



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Programme of Study : **Ph.D.**

Thesis Title : **Understanding NAC transcription Factor Mediated signaling in the Regulation of Growth and Abiotic Stress Tolerance in Cowpea**

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Thesis Submitted to the Department/ Center : **Department of Bioesciences and Bioengineering**

Date of completion of Thesis Viva-Voce Exam : **08-10-2021**

Key words for description of Thesis Work : Abiotic stress tolerance; Cowpea; Growth trade-off; NAC transcription factor; Yield improvement; Pulse crop

SHORT ABSTRACT

Abiotic stresses such as drought, salinity, and heat, account for $\geq 50\%$ yield loss. NAC transcription factors (TFs) have emerged as a powerful tool to manipulate central stress-signaling. However, the functional conservation of orthologous NAC genes is unpredictable, limiting their use in pulse genetic-engineering. Besides, transcriptional-reprogramming of stress-responses often cause yield-penalty. My Ph.D. thesis provides unique and strategic translational tool to develop stress-tolerance and yield improvement in pulses without growth trade-off. A genome-wide analysis was performed to annotate the unexplored NAC family in cowpea (VuNAC), which is an important pulse crop. The *in-silico* promoter analysis and integrative gene regulatory network (iGRN) indicated their crucial role in both stress and growth responses, governed by multi-tier signaling comprising of light, phytohormones, sugar, micronutrient, and stress-factors. Two potential genes (*VuNAC1* and *VuNAC2*) were cloned for molecular and biological functional study. The nuclear localization, DNA-binding assay, yeast-one and two hybrid, demonstrated the transactivation and dimerization abilities of the proteins. The genes showed prominent induction by dehydration, osmotic-stress, NaCl, aluminum, heat, cold, abscisic acid (ABA), methyl-jasmonate. The heterologous expression in yeast conferred tolerance to multiple stresses, improved cell-proliferation, longevity, and metabolic remodeling of vital energy-generating pathways, such as biosynthesis of ATP, vitamin B complex, methionine, and glutathione, giving preliminary insight into unique and versatile functions of VuNAC1/2. When expressed in Arabidopsis, the transgenic lines exhibited reduced ABA-hypersensitivity, enhanced growth and photosynthesis, and tolerance to severe drought, salinity, aluminum, cadmium, and H₂O₂ toxicity, under optimum and nutrient-deficit conditions.

Aiming to improve the stress-tolerance and agronomic traits in cowpea, we generated stable overexpressing transgenic lines. The gain-of-function phenotype manifested early germination and accelerated vegetative growth. The transgenic plants displayed improved morphological and physiological traits such as increased foliage biomass, leaf-size, stem-diameter, plant-height, nodule-abundance, flower-count, seed-production (~3.2-fold and ~2.1-fold), seed-weight, stomatal density, and photosynthetic parameters. Whereas, virus-induced transient suppression of genes impaired both vegetative growth and flowering, advocating their indispensability for basal plant-development. The transgenic lines exhibited improved tolerance to terminal drought, salinity, heat, and cold and recovered the yield by maintaining tissue water-status, Na^+/K^+ homeostasis, membrane-integrity, and accumulation of proline, ascorbate, and glutathione. The transcriptome analysis indicated activation of genes that synthesize key photosynthetic- and stress-responsive components, regulate nitrogen-uptake, cell-division, cell-wall biogenesis, ABA- and auxin-signaling, indicating consolidation of diverse plant processes by a single gene.

In conclusion, the bifunctional VuNAC1/2 TFs mediate favorable transcriptional cross-talk to regulate multiple stress-tolerance and agronomic traits, unlike their orthologs, hence are promising biotechnological tools for sustainable pulse improvement by overcoming yield penalty during stress-adaptation. The exotic VuNAC1/2 could also be applied to develop stress-tolerance and growth-vigor of sensitive and commercially valuable varieties of cash crops, like mung bean, pigeon pea, *etc.* Besides, the TFs are useful to engineer robust cell-factories, and whole-cell application strains to avoid poor productivity, metabolite-overflow, and enhanced production of commercial biomolecules, under fluctuating bioprocess conditions. Our study identified novel genetic engineering candidates to benefit agriculture as well as industry by aiding food- and nutritional-security under changing climate and adding value to production.