

Identification and characterization of *Sorghum bicolor* heme oxygenase genes and their role in conferring abiotic stress tolerance

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Background: Heme oxygenase (HO) is one of the stress responsive enzyme that possess antioxidant properties and is well characterized in animals. HO catalyses the oxidative degradation of heme to biliverdin (BV), carbon monoxide (CO) and iron (Fe). HO's are divided into the HO1 (HO1, HO3 & HO4) and HO2 subfamily and they play a major role in phytochrome chromophore biosynthesis. The role of HO's in plants has only been described in a few model plants including Arabidopsis, rice, wheat, cabbage and recently in sorghum (Mulaudzi et al., 2019), an important cereal crop. The aim of the study was to identify *Sorghum bicolor* HO's and characterize their role in conferring abiotic stress tolerance.

Method: Bioinformatics tools were used to identify and characterize *Sorghum bicolor* heme oxygenase (SbHOs) genes. Gene expression profile of SbHO genes was analysed under osmotic stress using quantitative real-time polymerase chain reaction towards understanding their biological roles in plants. HO1 was cloned into TOPO TA expression vector and expressed in *E. coli* BL21 Codon Plus followed by the spectrophotometric measurement of the activity of the recombinant protein.

Results: Based on bioinformatics analysis of 43 orthologs from 32 plant HOs, the HO signature motif (QAFICHFYNI/V) is conserved across all SbHO proteins and that they share more than 90 % sequence similarity with other cereals. Expression analysis of the SbHO genes revealed that they are constitutively expressed in leaves, stems and roots and their transcript levels were induced by osmotic stress. The expression level of SbHO1 revealed a 100-fold significant increase than HO2, HO3 & HO4 transcripts therefore, SbHO1 was characterized further. A 557 bp SbHO1 fragment was successfully amplified, and protein expression revealed a recombinant protein size of 25.1 kDa. Recombinant SbHO1 is active as shown by the formation of a SbHO1-heme complex at 405 nm and the degradation of heme to antioxidant BV at 610 nm.

Discussion and conclusion: This study identified and characterized four HO genes in sorghum and their expression pattern in response to osmotic stress. Results revealed highly conserved regions across all species based on gene structure, subcellular localization, signature motifs and phylogenetic analysis of the HO gene family members. Results also revealed that the SbHO genes are transcriptionally expressed and induced by osmotic stress suggesting a role in conferring stress tolerance. Enzymatic results revealed that SbHO1 is an active protein through its ability to produce an antioxidant. With knowledge from this research, transgenic and over-expressing lines can be generated for the development of high yield, stress-tolerant crops for sustainable agriculture.

Keywords: Heme oxygenase, osmotic stress, signature motif, stress tolerance, *Sorghum bicolor*.

References: Mulaudzi-Masuku, et al., (2019). Characterization and Expression Analysis of Heme Oxygenase Genes from *Sorghum bicolor*. Bioinformatics and Biology Insights. <https://doi.org/10.1177/1177932219860813>