

MSP2: A MESOCARP-SPECIFIC PROMOTER FOR PLANT GENETIC ENGINEERING

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INTRODUCTION

MPOB initiated the genetic engineering program in late 1980s to diversify the use and increase the economic value of palm oil. The aim of the program was to produce higher oleic acid content of the palm oil in the mesocarp. Subsequently, the genetic engineering program was expanded to include high contents of stearic, palmitoleic, ricinoleic, lycopene as well as biodegradable plastics (Parveez *et al.*, 2015).

IMPORTANCE OF PROMOTER

One of the prerequisite in any genetic engineering is the availability of a suitable promoter. To produce transgenic plants with the desired traits, the introduced gene carrying the trait of interest has to be functionally expressed for production of heterologous protein. Expression of the transgene is highly dependent on the promoter. The promoter is responsible to initiate and regulate the transcription process of the transgene, hence allowing gene expression (Porto *et al.*, 2013). Plant promoters are isolated based on the identification and characterization of genes expressed in a specific tissue or under physiological conditions. Tissue-specific promoter allows the expression of the transgene to be directed to a particular tissue where the promoter is active. In oil palm, to genetically engineer the palm to modify the mesocarp oil composition, a mesocarp-specific promoter is required. This is to ensure that the transgene is expressed in the fruit mesocarp and to avoid accumulation of the targeted products in other parts of the plant that could be detrimental to the plant. MSP1 is the first mesocarp-specific promoter isolated from oil palm and has been used in the MPOB genetic engineering program to drive the specific expression of the transgene to the mesocarp (Siti Nor Akmar and Zubaidah, 2008). As different promoters have different strengths and specificity to drive the expression of transgenes,

it was envisaged that more mesocarp-specific promoters are needed to drive the expression of the targeted genes specifically to the mesocarp. This is also one of the strategies to avoid homology dependent gene silencing due to multiple copies of the same promoter sequences inserted at the same loci.

FEATURES OF MESOCARP-SPECIFIC GENE (MSP2)

Full-length MSP2 gene was successfully isolated following its initial identification from a 17-week-old mesocarp cDNA library (Nurniwalis *et al.*, 2008; 2015). Gene expression analyses showed that the MSP2 gene was predominantly expressed in the mesocarp and the expression increased as fruits reached maturity, which also coincides with the oil synthesis period (*Figure 1*).

FEATURES OF MSP2 PROMOTER

Understanding of the MSP2 gene expression profile during fruit development and oil synthesis is useful for isolating its corresponding promoter for genetic manipulation. The MSP2 promoter is 765 bp long and contains sequences (TATA box, pyrimidine patch, and cis-acting regulatory elements) that makes up the important components of the promoter. The cis-acting regulatory elements also correspond to known transcriptional regulatory sequences to allow binding of transcription factors and RNA polymerase to initiate and regulate the expression and transcription process of the target gene.

Functionality of MSP2 promoter to direct the expression of transgenes to the mesocarp was determined using GUS transient assay. A vector construct containing the MSP2 promoter and the β -glucuronidase (GUS) reporter gene was successfully constructed (*Figure 2*) and was bombarded into mesocarp slices and leaf disks. The MSP2 promoter showed mesocarp-specific activity based on the detected GUS expression

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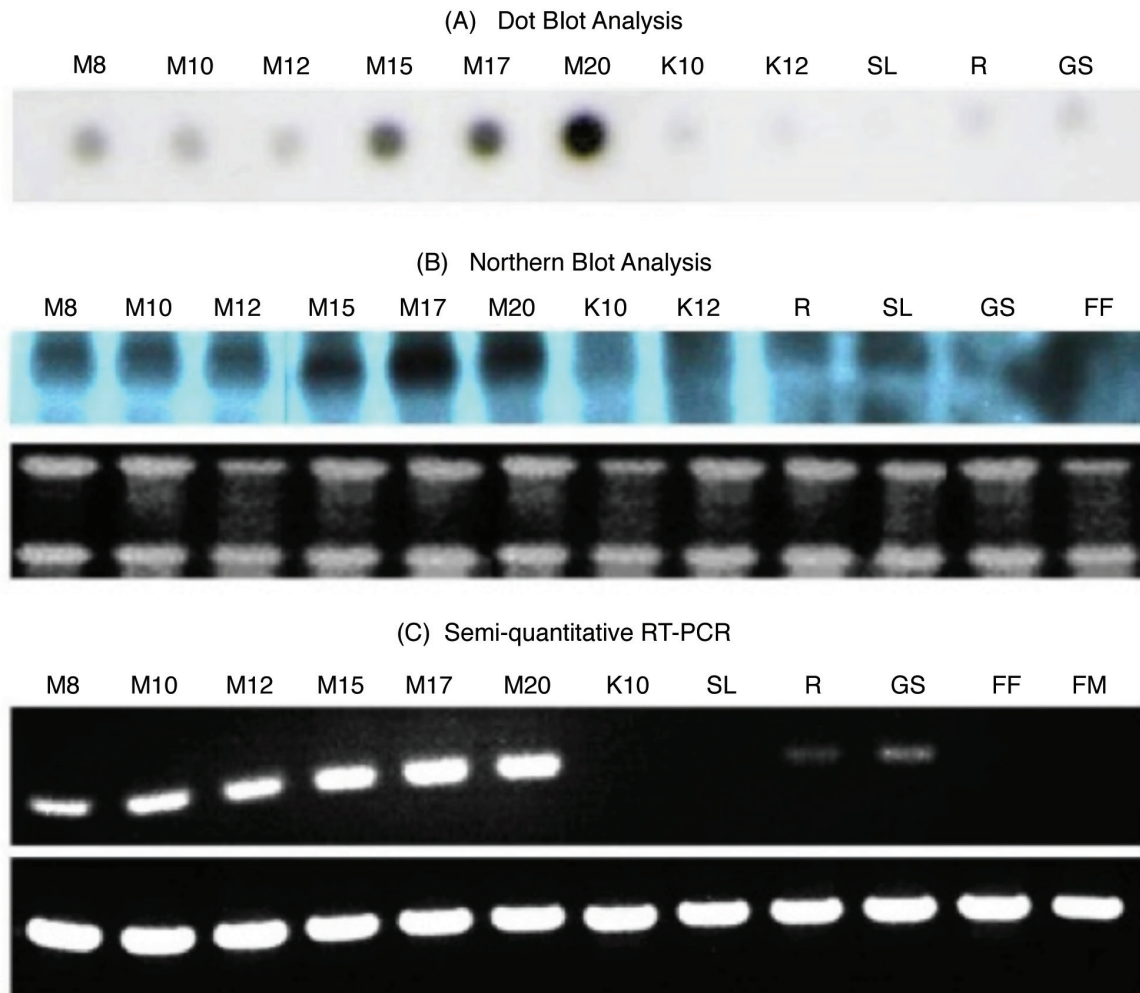


Figure 1. Expression patterns of MSP2 gene in various oil palm tissues as shown by the Dot Blot (A), Northern analysis (B) and RT-PCR (C). M = mesocarp, K = kernel, R = roots, SL = spear leaves, GS = germinated seedlings, FF = female inflorescence, FM = male inflorescence. Numbers in the mesocarp and kernel = developmental stages at WAA.

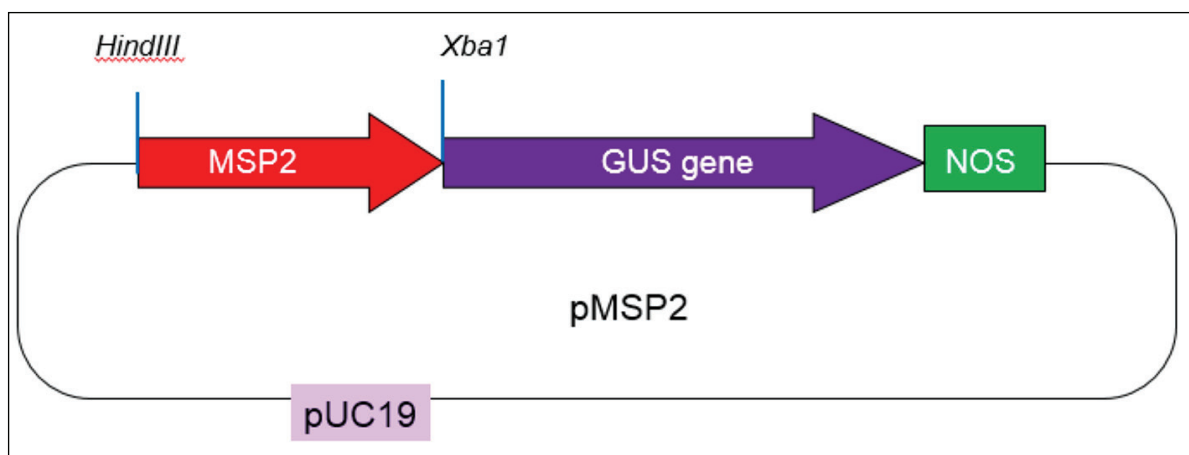


Figure 2. Transformation vector, pMSP2 containing MSP2 promoter driving reporter GUS gene. *NOS = nopaline synthase terminator.

(blue spots) only on the bombarded mesocarp slices especially in the vascular bundles and not on bombarded leaf tissue (Figure 3). This promoter could direct expression of foreign genes to the

mesocarp tissue of plants hence, can potentially / subsequently be used for genetic modification in oil palm for the focus product in the mesocarp.

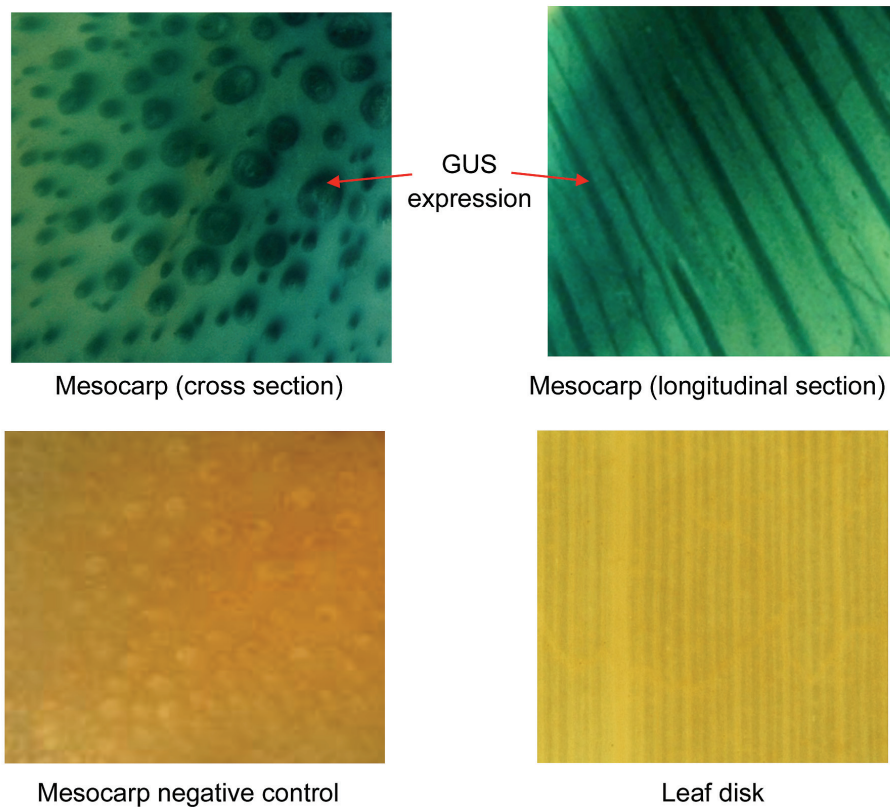


Figure 3. An *FLL1pro*: *GUS* expression profile in the mesocarp and leaf tissues of oil palm.

BENEFIT OF MSP2 PROMOTER

In addition to MSP1 promoter, MSP2 promoter from oil palm can also be used as an alternative to drive the expression of the transgene carrying the desired traits to the mesocarp. This is especially targeted towards producing transgenic palms for specialty oils and other novel and value added products. MSP2 promoter can also be applied to express genes in mesocarp tissues of other fruits containing mesocarp such as tomato, peach and avocado.

WHO WILL BENEFIT

The MSP2 promoter and vector construct is readily available to interested parties. The MSP2 promoter should be of interest to molecular biologists in research institutions, universities, research-based companies and the oil palm industry. These communities can benefit from the use the MSP2 promoter to drive the expression of transgenes to the mesocarp either for their R&D work or commercial scale production of the trait of interest in the mesocarp tissue of the plant.

INTELLECTUAL PROPERTY

Application for patent has been filed for MSP2 promoter isolated from oil palm.

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