线虫侵染松树体内 *CaMBPCXY165* 基因的表达模式研究

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摘 要：松材线虫病现已成为我国有史以来森林最危险的生物灾害之一，但对于该病的致病机理一直存在争议。细胞内 Ca^{2+}浓度的瞬时升高是触发植物防御反应的早期信号，植物细胞内的 Ca^{2+}对于不同的刺激会产生不同的时空动态，伴随松材线虫病的发展，薄壁细胞内游离 Ca^{2+}浓度变化具有“低-高-低-高”的特征，而细胞内 Ca^{2+}不同的时空动态进而被多种细胞内 Ca^{2+}传感蛋白如钙调素（calmodulin，CaM）等所破译，可能与下游的钙调素结合蛋白（CaMBPs）结合，进而引起细胞反应。胞内 Ca^{2+}/CaM 浓度的改变对于防卫反应如防卫相关基因的诱导以及细胞程序性死亡是必须的，因此对松树——线虫互作早期的 CaMBPs 分布变化以及表达量的研究，对于阐释 CaMBPs 蛋白及其所在的“Ca^{2+}-CaM-CaMBP”信号途径在松材线虫病发生发展过程中的调控机制具有重要意义。

本研究通过建立线虫侵染马尾松差减文库，并筛选获得 *CaMBP*CXY165 基因的 EST 序列。同时采用强致病力松材线虫虫株 NXY61 侵染 1 年生马尾松苗。发现：接种线虫悬浮液 24h 时，距接种点 2-4cm 处明显检测到线虫，确定线虫已侵染寄主体内，而此时茎干处的 *CaMBP*CXY165 基因表达量下降明显，较松树根、叶器官更易响应线虫侵染；在线虫侵染早期的 1h、6h、24h、48h、72h 时间点，6-48h 时间段，接种点处线虫数量呈明显上升趋势，48h-72h 时间段，接种点处线虫数量呈下降趋势，而茎干处 *CaMBP*CXY165 基因在这 5 个时间点响应线虫侵染的表达量处于持续下调的趋势；而且 6h、24h、48h 三个时间点的表达量在 5%显著水平上差异不显著，但它们与线虫侵染 1h、72h 时间点的表达量存在显著差异。总之，松树体内 *CaMBP*CXY165 基因在线虫侵染早期的表达量变化响应了线虫侵染，而且其时空表达模式符合线虫在松树体内的迁移规律；由本研究结果可知，松材体内的 *CaMBP*CXY165 基因表达响应线虫侵染，而且该蛋白可能在松材线虫病发生发展过程中起到关键作用；同时本研究揭示了“Ca^{2+}-CaM-CaMBP”信号途径在松材线虫和寄主松树互作早

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期寄主抗病反应中可能发挥了重要作用。

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Research on the Expression Pattern of Gene *CaMBPCXY165* in Pinus infected by Pine Wood Nematode

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Abstract: Pine wood wilt has become one of the most dangerous biological disasters to China forest, but its pathogenic mechanism has been controversial. The transiently elevation of intercellular Ca^{2+} is the early singal to trigger plant defense response, the different spatio-temporal dynamics will be produced when intercellular Ca^{2+} meeting different stimulus; accompanying with pine wood wilt development, the feature of concentration changes of free Ca^{2+} in parenchyma cells was "low-high-low-high", and the different spatio-temporal dynamics of intracellular Ca^{2+} are deciphered by a variety of intracellular Ca^{2+} sensor proteins such as calmodulin (CaM), further bind the downstream calmodulin binding protein (CaMBPs), result in different cell reactions. The concentration changes of intracellular Ca^{2+}/CaM is a must in host defensive reactions such as defense related genes induced and programmed cell death. Therefore, the reseach on the distribution and expression changes of CaMBPs early interaction between pine wood nematode and host pine has an important significance to interpretate the control mechanism of the protein CaMBPs and signal pathway "Ca^{2+}-CaM-CaMBP" on pine wood wilt occurrence and development.

The EST sequence of Gene *CaMBP*CXY165 was screened from *Pinus massoniana* SSH cDNA library inoculated with *Bursaphelenchus xylophilus* in the study. The found from the observation result of one year pine infected by strong virulence pine wood nematode strains NXY61: at 24h after the pine vaccinated with NXY61, nematodes were detected from stem 2-4cm below vaccination piont, it was identified that the nematodes infected into host body; at this time the expression level of Gene *CaMBP*CXY165 in stem decreased more obviously, was more

responsive to nematodes infection than that in root and leaf. At the early time such as 1h, 6h, 24h, 48h, 72h after nematodes infection, nematode population in the inoculation point of pinus showed an upward trend during 6h-48h time period, while decline trend during 48h-72h time period; The expression quantity of Gene *CaMBP*CXY165 in stem was continued downward trend at the 5 time point response to nematode infection; between at the three time points 6h, 24h, 48h, the expression quantity was not significantly different at the 5% significance level, but they were significantly different with that at 1h, 72h. In short, expression quantity changes of Gene *CaMBP*CXY165 in pinus at the early time responded to nematode infection, the spatio-temporal expression patterns of the gene consistented with the nematodes migration in pine; the research results reveals that the expression of Gene *CaMBP*CXY165 responded to nematode invasion, and the protein might play a key role during the process of pine wilt disease occurrence and development; the signal pathway "Ca^{2+}-CaM-CaMBP" might play an important role on host defense responses early interaction between pine wood nematode and host pine.

Key Words: Expression Pattern; *CaMBP*; Nematode migration; Host defense responses; Pine Wood Wilt;