PRÊMIO - BIOTECNOLOGIA E GENÔMICA

EXPLORING MQSR TOXIN AS NEW APPROACH FOR CANDIDATUS LIBERIBATER ASIATICUS CONTROL

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Prokaryotic Toxin-Antitoxin (TA) systems encode a stable toxin, that disrupts cellular functions, and its labile cognate antitoxin in the same operon. TA systems can restrict bacterial growth in response to environmental stresses. Therefore, the toxins from TA systems are good candidates for developing novel antimicrobials due to the essential bacterial processes they target. Previous studies of our research group showed that the MqsR toxin is associated with arrested growth and reduced metabolism of X. fastidiosa and Xanthomonas citri subsp. citri, which causes CVC and citrus canker disease, respectively. Thus, we hypothesized that this toxin if expressed in phloem cells could inhibit the Candidatus Liberibacter asiaticus (CLas) growth, the causal agent of Huanglongbing (HLB), the most devastating disease in citrus nowadays. To verify such hypothesis, we built a vector using the phloem-specific AtSUC2 promoter (sucrose transporter) to drive the mqsR expression directly in phloem and companion cells in Citrus sinensis Hamlin variety. Epicotyls were used for transformation with Agrobacterium tumefaciens

EHA105 carrying the construction AtSUC2:mqsR containing the reporter gene uidA (ß-glucuronidase). Three transgenic lines, HSUCM1, HSUCM2 and HSUCM3 were obtained and transformation was confirmed by histochemical activity (ß-glucuronidase, GUS) and PCR, using specific primers for mqsR. Gene expression was verified by RT-quantitative PCR and the toxin production in the transgenic lines was confirmed by immunoblotting using specific antibody raised against MqsR. The expression of mqsR was similar in all transgenic lines. However, HSUCM3 produced more toxin than HSUCM1 and HSUCM2. Afterward, these plants were propagated, and inoculated with budwoods from symptomatic plants with positive CLas amplification by qPCR. The plants are maintained in growth chamber under the following condition: 26°C (6 a.m.), 27°C (9 a.m.), 28°C (12 a.m.), 25°C (6 p.m.) and dark period is kept at 24°C, with a photoperiod of 12 hours to accelerate the symptoms development. Samples from these plants were collected 60, 90 and 120 days after inoculation (DAI) for DNA isolation and qPCR. The 60 and 90 DAI samples were negative for CLas in qPCR. However, some samples collected at 120 DAI were positive to CLas, even though they are still symptom less. The plants will be evaluated for 150 and 180 DAI for bacterial titer to assess tolerance levels to HLB and symptoms severity over time.