INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	Vijya Laxmi
Roll Number	:	11610614
Programme of Study	:	Ph. D.

Thesis Title: Understanding of calcium signaling pathway mediated by calmodulin and related proteins in *Neurospora crassa*

Name of Thesis Supervisor(s)	:	Dr. Ranjan Tamuli Prof. Utpal Bora
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SHORT ABSTRACT

In this study, I investigated the cellular functions of calmodulin (CaM) and related calcium (Ca^{2+}) signaling proteins in *Neurospora crassa*. The CaM in *N. crassa* is essential for viability, therefore, I studied its function using the CaM antagonists, trifluoperazine (TFP) and chlorpromazine (CPZ). I found that the addition of CaM antagonists inhibit growth, hyphae branching, and sexual development in *N. crassa.* I also studied Ca^{2+} -ATPases that are important targets of CaM and maintain Ca^{2+} homeostasis in cells. Double mutant of the Ca^{2+} -ATPases, encoded by *trm-9* and *nca-2*, exhibited a growth defect, less pigmentation, reduced survival in Ca^{2+} stress and induced heat shock temperature in *N. crassa*. In addition, I isolated mutants of the calmodulin gene (*cmd*) using repeat-induced point mutation to identify the important amino acid residues. One of the eight cmd^{RIP} mutants isolated, the $\Delta pan\text{-}2::P_{tcu\text{-}1}::cmd^{RIP}$(26); *mat A* strain possess missense mutations, showed a decreased carotenoid accumulation, severe defect in growth as well as reduction in viability upon ultraviolet (UV) irradiation. Moreover, crosses inducing meiotic silencing of *cmd* resulted in a barren phenotype, suggesting its role in sexual development in *N. crassa*. Furthermore, I performed site-directed mutagenesis of the calcium/calmodulin-dependent kinase-2 (Ca^{2+}/CaMK-2) phosphorylation sites corresponding to serine

247 and threonine 267. The *camk-2*^{S247A} and the *camk-2*^{T267A} mutants in a homozygous cross, or in a heterozygous cross with a Δ*camk-2* mutant, showed an intermediate phenotype, indicating requirement of these phosphorylation sites for full fertility. Additionally, fold change expression level of *cmd, trm-9, nca-2* and some others related Ca^{2+} signaling genes using Real-Time PCR indicated a complex interaction pattern among Ca^{2+}-signaling genes. Therefore, this study revealed role of the CaM and related proteins in vegetative and sexual development in *N. crassa*.