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DEVELOPMENT AND EVALUATION OF A NEW BINARY VECTOR FOR GENE EXPRESSION IN HIGHER PLANTS

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ABSTRACT

The binary vector is an essential component in *Agrobacterium*-mediated transformation. It carries the DNA fragments to be delivered into the plant genome. Many binary vectors have been developed for different transformation experiments. Some of the binary vectors are commercially available. Most of the binary vectors make use of the viral-derived CaMV 35S promoter and the NOS terminator from bacteria to regulate the expression of the transgene. Besides, the binary vector also contains a selectable marker gene for transformant selection. Using viral-derived regulatory elements has posed particular problems in transgenic plants and raised some biosafety concerns. Therefore, a binary vector containing the plant-derived gene regulatory elements is deemed required. In this study, a new binary vector named pJASIN101 was developed using the backbone of the pMDC162 vector and evaluated in model plants through transient transformation experiments. The 10,404-bp pJASIN101 vector consists of the *EgPAL2* promoter from oil palm and the *NbACT* terminator from *Nicotiana benthamiana* for transgene expression. For selection purposes, GFP was utilized as a selectable marker and regulated by the *AtSCPL30* promoter and heat shock protein 18.2 (HSP) terminator from *Arabidopsis*. To evaluate the pJASIN101 vector, the *GUS* gene was cloned into the vector, yielding the pJASIN101-GUS construct. *Agrobacterium* carrying the pJASIN101-GUS construct was infiltrated into *Nicotiana benthamiana* and *Petunia* sp. leaf tissue for transient expression of the *GUS* gene. *GUS* staining was performed on the infiltrated tissues four days after agroinfiltration. As anticipated, the infiltrated regions turned blue after *GUS* staining. In addition, the infiltrated tissue emitted green fluorescence when visualized under a fluorescence microscope. The results indicated that the construct is well functioning in plant cells. Therefore, the pJASIN101 vector developed in this study could be used for plant transformation. When using the pJASIN101 vector in transformation, no viral or bacterial-derived regulatory elements will be transferred into the plant genome. The availability of this new binary vector permits the production of transgenic plants for functional study and trait improvement purposes without introducing any viral or bacterial-derived DNA elements into the transformants.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Crop improvement through conventional breeding relies on the gene and allele present in a gene pool. In conventional breeding, the exchange of genetic materials among different organisms is limited to the closely related species (Ahmar et al., 2020). At the same time, undesirable genes can be transferred along with the desirable gene. The conventional breeding also takes a long period to achieve desired results (Caruso et al., 2020). In contrast, genetic engineering allows the transfer of genetic materials among organisms from different kingdoms, expediting the crop improvement process and development of new cultivars. Genetic engineering is also frequently known as genetic modification (Oliveira et al., 2017). Development of genetically modified (GM) crops often involves the introduction of a foreign gene into a wild-type plant. The phenotypic or metabolic alterations observed in the GM crops are attributed to the expression of the foreign gene inserted (Bedair & Glenn, 2020). Hence, the expression level and the behaviour of the transgene is the main concern in developing GM crop.

Delivery of a foreign gene to a plant is frequently achieved by the *Agrobacterium*-mediated transformation method (Hwang et al., 2017). In *Agrobacterium*-mediated transformation, the soil-borne bacterium transfers a portion of its DNA located on the Ti Plasmid to a plant cell (Risha Amilia & Muhammad Imam, 2020). Development of transgenic research has led to the production of binary vector. The binary vectors developed allow efficient manipulation of the plasmid for high level expression of a transgene (Sheludko, 2008). The binary vector contains a promoter to govern the expression of a transgene spatially and temporally. Overexpression of the gene of interest (GOI) requires a strong constitutive promoter (Lee & Gelvin, 2008).