

INTISARI

Molecular farming merupakan teknik pemanfaatan potensi tanaman sebagai pabrik biologi untuk memproduksi dan mengkomersialisasikan produk biofarmasi. *Molecular farming* perlu diinisiasi di Indonesia karena memiliki potensi swasembada produk biofarmasi di masa mendatang. Tujuan studi ini adalah untuk menjadi inisiasi pengembangan *molecular farming* di Indonesia dengan mengkonstruksi gen *nucleocapsid* (NC) SARS-CoV-2 pada vektor biner pRI101AN (pRI101AN-NC), dan mengintroduksi konstruk tersebut ke dalam genom tanaman tembakau (*Nicotiana tabacum*) melalui vektor *Agrobacterium tumefaciens* strain GV3101 sehingga nantinya protein NC dapat diekspresikan pada tanaman *N. tabacum*. Konstruksi gen dimulai dari amplifikasi PCR gen NC menggunakan sepasangan primer spesifik dengan penambahan restriksi enzim *Bam*HI dan *Sac*I, kloning gen tersebut akan di potong dengan enzim restriksi kemudian akan di ligase ke vektor biner pRI101AN, transformasi pRI101AN-NC ke sel kompeten *Escherichia coli* DH5 α , dan transformasi konstruk ke *A. tumefaciens* GV3101. Pada penelitian ini, konfirmasi keberhasilan konstruksi pRI101AN-NC dilakukan melalui analisis molekuler menggunakan PCR, enzim restriksi, dan *Sanger sequencing*. Transformasi pada *N. tabacum* akan dilakukan secara *transient expression* menggunakan sistem agroinfiltrasi. Agroinfiltrasi merupakan metode transformasi genetik injeksi pada daun tembakau dengan suspensi *A. tumefaciens* yang mengandung konstruk pRI101AN-NC. Hasil penelitian menunjukkan bahwa gen NC berhasil dikonstruksi ke dalam vektor pRI101AN dan ditransformasikan ke tanaman tembakau. Tanaman klon positif yang membawa gen NC telah dikonfirmasi dengan PCR menggunakan primer spesifik yang menghasilkan pita DNA berukuran 1000 bp. Hasil ini menjadi dasar penting bagi awal pengembangan metode *molecular farming* di Indonesia.

Kata kunci : Agroinfiltrasi; *molecular farming*; nucleocapsid SARS-CoV-2; *Nicotiana tabacum*; transformasi genetik tanaman.

ABSTRACT

Molecular farming is a technique of utilizing plant as a biological factory to produce commercialize biopharmaceutical products. Development of Molecular farming in Indonesia needs to be explored since it has the potential for self-sufficiency in biopharmaceutical products. The purpose of this study is to initiate the development of molecular farming in Indonesia by constructing the nucleocapsid (NC) gene of SARS-CoV-2 into the binary vector pRI101AN (pRI101AN-NC), and introducing this construct into the genome of tobacco plants (*Nicotiana tabacum*) through the vector *Agrobacterium tumefaciens* strain GV3101 so the NC protein is expressed into *N. tabacum*. The gene construction have is been carried out by adding which include PCR amplification of the NC gene using a specific primary pair with the fragment of *BamHI* and *SacI* restriction enzyme. The amplified gene was cut with the enzyme restrictions then it was ligased to the binary vector pRI101AN. Genetic transformation of pRI101AN-NC was transfered competent cells of *Escherichia coli* DH5 α , and constructed into *A. tumefaciens* GV3101. In this study, the confirmation of the succeeded construction was conducted by PCR, restriction enzymes, and Sanger sequencing. Plant genetic transformation was transiently expressed by agroinfiltration system. Agroinfiltration is a plant genetic transformation method that using *Agrobacterium* mediated system. carried out in plants using syringes on tobacco leaf with *A. tumefaciens* suspension containing the construct pRI101AN-NC. The results showed that the NC gene was successfully constructed into the pRI101AN vector and transformed into tobacco plants. Positive cloned were confirmed by PCR using a specific primer and the band size of 1000 bp was detected. This result is important for the development of plant-based molecular farming in Indonesia.

Keywords: Agroinfiltration, molecular farming, nucleocapsid SARS-CoV-2, *Nicotiana tabacum*, genetic transformation of plants.