

Isolation and Characterization of FingerMillet (*Eleusine coracana* L.) bZIP Transcription Factor

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Abstract

Finger millet (Eleusine coracana L.) is an allotetraploid with a basic chromosome number of 9 and genome composition AABB (2n = 4x = 36). This crop is adapted to a wide range of environments and can withstand significant levels of drought, heat stress and salinity. It is relatively resistant to water logging and has few serious diseases. The crop has ability to grow under marginal environments and rainfed conditions. It recognised as nutri-cereal and climate resilient crop. Abiotic stresses (Drought, Heat, Salt, and Cold) are major threats to the crop plants regarding growth and productivity. Among these abiotic stresses, heat stress is one of the major factors that affect the plant growth and productivity to a greater extent. To understand the underlying mechanism of heat stress tolerance and to identify genes involved in it, suppressive subtractive hybridization has been performed for heat stressed tissues of Ragi seedlings and identified a novel bZIP transcription factor gene. In plants transcription factors of bZIP family are regulators of abiotic and biotic stress responses. Genetic, molecular and biochemical analyses indicate that bZIP transcription factors regulate important plant processes such as organ and tissue differentiation, cell elongation, nitrogen/carbon balance control, pathogen defence, energy metabolism, unfolded protein response, hormone and sugar signalling, light response, osmotic control and seed storage protein gene regulation. The expression of bZIP transcription factor encoding gene has been analysed by qRT-PCR. In order to obtain full length sequence of this gene 5'RACE (Rapid amplification of cDNA ends) was performed and the fragments obtained after 5'RACE was cloned in pGEMT-Easy vector. Sequencing result showed that the clones contain two alleles, with respective to the genome composition (AABB).The difference between two alleles is 17 amino acids at protein level and the major domains present in each allele are BRLZ and transmembrane domains.The sequences of two alleles are submitted to the NCBI and the accession numbers are KF245640 and KF245641. The full length bZIP transcription factor was cloned in binary vector pCAMBIA2300 and mobilized in to Agrobacterium strain EHA105 for tobacco transformation to characterize it for stress tolerance.

The millets are a group of highly variable small-seeded grasses, widely grown around the world as cereal crops or grains for both human food and fodder. They do not form a taxonomic group, but rather a functional or agronomic one. Millets are important crops in the semi-arid tropics of Asia and Africa (especially in India, Nigeria, and Niger), with 97% of millet production in countries. The crop is favoured due to its productivity and short growing season under dry, high temperature conditions. Millets are indigenous to many parts of the world; millets most likely had an evolutionary origin in tropical western Africa, as that is where the greatest number of both wild and cultivated forms exists. Millets have been important food staples in human history, particularly in Asia and Africa, and they have been in cultivation in East Asia for the last 10,000 years. Finger Millet (*Eleusine coracana* L.) commonly called Ragi, is an annual plant widely grown as a cereal in the arid areas of Africa and Asia. *E. coracana* is native to the Highlands. It is very adaptable to higher elevations and is grown in the Himalaya up to 2,300

metres in elevation. Finger millet (*Eleusine coracana* L.) is an allotetraploid with a basic chromosome number of 9 and genome composition AABB ($2n = 4x = 36$). This crop is adapted to a wide range of environments and can withstand significant levels of drought, heat and salinity. It is relatively resistant to water logging and has few serious diseases. The characteristic genetic traits of high nutritional value and drought tolerance make it a particularly attractive system to study gene expression from the standpoint of understanding osmoregulation and storage proteins. It can also serve as a gene pool for various important characters and disease resistant genes. Protein content in finger millet ranges from 7 to 14 per cent with a high methionine, and all the essential amino acids which are lacking in grain based foods like Rice and Sorghum (National Research Council, 1996).

Apart from the major nutrients each 100 grams of finger millet contains 12.6 mg iron, 410 mg calcium and 290 mg phosphorus. Because of its low glycemic index with high fibre, it is also recommended for diabetic patients. Consumption of finger millet prevents Constipation and lowers cholesterol. Finger millet is used medicinally, e.g. the seed as a prophylaxis for dysentery. In southern Africa the juice of a mixture of finger millet leaves and leaves of *Plumbago zeylanica* L. are taken as an internal remedy for leprosy. Because of these high nutritional and medicinal properties, it has proved to be a “**super cereal**”. Abiotic stresses (Drought, Heat, Salt, and Cold) are major threats to the crop plants regarding growth and productivity. Among these abiotic stresses, heat stress is one of the major factors that affect the plant growth and productivity to a greater extent. Plant responses to heat stress vary with the degree and duration of heat stress and the plant type. Heat stress is now a major concern for crop production and approaches for sustaining high yields of crop plants under heat stress are important agricultural goals. The global air temperature is predicted to rise by 0.2 °C per decade, which will lead to temperatures 1.8–4.0°C higher than the current level by 2100. This prediction is creating apprehension among scientists, as heat stress has known effects on the life processes of organisms, acting directly or through the modification of surrounding environmental components. Plants in particular as sessile organisms cannot move to more favourable environments; consequently plant growth and developmental processes are substantially affected often lethally by high temperature stress. Plants possess a number of adaptive, avoidance, or acclimation mechanisms to cope with heat stress situations. In addition, major tolerance mechanisms that employ are ion transporters, osmoprotectants, antioxidants, and other factors involved in signalling cascades. Transcriptional control is activated to stress, depends on the ability to perceive the heat stress stimulus, generate and transmit the signal, and initiate appropriate physiological and biochemical changes.

To understand the underlying mechanism of heat stress tolerance and to identify genes involved in it, suppressive subtractive hybridization has been performed for heat stressed tissues of Ragi seedlings and identified a novel *bZIP* transcription factor gene.

A number of families of transcription factors (TFs), each containing a distinct type of DNA binding domains, such as WRKY, ERF, bZIP and MYB, involved in stress-responsive gene regulation have been identified. Basic leucine zipper (bZIP) is one of the largest TF families in plants, which was characterized by a basic domain responsible for sequence-specific DNA binding, adjacent heptad leucine repeat and the leucine zipper. In plants transcription factors of *bZIP* family are regulators of abiotic and biotic stress responses. Genetic, molecular and biochemical analyses indicate that *bZIP* transcription factors regulate important plant processes such as organ and tissue differentiation, cell elongation, nitrogen/carbon balance control,

pathogen defence, energy metabolism, unfolded protein response, hormone and sugar signalling, light response, osmotic control and seed storage protein gene regulation. The expression of *bZIP* transcription factor encoding gene has been analysed by qRT-PCR. In order to obtain full length sequence of this gene 5'RACE (Rapid amplification of cDNA ends) was performed and the fragments obtained after 5'RACE was cloned in pGEMT-Easy vector. Sequencing result showed that the clones contain two alleles, with respective to the genome composition (AABB). The major domains present in each allele are BRLZ and transmembrane domains. The sequences of two alleles are submitted to the. The full length *bZIP* transcription factor was cloned in binary vector pCAMBIA2300 and mobilized in to *Agrobacterium* strain for tobacco transformation to characterize it for stress tolerance.

