ABSTRACT

TRI JOKO SANTOSO. Identification of Indonesian Begomoviruses in Tomato and Genetic Diversity Analysis of AV1 Gene as well Its Use for Developing Virus Resistant Plant. Under directions of SUDARSONO, HAJRIL ASWIDINNOOR, SRI HENDRASTUTI HIDAYAT, and MUHAMMAD HERMAN.

Tomato (*Lycopersicon esculentum*, Mill) is one of the most important vegetables in Indonesia, both economically and nutritionally. Production, however, is severely hampered by a leaf curl disease caused by Tomato (yellow) leaf curl virus (TYLCV/ToLCV), one of members of the genus *Begomovirus*, the family *Geminiviridae*. Recently, there is no effectively way to control this disease. The use of resistant tomato plants is undoubtedly the best way to control Begomovirus. Genetic engineering technologies give the opportunity to develop transgenic tomatoes resistant to Begomovirus through pathogen derived resistance (PDR) approach. Begomovirus *AV1* gene is a gene expressing coat protein which responsible for particle encapsidation and have a role in specificity determinant of virus transmission and symptom development. The objectives of this research were (1) to detect Begomoviruses infecting tomato in several of tomato production areas of East Java, Central Java, Special Province of Jogjakarta and West Java by using PCR technique. (2) to analyze genetic diversity of Begomovirus isolates infecting tomato based on the PCR-RFLP technique, (3) to identify and analyze the genetic diversity of Begomovirus isolates infecting tomato based on nucleic acid and amino acid of *AV1* gene, (4) to construct the *AV1* gene of Begomovirus into pBI121 expression vector plasmid and generate tobacco transformants through *A. tumefaciens*-mediated transformation with *AV1* gene cassette, (5) to obtain transgenic tobacco plants carrying *AV1* gene and resistant to Begomovirus, (6) to generate tomato lines carrying resistance against Begomovirus (TYLCV) combined with resistance to CMV through conventional breeding program. The results of this research showed that the symptomed plants collected from several tomato production areas of East Java, Central Java, Special Province of Jogjakarta and West Java indicated that those plants have been infected by *Begomovirus* following PCR detection using a pair of degenerate primers. Phylogenetic analysis based on the PCR-RFLP technique showed that the eight *Begomovirus* isolates were divided into three different groups. Meanwhile, identity of nucleic and amino acid of *AV1* gene among Begomoviruses indicated that the isolates determined in this research were Indonesian isolates of AYVV and phylogenetic analysis of the eight Begomovirus isolates based on the nucleotide and predicted amino acid sequence analysis of *AV1* gene indicated they belonged into two different clades. In the experiments of genetic transformation, results of the experiments showed that (i) Indonesian Begomovirus *AV1* gene was successfully amplified and inserted in pBI121 expression vector plasmid, (ii) tobacco transformants carrying kanamycin-resistant gene (*nptII* gene) were regenerated and established in glasshouse, (iii) there was a positive correlation between the presence of the *AV1* gene in T0 generation putative transgenic tobacco plants and the resistant phenotype to Begomovirus, (iv) transgenic plants with a single copy integration of the transgene exhibited more resistant than the multiple copy one and non transgenic plant. The resistance phenotype of *AV1* gene expression was indicated with no symptom in T0 generation putative