E. mail: pushpa.lohani@ yahoo.com

Received: 15-11-2016
Revised: 25-11-2016
Accepted: 14-12-2016
Available online: 01-01-2017

situation, it is imperative to search for an alternative source either in the form of modified cultural practices or genetic modifications per se of crops that could better adapt to such stress situations and bridge the gap between demand and supply of food grains. C4 plants like millets, maize, sorghum perform well under stress condition. In general, abiotic stress often causes a series of morphological, physiological, biochemical and molecular changes that affect plant growth, development and productivity. Drought, salinity, extreme temperatures (cold and heat) and oxidative stress are often interrelated. These conditions either alone or in combination induce cellular damage. Abiotic stress such as drought leads to accumulation of ABA endogenously. Accumulation of ABA triggers ABA dependent signalling pathway. Accumulation of ABA causes up regulation and down regulation of ABA responsive gene. This up regulation and down regulation occurs due to a number of factors; among these transcription factors (TFs) and RNA binding proteins are of great importance.

Finger millet (*Elucine coracana*) is one such crop which is well known for its hardiness and cultivation on marginal land deprived of inputs like irrigation. Therefore it can be used for fishing genes responsible for drought tolerance. Once such genes are identified, these can be isolated and integrated into the genome of other crops like wheat and rice using recombinant DNA technology to tailor abiotic stress resilient crop. Therefore, the molecular exploration of finger millet, an underutilized but drought tolerant and nutritionally rich crop would hold a high promise in the present global warming scenario.

Drought responsive Myb proteins are TFs (Transcription factors) composed of one, two, or three imperfect helix-turn helix repeats that recognize the major groove of DNA and are thought to play an important role in imparting drought tolerance to plants (Yanhui et al., 2006). Therefore, investigating the pathway underlying drought tolerance in plants is important and finger millet can be taken as a model plant for this purpose. EcMyb, homologue of a drought responsive Myb gene has been shown to express in *Eleusine coracana* and its expression increased with the severity of drought (Salvi et al., 2012). The present study was carried out to investigate the expression pathway of EcMyb gene in seedling stage of *Eleusine coracana* under drought stress and to determine whether it followed the ABA dependent pathway.

2. MATERIALS AND METHODS

2.1. Plant material

Two contrasting genotypes of finger millet viz. PES-400 & PRM-6107 were used. The former was drought susceptible and the latter was drought tolerant (Rai et al., 2009).

Seeds were sown in pots filled with garden soil, peat moss and vermicompost in 3:1:1 ratio and plants were grown in a poly house. Uniform germinated seedlings were transplanted into plastic foam frames suspended in a plastic tray containing an aerated nutrient solution (Sonoda et al., 2003). The pH of the solution was maintained at 5.8. The seedlings were grown in nutrient solution for 4 days before subjecting them to experimental conditions.

2.2. ABA treatment

Both the contrasting genotypes were subjected to ABA treatments of different concentrations (25, 50 and 100 μM) by mixing with the nutrient solution. The pH of the ABA solution was adjusted to 6.5 by adding HCl or NaOH. The treatment was given through roots. No ABA treatment was given to control.

2.3. PEG treatment

In a separate experiment, drought was imposed to the seedlings using PEG (15 % polyethylene glycol having a molecular weight 8000 MW) in the nutrient solution. Plantlets were subjected to drought by immersing the roots of both the genotypes in the nutrient solution containing PEG. Control was not subjected to PEG treatment.

2.4. Primer designing

Primers were designed against two genes, EcMyb and NCED. The target specific internal primer was designed for EcMyb1 using NCBI data base (accession no. JN107890.1) and online PRIMER3 tool. Since no information was available regarding NCED genes of finger millets at NCBI, homologous sequences from other species like Zea mays, Oryza sativa, Sorghum bicolor, Setaria italica, Triticum asetivum and Hordeum vulgare present in NCBI data base were downloaded. These sequences were aligned using on-line bioinformatics tool CLUSTALW. Based on homology and conserved region, degenerate primers were designed. Tubulin primers were used as control. Primers were custom synthesised by IDT (Integrated DNA Technologies).

2.5. RNA isolation and semi quantitative expression analysis

An RNase free environment was created prior to RNA isolation. Tips and tubes of diffetablrent sizes were thoroughly treated with 0.1% DEPC (Diethyl pyrocarbonate) water and then completely dried in oven and autoclaved. Aluminium foil and tissue paper to be used were also autoclaved. The pestle and mortar were dipped in 0.1% DEPC water overnight, wiped with chloroform , autoclaved and subsequently kept at 0 °C to cool it before grinding the samples. Workbench was also cleaned with DEPC water.

In order to study the expression of genes (EcMyb and NCED), total RNA was isolated from leaves using RNA isolation solution (invitrogen) and digested with RNase-free DNase I to eliminate genomic DNA contamination of the desired gene. Isolated and purified RNA was quantified by nano-drop instrument. From quantified RNA, 2 µg RNA was reverse transcribed using cDNA synthesis kit of Fermentas according to the manufacturer's instructions. The transcript level of each gene was measured using semi quantitative PCR in a total volume of 25 µL reactions. The amplification program was as follows: Initial denaturation at 94 °C for 3 min, and then 32 cycle of sequential step at 94 °C for 1 min, 55 °C for 30 sec, 72 °C for 30 sec and the last reaction cycle was at 72 °C for 7 min.

The expression levels of EcMyb gene and NCED gene were analysed by densitometry analysis on gel using geldoc system known as Semi quantitative expression analysis.

3. RESULTS AND DISCUSSION

In PEG treated genotype PRM 6107, the expression of EcMyb gene was observed and it increased with increase in duration of exposure of PEG. In other words, the expression of Ecmyb gene increased in drought tolerant genotype with increase in severity of drought. There was no expression of EcMyb gene in drought sensitive genotype PES on exposure of PEG. PEG was applied to mimic the drought stress and as PRM 6107 is drought tolerant genotype so its tolerant phenotype was positively correlated with the expression of EcMyb while PES 400, a drought sensitive genotype failed to accumulate an optimum level of EcMyb gene product to combat drought.

In order to investigate their ABA dependency, the expression of EcMyb gene was observed in response to ABA under unstressed condition and it was found that the gene was expressed in both the genotypes even without any drought stress. The expression of EcMyb gene was found to increase with increase in concentration as well as duration of exposure to ABA in both the genotypes. It indicates that expression of EcMyb gene is ABA dependent and it may be possible that in sensitive genotype ABA did not accumulate to such a level so as to induce the expression EcMyb gene.

The expression of genes encoding the first two enzymes of the ABA biosynthetic pathway, zeaxanthin epoxidase (ZEP) and 9-cis-epoxycarotenoid dioxygenase (NCED), is significantly induced by stress conditions in order to increase drought tolerance (Tan et al., 2003; Moumeni et al., 2011; Ye et al., 2011). The expression of ZEP, MCSU and AAO3 in Arabidopsis (Xiong et al., 2002) and of AhNCED1 in peanut (Guo et al., 2009) was significantly up-regulated by exogenous ABA.

The expression analysis of NCED gene showed that there was a slight increase in expression from 1st to 7th day in drought tolerant genotype, PRM 6107 after PEG treatment while, only slight expression was observed in drought sensitive genotype, PES 400 after 5th and 7th day. It means that ABA accumulation in sensitive genotype was less than tolerant genotype, which may not be sufficient to increase the expression of EcMyb gene. Co-expression of NCED gene and EcMyb gene in PRM 6107 indicates that EcMyb gene which is drought responsive is expressed when optimum amount of ABA accumulates in the plant.

3.1. Effect of PEG treatment on EcMyb and NCED gene expression

A gradual increment in the expression level of EcMyb was recorded in PRM 6107 right from the first day of PEG treatment that created a physiological drought condition in the plant. The expression reached its plateau at 5th day and was constant up to 7th day of PEG treatment (Figures 1 and 2). However no expression of EcMyb gene was observed in PES 400 under the same experimental condition. To further investigate whether regulation of EcMyb is ABA dependent or not, the level of NCED transcript accumulation was tracked which indicated the endogenous ABA accumulation. The expression of NCED gene increased from 1st day to 7th day in the tolerant genotype. In sensitive genotype expression of NCED was observed at 5th and 7th days of PEG treatment (Figures 3 and 4). The expression of NCED and EcMyb gene was positively co-related with each other (Figure 5). This result indicates that accumulation of EcMyb gene product is ABA dependent.

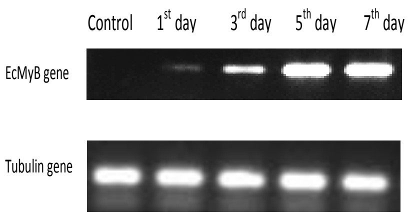

Figure 1. EcMyb gene expression after PEG treatment in PRM 6107 genotype.

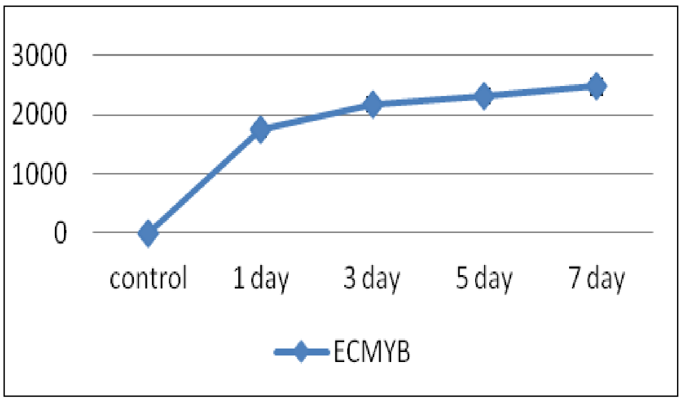

Figure 2. Graphical representation of EcMyb gene expression after densiometry analysis.

3.2. Effect of ABA treatment on EcMyb and NCED gene expression

The results (Figures 1, 2, 3 and 4) showed that the expression of EcMyb gene took place only in PRM6107 genotype which also showed optimum expression of NCED gene. Therefore, it could be thought expression of EcMyb gene follow ABA dependent signalling pathway. However, it is not conclusive in nature as drought results in manifestation of a concerted set of reactions within plant system and genes other than NCED might have contributed in endogenous accumulation of EcMyb. Co-expression based assay is not sufficient to confirm such results. Therefore second set of experiment was designed where tolerant and susceptible genotypes were treated exogenously with ABA under optimal moisture condition (unstressed condition).

Plantlets subjected to varying concentration (25, 50 and 100 μM) of exogenous ABA under unstressed condition showed the expression of EcMyb gene in both the tolerant as well as sensitive genotypes. The expression of EcMyb was observed in drought tolerant genotype PRM 6107 for all the concentrations of ABA at an early stage ie 6 h after ABA treatment (Figures 6 and 7). However the expression of the same was deferred until 12 h of treatment imposition with low level of expression at 25 μM and 50 μM concentration of exogenous ABA treatment in PES 400 (Figure: 8 and 9). However, a strong and quick (6 h) expression was reported at higher dose (100 μM) of ABA treatment in PES 400.

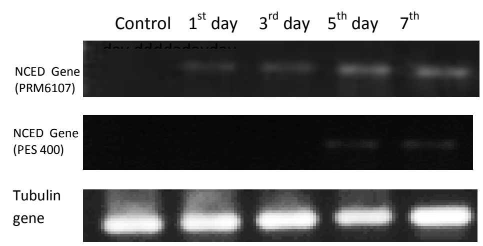

Figure 3. NCED gene expression after PEG treatment in PRM 6107 and PES 400 genotype.

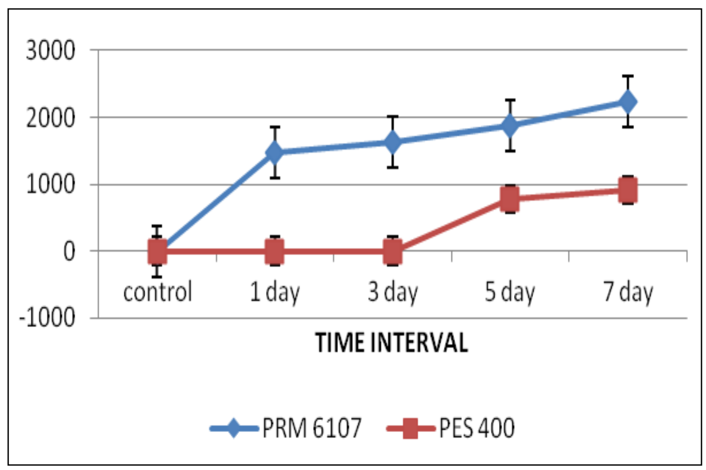

Figure 4. NCED gene expression after PEG treatment in PRM 6107 genotype.

NCED (9-cis epoxy carotenoid dioxygenase) is a key gene in ABA biosynthesis pathway and expressed towards the early stages of the pathway. To increase drought tolerance, the expression of genes encoding the first two enzymes of the ABA biosynthetic pathway i.e. zeaxanthin epoxidase (ZEP) and 9-cis epoxy carotenoid dioxygenase (NCED) is significantly induced by stress conditions (Tan et al., 2003; Moumeni et al., 2011; Ye et al., 2011).

In response to exogenous ABA, no expression of NCED was found in any genotype at any concentration and duration of exposure. This might have taken place because of feedback inhibition. We applied ABA under unstressed condition; therefore only basal level of expression was observed which was not sufficient to be tracked under gel. Also the exogenous ABA which was provided in nutrient media through root might have led to suppression of endogenous NCED gene. Similar results were reported for NCED gene expression in response to exogenous ABA in tomato (Thompson et al., 2000) and cowpea plants (Iuchi et al., 2000). However response of ABA biosynthetic genes to exogenous ABA may vary among plant species (Xiong and Zhu, 2003).

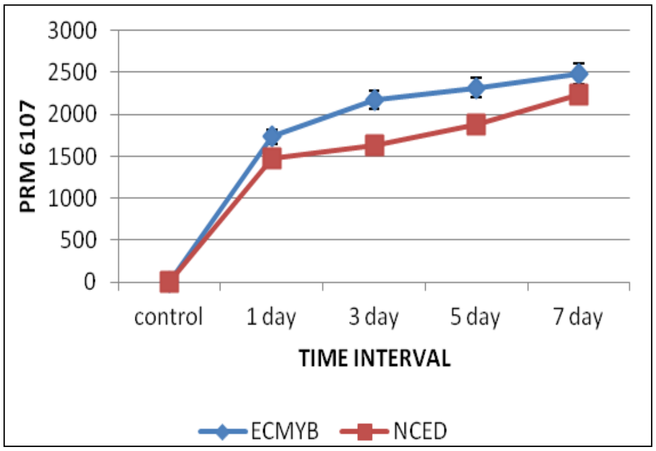

Figure 5. Correlation between expression of NCED and EcMyb gene in PRM 6107.

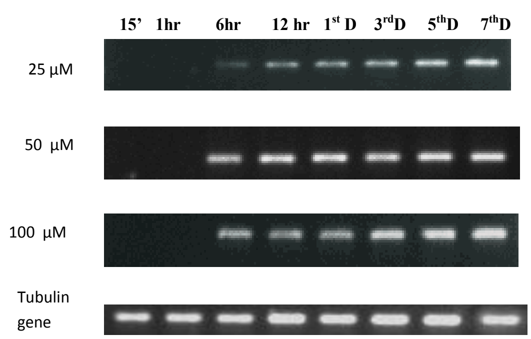

Figure 6. EcMyb gene expression in PRM 6107 under different concentration of ABA.

3.3. Root length

Drought is a complex trait that can be avoided by alteration in morphological and physiological characters. Root and shoot length (root: shoot ratio) plays an important role in avoiding drought stress (Hoogenboom et al., 1987; Guoxiong et al., 2002). Root plays an important role in drought avoidance by uptake of water from soil. Therefore plant genotypes with higher root growth have higher ability to avoid drought (Leishman and Westoby, 1994). An increased level of osmotic stress led to higher root to shoot ratio (Bajji et al., 2000). Root and shoot growth is regulated by ethylene accumulation via ABA. Arabidopsis root growth was found to be inhibited by ABA due to ethylene accumulation (Xingju et al., 2014).

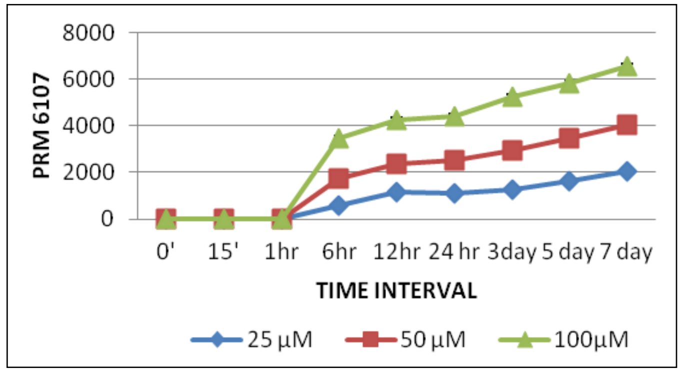

Figure 7. Densiometric analysis for EcMyb gene expression in PRM 6107 under different concentration of ABA.

Table 1. Effect of drought and ABA treatment on root length of seedlings of *Eleucine coracana*.

		Variety	0 day	1ST day	3rd day	5th day	7th day
PEG		PRM 6107	7.5	7.5	8.2	8.2	8.3
		control	6.5	7.3	7.7	8	8.9
		PES 400	7	7.3	7.3	7.3	6.2
		control	7.5	7.8	8	8.3	8.5
ABA	25 µM	PRM 6107	18	18	18.2	18.3	18.5
		PES 400	19.5	19.5	19.6	19.7	19.9
	50 µM	PRM 6107	20.5	20.5	21	21	21.3
		PES 400	23.1	23.1	23.3	23.5	23.6
	100 µM	PRM 6107	19.5	19.5	20	20.5	21
		PES 400	14.5	14.5	15	15.2	15.4

In the present study , increase in root length in both the drought tolerant and sensitive genotypes was normal in controlled condition. In PRM 6107, root length increased up to 5th day of exposure to PEG and after that it did not increase further (Table 1). In PES 400, root length was maintained up to 3rd day of PEG treatment after which it also did not increase . This might be possibly due to wilt induced by PEG (8000) as the experiment was performed in hydroponic condition and PEG created physiological drought. Increase in root length up to 5th day might be due to the avoidance mechanism of the plant after which the accumulation of stress component overtook the avoidance mechanism resulting in no increase in root length after 5th day. PEG induces lignification of roots which causes wilting of roots and hence leads to reduced root length (Hongtao et al., 2014).

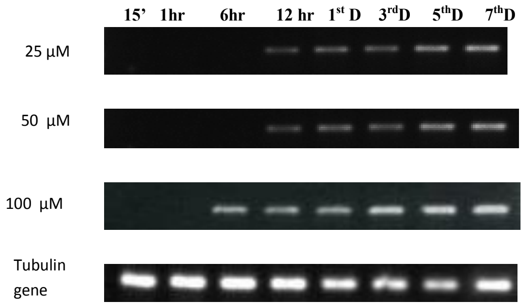

Figure 8. EcMyb gene expression in PES 400 under different concentration of ABA.

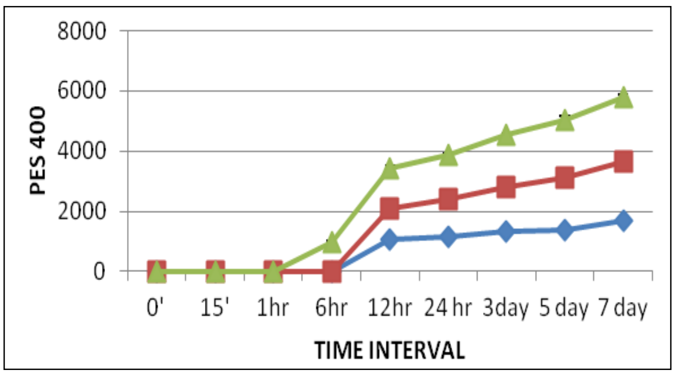

Figure 9. Densiometric analysis for EcMyb gene expression in PES 400 under different concentration of ABA.

Table 2. Effect of drought and ABA treatment on shoot length of seedlings of *Eleucine coracana*.

		Variety	0 day	1ST day	3rd day	5th day	7th day
PEG		PRM 6107	13.3	13.5	13.0	13.0	13.0
		control	15	15.2	15.5	16	16.3
		PES 400	15	15.0	15.0	14.9	14.9
		control	14	14.1	15	15.4	16
ABA	25 µM	PRM 6107	20	20	20.2	20	19.9
		PES 400	21.5	21.5	21.6	21.5	21.5
	50 µM	PRM 6107	20.5	20.6	20.7	20.7	20.6
		PES 400	19.5	19.5	19.5	19.5	19.5
	100 µM	PRM 6107	19.5	19.5	19.8	19.7	19.6
		PES 400	21.3	21.3	21.4	21.3	21.1

Both the genotypes had similar variation in root length at various concentration of ABA applied under unstressed condition and no adverse effect on root length was recorded. Similar results were reported in wheat and maize (Moosavi et al., 2005). However the correlation of ABA concentration and root length varied in different studies. It was found to increases with increase in concentration of ABA applied in maize (Mulkey et al., 1983; Pilet and Saugy, 1987) whereas a reverse trend was reported in wheat (Jones et al., 1987) and maize (Pilet and Chanson, 1981). Effect of ABA on soybean seedling was found to similar like drought (Creelman et al., 1990).

3.4. Shoot length

Shoot length for both genotypes was found to decrease in response to the PEG treatment since first day. However, the decrease was more prominent for sensitive one (Table 2). Treatment with ABA led to decrease in overall shoot length in comparison to control in both the genotypes at all the concentrations but this decrease was noticed only after 3rd day of treatment. However, shoot length decreased in response to both the treatments but treatment with PEG caused loss of turgidity in whole plant while exposure to different concentrations of ABA led to wilting of tip only. Wilting in case of PEG treatment occurred due to loss of turgidity as seedlings failed to take water from solution in presence of PEG. Similar result was reported in tomato where ABA was applied exogenously at seedling stage (Basak et al., 2012). In another study it was reported that ABA accumulated during water stress inhibits shoot growth to reduced water loss (Saab et al., 1990 ; Sharp and LeNoble, 2002). However, some studies showed a positive correlation between ABA applied through soil and shoot growth as ABA restricts the ethylene production responsible for shoot growth inhibition (Abeles et al., 1992; Hussain et al., 2000).

3.5. Relative water content

Relative water content (RWC) was found to decrease in both the genotypes upon drought treatment however; this decrease was more prominent for sensitive genotype than tolerant one. Drought is sensed by the roots in soil and ABA gets accumulated in root from where it is transported to the leaf through xylem (Davies and Zhang, 1991) where it regulates stomatal conductance so that water deficit in shoot region can be avoided by transpiration. Therefore endogenous accumulation of ABA initially plays main role in avoiding water stress. Simultaneously water in nutrient solution is also limited to be taken up by the plant because of PEG which ultimately leads to low water availability in leaves. Production of osmolytes like proline is also induced under drought which reduces relative water content of plant under drought.

Table 3. Effect of drought and ABA treatment on relative water content of seedlings of *Eleucine coracana*

		Variety	0 day	1ST day	3rd day	5th day	7th day
PEG		PRM 6107	101.5	97.2	90.5	79.3	65.1
		control	97.1	97.6	98.1	98.2	98.9
		PES 400	97.2	91.0	82.2	72.1	53.4
		control	95.0	95.3	95.8	95.5	95.6
ABA	25 µM	PRM 6107	95.0	95.4	98.8	103.9	84.3
		PES 400	89.9	90.3	97.0	99.4	86.7
	50 µM	PRM 6107	99.3	100.5	102.2	107.6	79.1
		PES 400	94.9	95.0	96.5	98.3	88.6
	100 µM	PRM 6107	87.9	90.2	99.0	104.6	66.9
		PES 400	82.8	85.0	98.0	99.5	93.6

Relative water content (RWC) of ABA treated plants increased initially slightly but with increasing duration of exposure after 5th day of treatment it decreased drastically (Table 3). ABA might have caused closure of stomata and thus reduced transpirational loss of water initially resulting in an increase in relative water content (Hussain et al., 2012). Later on the reduction of RWC might occur due to reduced water transport through the roots to the leaves due to presence of ABA in nutrient solution (Manuel and Marc, 2011). Similar results were reported by (Xing et al. 2002). This negative correlation of ABA concentration and relative water content showed that it mimics the water stress condition.

On the other hand RWC may increase in response to ABA as there are reports that ABA transiently increases the opening of water channels like Aquaporins (Hose et al.,. 2000; Mahdieh and Mostajeran, 2009). But the expression is only transient and prolonged exposure and high concentration of ABA limits the root conductance which reduces the water uptake (Fiscus, 1981). One of the possible reasons for temporary increase in RWC may be increased water uptake through its impact on aquaporins and then limitation by morphological changes to the roots i.e. suberization as a means of protecting the plant from drought stress . RWC of tolerant genotype was found to be higher than sensitive one but the reduction in tolerant genotype was also found to be higher and RWC of tolerant genotype at 7th day was lower than that of sensitive one. The possible reason behind this may be relatively higher sensitivity of tolerant genotype to ABA.

3.6. Chlorophyll content

Chlorophyll is a light absorbing pigment that plays a critical role in photosynthesis and therefore is an important component to be considered during stress, especially drought. Chlorophyll content was found to decrease with increased exposure to PEG in both the genotype (Table 4). However the extent of decrease in chlorophyll was more in sensitive genotype. Water deficit may trigger the production of reactive oxygen species (ROS) which in turn may lead to lipid peroxidation and consequently reduction in chlorophyll content (Kumar et al., 2011).

Table 4. Effect of drought and ABA treatment on chlorophyll content of seedlings of *Eleucine coracana*.

		Vari-ety	0 day	1st day	3rd day	5th day	7th day
PEG		PRM 6107	0.217	0.207	0.193	0.185	0.177
		control	0.198	0.198	0.198	0.200	0.201
		PES 400	0.182	0.180	0.139	0.108	0.054
		control	0.272	0.274	0.278	0.279	0.301
ABA	25 µM	PRM 6107	0.151	0.156	0.161	0.162	0.151
		PES 400	0.130	0.131	0.146	0.146	0.140
	50 µM	PRM 6107	0.145	0.146	0.158	0.161	0.157
		PES 400	0.136	0.136	0.148	0.148	0.147
	100 µM	PRM 6107	0.160	0.161	0.198	0.193	0.190
		PES 400	0.152	0.152	0.148	0.147	0.146

Cytokinin is a phytohormone that plays role in chlorophyll accumulation and keeps plant green and ABA being antagonist of cytokinin (Kulaeva, 1982) may interfere with chlorophyll accumulation on prolonged exposure (Fletcher and McCullach, 1971; Fletcher et al., 1973; Lew and Tsuji, 1982; Reiss and Beale, 1995). This may cause chlorosis and leaf abscission when high concentrations of ABA are applied (Blanchard et al., 2007; Kim and Iersel, 2011; Waterland et al., 2010). ABA drenches may induce wilting, even if the plants are well watered (Barrett et al., 2009).

4. CONCLUSIONS

ABA plays an important role in regulating drought response by inducing drought responsive genes like EcMyb whose product accumulate under drought condition in tolerant genotype PRM 6107 whereas no expression was observed in sensitive genotype PES 400. This gene was expressed in both the genotypes in presence of ABA which indicates that ABA regulates the expression of EcMyb in both the genotypes . Thus, the expression of EcMyb gene followed ABA dependent signalling pathway under drought condition. Difference in the responses of two genotypes under drought condition occured due to differential accumulation of ABA. In sensitive cultivar, lower accumulation of ABA occurred which was basal and not sufficient to induce stress tolerance response under drought condition. Therefore, ABA may act as biochemical marker for varietal characterisation of tolerant and resistant genotypes under drought condition and only cloning of EcMyb gene is not sufficient to obtain drought tolerance traits. Sometimes gene may be present but due to lack of proper signalling, cloned gene may not be expressed. In future, for transgenic approach involving transfer of EcMyb gene from underutilised crop like finger millet to cereals grain like rice, wheat and maize, we will have to focus on cloning and also over expression of ABA for proper expression of stress related EcMyb gene.

REFERENCES

Abeles, F.B., Morgan, P.W. and Saltveit Jr., M.E. (1992) Ethylene in plant biology, 2nd ed. San Diego: Academic Press.

Bajji, M., Lutts, S. and Kinet, J.M. (2000) Physiological changes after exposure to and recovery from polyethylene glycol-induced water deficit in roots and leaves of durum wheat (*Triticum durum* Desf.) cultivars differing in drought resistance. Journal of Plant Physiology, 157, 100-108.

Barrett, J.E., Bartuska, C.A. and Clark, D.G. (2009) Effects of s-ABA on ethylene insensitive petunias. Proceedings of Plant Growth Regulation , 36, 104-113.

Basak, H., Demir, K. and Doganlar, Z.B. (2012) The effect of abscisic acid application on root-shoot length and some antioxidant enzyme activities of two different tomato seedlings. The Journal of Animal & Plant Sciences, 22, 695-703.

Blanchard, M.G., Newton, L.A., Runkle, E.S. and Woolard, D. (2007) Exogenous applications of abscisic acid improved the postharvest drought tolerance of several annual bedding plants. Acta Horticulture, 755, 127–132.

Creelman, R.A., Mason, H.S., Bensen, R. J., Boyer, J.S. and Mullet, J.E. (1990) Water deficit and abscisic acid cause differential inhibition of shoot versus root growth in soybean seedlings: Analysis of growth, sugar accumulation, and gene expression. Plant Physiology, 92, 205-214.

Davies, W.J. and Zhang, J. (1991) Root signal and the regulation of growth and developments of plants in drying soil. Annual Reiview of Plant Physiology and Molecular Biology, 42, 55-76.

Fiscus, E.L. (1981) Effects of Abscisic acid on the hydraulic conductance of and the total ion transport through Phaseolus root systems. Plant Physiology, 68, 169–174.

Fletcher, R.A. and McCullagh, D. (1971) Benzyladenine as a regulator of chlorophyll synthesis in cucumber cotyledons. Canadian Journal of Botany, 49, 2197-2201.

Fletcher, R.A., Teo, C. and Ali, A. (1973) Stimulation of chlorophyll synthesis in cucumber cotyledons by benzyladenine. Canadian Journal of Botany, 51, 937-939.

Guo, D.L., Liang, J.H. and Li, L. (2009) Abscisic acid (ABA) inhibition of lateral root formation involves endogenous ABA biosynthesis in *Arachis hypogaea* L. Plant Growth Regulations, 58, 173–179.

Guoxiong, C., Krugman, T., Fahima, T., Korol, A.B. and Nevo, E. (2002) Comparative study on morphological and physiological traits related to drought resistance between xeric and mesic Hordeum spontaneum lines in Isreal. Barley Genetics Newsletter, 32, 22-33.

Hongtao, J., Ling, L., Kexue, L., Qingen, X., Zhijuan, W., Xuhua, Z. and Xia, L. (2014) PEG-mediated osmotic stress induces premature differentiation of the root apical meristem and outgrowth of lateral roots in wheat. Journal of Experimental Botany, 65, 4863-4872

Hoogenboom, G., Huck, M.G. and Peterson, C.M. (1987) Root growth rate of soybean as affected by drought stress. Journal of Agronomy, 79, 607-614.

Hose, E., Steudle, E. and Hartung, W. (2000) Abscisic acid and hydraulic conductivity of maize roots: A study using cell- and root-pressure probes. Planta, 211, 874–882.

Hussain, A., Black, C.R., Taylor, I.B. and Roberts, J.A. (2000) Does an antagonistic relationship between ABA and ethylene mediate shoot growth when tomato (*Lycopersicon esculentum* Mill.) plants encounter compacted soil? Plant cell and Environment, 23, 1217-1226.

Hussain, S., Ali, A., Ibrahim, M., Saleem, M.F., Alias, M.A. and Bukhsh, H.A. (2012) Exogenous application of abscisic acid for drought tolerance in sunflower (*Helianthus Annuus* L.). The Journal of Animal and Plant Sciences , 22, 806-826.

Iuchi, S., Kobayashi, M., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2000) A stress inducible gene for 9-cis-epoxycarotenoid dioxygenase involved in abscisic acid biosynthesis under water stress in drought-tolerant cowpea. Plant Physiology, 123, 553–562.

Jones, H., Leigh, R.A., Tomos, A.D. and Wyn Jones, R.G. (1987) The effect of abscisic acid on cell turgor pressures, solute content and growth of wheat roots. Planta, 170, 190-197.

Kim, J. and Iersel, M.W.V. (2011) ABA drenches induce stomatal closure and prolong shelf life of *Salvia splendens*. Scientia Horticulturae, 127, 420–423.

Kulaeva, O.N. (1982) Hormonal regulation of physiological processes in plant at the level of RNA and protein synthesis. 41st Timiryazev Memorial Lecture, Nauka, Moskva.

Kumar, R.R., Kumar, K. and Naik, G.R. (2011) Effect of polyethylene glycol induced water stress on physiological and biochemical responses in Pigeonpea (*Cajanus cajan* L. Millsp.). Recent Research in Science and Technology , 3, 148-152.

Leishman, M.R. and Westoby, M. (1994) The role of seed size in seedling establishment in dry soil conditions - experimental evidence from semi-arid species. Journal of Ecology, 82, 249-258.

Lew, R. and Tsuji, H. (1982) Effect of benzyladenine treatment duration on d-aminolevulinic acid accumulation in the dark, chlorophyll lag phase abolition, and long-term chlorophyll production in excised cotyledons of dark-grown seedlings. Plant Physiology, 69, 663-667.

Mahdieh, M. and Mostajeran, A. (2009) Abscisic acid regulates root hydraulic conductance via aquaporin expression modulation in *Nicotiana tabacum*. Journal of Plant Physiology, 166, 1993–2003.

Manuel, G.A. and Marc, W.I. (2011) Concentrated exogenous abscisic acid drenches reduce root hydraulic conductance and cause wilting in Tomato. Hortscience, 46, 1640–1645.

Moosavi, S.S., Samadi, B.Y., Zali, A., Ghannadha, M., Omidi, M. and Naghavi, M. (2005) Investigation the effect of Abscisic acid on drought stress induction in wheat seedling. Czech Journal of Genetics and Plant Breeding , 41, 127-137.

Moumeni, A., Satoh, K., Kondoh, H., Asano, T., Hosaka, A., Venu Prasad, R., Serraj, R., Kumar, A., Leung, H. and Kikuchi, S. (2011) Comparative analysis of root transcriptome profiles of two pairs of drought tolerant and susceptible rice near isogenic lines under different drought stress. BMC Plant Biology, 11, 174–191.

Mulkey, T.J., Evans, M.L. and Kuzmanoff, K.M. (1983) The kinetics of abscisic acid action on root growth and gravitropism. Planta, 157, 150-157.

Nelson, and Gerald, C. (2010) Food security, farming, and climate change to 2050: scenarios, results, policy options. Washington, D.C.: International Food Policy Research Institute.

Pilet, P.E. and Chanson, A. (1981) Effect of abscisic acid on maize root growth: A critical examination. Plant Science Letters, 21 ,99-106.

Pilet, P.E. and Saugy, M. (1987) Effect on root growth of endogenous and applied IAA and ABA: A critical reexamination. Plant Physiology, 83, 33-38.

Rai, S, Gaur A.K., Guru S.K., Agrawal S. and Arora S. (2009) In vitro evaluation of relative tolerance of *Eleucine coracana* Genotypes against water deficit stress. Indian Journal of Plant Physiology, 14, 82-87.

Reiss, C. and Beale, S.I. (1995) External calcium requirements for light induction of chlorophyll accumulation and its enhancement by red light and cytokinin pretreatment in excised etiolated cucumber cotyledons. Planta, 196, 635-641.

Saab, I.N., Sharp, R.E., Pritchard, J. and Voetberg, G.S. (1990) Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. Plant Physioliology, 93, 1329-1336.

Salvi, P., Arora S. and Lohani P. (2012) Expression of MYB transcription factor in *Eleusine coracana*: towards making of drought tolerant plants. LAP Lambert Academic Publishing AG & Co KG ISBN 9783848482917.

Sharp, R.E. and LeNoble, M.E. (2002) ABA, ethylene and the control of shoot and root growth under water stress. Journal of Experimental Botany, 53, 33-37.

Sonoda, Y., Ikeda, A., Saiki, S., Wiren, N.V., Yamaya, T. and Yamaguchi, J. (2003) Distinct expression and function of three ammonium transporter genes (OsAMT1;1–1;3). Plant and Cell Physiology, 44, 726–734.

Tan, B.C., Joseph, L.M., Deng, W.T., Liu, L.J., Li, Q.B., Cline, K. and McCarty, D.R. (2003) Molecular characterization of the *Arabidopsis* 9-cis epoxycarotenoid dioxygenase gene family. Plant Journal, 35, 44–56.

Thompson, A.J., Jackson, A.C., Parker, R.A., Morpeth, D.R., Burbidge, A. and Taylor, I.B. (2000) Abscisic acid biosynthesis in tomato: regulation of zeaxanthin epoxidase and 9-cis-epoxycarotenoid dioxygenase mRNAs by light/dark cycles, water stress and abscisic acid. Plant Molecular Biology, 42, 833–845.

United Nations (2011). World Population Prospects: The 2010 Revision.

Waterland, N.L., Finer, J.J. and Jones, M.L. (2010) Benzyladenine and gibberellic acid application prevents abscisic acid-induced leaf chlorosis in pansy and viola. Horticulture Science, 45, 925–933.

Xing, X., Zheng, G., Xi-Ping, D. and Medrano, H. (2002) Effects of exogenous abscisic acid and water stress on the growth response of subterranean clover of different genotypes. Acta Botanica Sinica, 44, 1425-1431.

Xingju, L., Zhizhong, C., Junping, G. and Zhizhong, G. (2014) Abscisic acid inhibits root growth in *Arabidopsis* through ethylene biosynthesis. The Plant Journal, 79, 44–55.

Xiong, L.M. and Zhu, J.K. (2003) Regulation of Abscisic Acid biosynthesis. Plant Physiology, 133, 29–36.

Xiong, L., Lee, H., Ishitani, M. and Zhu, J. K. (2002) Regulation of osmotic stress responsive gene expression by the LOS6/ABA1 locus in *Arabidopsis*. Journal of Biological Chemistry, 277, 8588–8596.

Yanhui, C., Xiaoyuan, Y., Kun, H., Meihua, L., Jigang, L., Zhaofeng, G., Zhiqiang, L., Yunfei, Z., Xiaoxiao, W., Xiaoming, Q., Yunping, S., Xiaohui, D., Jingchu, L., Xing-Wang, D., Zhangliang, C., Hongya, G. and Li-Jia, Q. (2006) The MYB transcription factor superfamily of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB family. Plant Molecular Biology, 60, 107–124.

Ye, N.H., Zhu, G.H., Liu, Y.G., Li, Y.X. and Zhang, J.H. (2011) ABA controls H_2O_2 accumulation through the induction of OsCATB in rice leaves under water stress. Plant Cell Physiology, 52, 689-698.