DNA markers for assisted selection of cassava resistant to cassava mosaic disease (CMD)

Cassava (*Manihot esculenta*) is an important crop cultivated in tropical and subtropical regions. The stable production of cassava is crucial for food security, as its starch is used in both food and industrial applications. However, cassava mosaic disease (CMD), a viral disease, has significantly hampered cassava productivity, particularly in Africa, India, and recently in Southeast Asia. Developing CMD-resistant cassava varieties is essential, but traditional breeding methods are time-consuming and costly. Therefore, there is a need for DNA markers that can identify the mutations providing CMD resistance to accelerate and reduce the cost of breeding resistant varieties.

As a result, the authors have confirmed mutations in the DNA polymerase δ subunit 1 (*MePOLD1*) gene, associated with CMD resistance, in cassava varieties and lines maintained by the Agricultural Genetics Institute (AGI) in Vietnam. These mutations, G680V and L685F, were found in lines introduced from the International Center for Tropical Agriculture (CIAT) in Colombia and the International Institute of Tropical Agriculture (IITA) in Nigeria, respectively. The developed DNA markers can distinguish these *POLD1* genotypes using two methods: the dCAPS method, which is cost-effective, and the KASP method, which is suitable for high-throughput screening. Field trials in CMD-infected areas showed that cassava lines with the G680V mutation exhibited significantly less severe symptoms compared to non-mutated lines, demonstrating the effectiveness of the DNA markers.

In terms of applications, the developed DNA markers enable the efficient selection of CMD-resistant cassava, thereby facilitating the breeding of varieties suited to conditions in Southeast Asia. These tools can be applied not only to combat CMD in Southeast Asia but also to develop resistant varieties against CMD races prevalent in Africa.

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A Southeast Asian variety
(CMD-susceptible)

C-33
(CMD-resistant)

Fig. 1. Cassava mosaic disease-resistant varieties

Cultivation of cassava in CMD-infected fields in Vietnam for three months. While the typical Asian variety exhibits CMD symptoms, the C-33 line grows without any disease symptoms. Photo provided by Thuy, C. T. (CIAT).

Table 1. Mutations in the *PLOD1* genes in CMD-resistant varieties and lines

Variety/Line	CMD susceptibility	Mutations in *POLD1* and corresponding amino acid replacements			
		680th amino acid		685th amino acid	
		Codon	Amino acid	Codon	Amino acid
Asian varieties	Susceptibility	G G T	G	T T G	L
CIAT lines from Colombia	Resistance	G T T	V	T T G	L
IITA varieties from Nigeria	Resistance	G G T	G	T T C	F

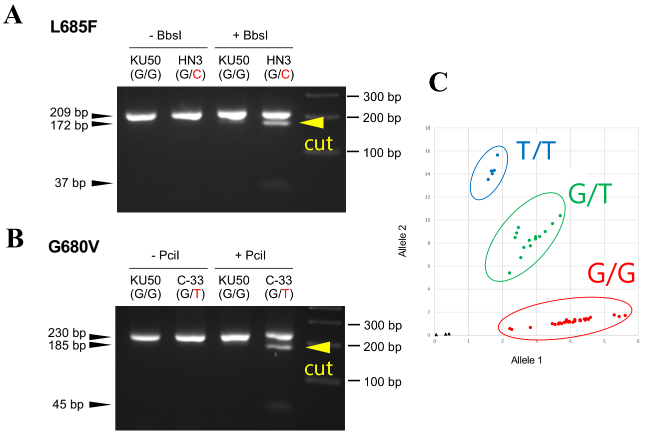

Fig. 2. Identification of *POLD1* mutations using DNA markers

(A, B) The dCAPS method creates restriction enzyme sites into PCR products, identifying *POLD1* gene mutations in WT (KU50), L685F (HN3), and G680V (C-33). PCR products treated with BbsI (A) or PciI (B) show WT if not cut, mutant type if cut. (C) The KASP assay rapidly identifies WT and G680V mutant *POLD1* genes, classifying individuals into WT G/G, heterozygous G/T, and homozygous T/T.

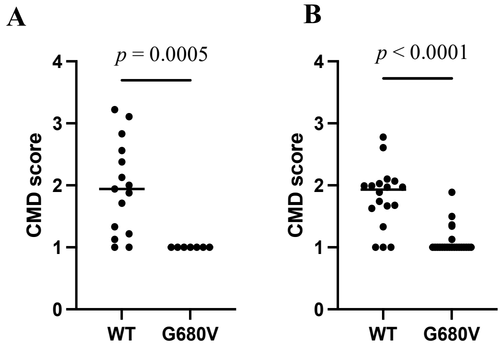

Fig. 3. Association between *POLD1* genotype and CMD resistance

(A, B) Observed CMD symptoms in progeny of G680V-resistant lines crossed with Southeast Asian (A) or South American (B) varieties after 3 months in CMD-infected fields. CMD symptom score 1 indicates no symptoms; higher scores indicate more severe symptoms. Significant differences in CMD scores were found between normal and G680V *POLD1* genes using the dCAPS method.

Reference: Tokunaga et al. (2025) *Breeding Science* (in press).