Soybean *PMI* genes as a selectable marker for corn and rice transformation

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PMI gene from *E. coli* has been successfully used in crop transformations of corn, rice, wheat and many other crops (Gui H.P., Plant Cell Report, 33:1081-1090, 2014; D. Negrotto, Plant Cell Report, 19(8): 798-803,2000). Its mode of action can enable transformed cells to utilize mannose instead of sucrose as carbon source so that it has been termed as a positive selection marker with higher transformation frequency (TF). To test whether *PMI* genes originated from crops will be effective in plant genetic transformation, we searched online (NCBI Blast & Phytozome) with the reference of Peach PMI amino acid and obtained many confirmed / hypothetical *PMI* genes from different sources (Table 1). Two of soybean *PMI* genes driven by maize ubiquitin promoter were tested in corn and rice transformation. The sequences of the two genes were optimized by using rice code optimization used in our tests.

Rice IR58025B was transformed by *Agrobacteria* EHA101containing vectors with the soybean *PMI* selectable markers. Both *Glyma.18G296300.1* and *Glyma.13G045900* were tested as selectable marker to compare with *PMI* gene from *E.coli*. Results show that similar transformation frequencies are obtained in rice with soybean *PMI* genes as a selectable marker, comparing to *E.coli PMI* gene (Figure 1). In corn transformation, soybean *PMI* gene *Glyma.18G296300.1* was tested. A corn inbred line was transformed with *Agrobacteria* LBA4404 containing vectors with *Glyma.18G296300.1* and *E.coli PMI* as a control. Data shows that the average corn transformation frequency of using soybean *PMI* gene as selectable marker is 26.3%, which is similar with the frequency of using *E.coli PMI* gene (Table 2).

The function of these plant originated *PMI* genes is not well understood. It is known soybean is less sensitive to mannose selection, where the *PMI* gene does not work well as a selection marker. Its active *PMI* genes might provide such tolerance to mannose in soybean. Furthermore, *PMI* genes from corn, rice and arabidopsis will be tested in corn and rice transformation in our future study. We anticipate a high transformation frequency by using *PMI* genes of rice, corn and Arabidopsis as selectable marker could be achieved since both rice and corn are very sensitive to mannose selection.

Table 1 *PMI* genes from different plants with PMI amino acid from peach as a reference

Sources	Gene Accession	Query cover	Identity
Rice	ABA94585.1	97%	56%

	NP_001041907.1	95%	59%
	NP_001063081.2	95%	50%
Corn	ACG45229.1	95%	58%
	NP_001140535.1	95%	58%
	NP_001140476.1	95%	58%
	NP_001147755.1	94%	57%
	AFW60447.1	94%	57%
Soybean	Glyma.18G296300.1	96%	69%
	Glyma.08G365900.1	96%	68%
	Glyma.13G045700	98%	64%
	Glyma.19G049300	88%	59%
Arabidopsis	NP_186906.1	94%	64%
	BAD44147.1	95%	60%
	NP_176878.1	95%	61%
Escherichia coli K-12	WP_001170664.1	72%	35%

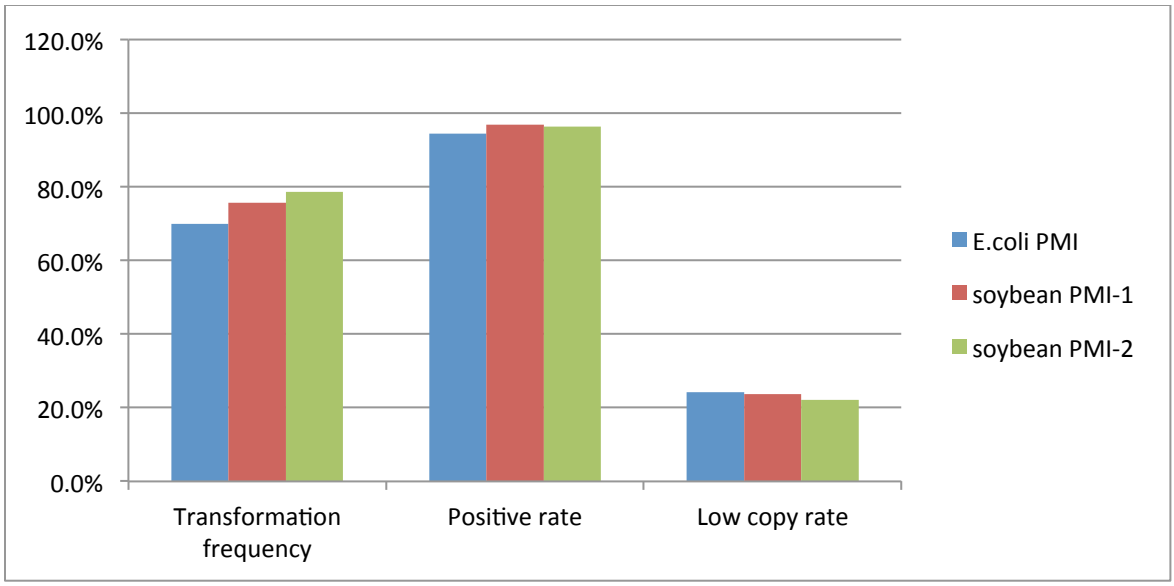

Figure 1: Rice transformation frequency with soybean *PMI* as selectable marker. E.coli PMI indicates *E.coli PMI* gene as selectable marker; Soybean PMI-1 indicates soybean *PMI* gene of Glyma.18G296300.1 as selectable marker; Soybean PMI-2 indicates soybean *PMI* gene of Glyma.13G045900 as selectable marker; Transformation frequencies (TF) defined as number of events generated among 100 explants inoculated; The positive rate is defined as the percentage of positive events among total events generated; Low copy rate is defined as the percentage of low copy events (1-2 copy) among total positive events. Data generated by three repeats with 200 explants per treatment per repeat.

Table 2. Corn transformation frequency with soybean *PMI* as a selectable marker

Selectable marker	# Explants inoculated	# of plants regenerated	Transformation frequency*	Low copy rate
E.coli PMI	4782	1632	30.2%	34.5%
Soybean *PMI*-1	608	170	26.3%	28.5%

*Transformation frequencies (TF) defined as number of events generated among 100 explants inoculated. TF Data among the two selectable markers is with no significant difference from each other. Low copy rate is defined as the percentage of low copy events among total positive events.