



NARDI Fundulea

EXPLORING ZMVTE4 POLYMORPHISM IN LOCAL MAIZE GERMPLASM

Conțescu Elena-Laura*, Ciucă Matilda, Horhocea Daniela, Cristina Daniel,Turcu Alina, Ionescu Violeta
National Agricultural Research and Development Institute, Fundulea

ABSTRACT- Tocopherol content in maize kernels is influenced by genetic variation at the *ZmVTE4* locus. This study investigated local maize populations using InDel118 and InDel7 markers to identify alleles associated with higher tocopherol levels. Favorable alleles (373 bp and 160 bp) were detected in several genotypes, while a distinct ~180 bp fragment observed for InDel7 in the Portocaliu 1 population indicates a previously unreported polymorphism. Molecular screening identified Românesc de Studina and several modern inbred lines as promising sources for enhanced α -tocopherol content. These results highlight the effectiveness of marker-assisted selection for improving nutritional traits in maize germplasm.

INTRODUCTION

Maize ranks among the world’s most important crops, serving human nutrition, animal feed, and various industrial purposes, including bioenergy. While research has traditionally prioritized yield and stress resilience, grain quality has become increasingly critical due to evolving market demands and stricter nutritional standards. Grain quality involves traits like kernel composition, hardness, and how easily kernels break. These traits are shaped by both genetics and the environment- factors like climate, soil, and farming practices. Furthermore, the interaction between genotype and environment represents a key determinant of grain quality stability across diverse production conditions.

Chemical composition - maize kernels are made up of 8-14% protein, mostly zein, which is rich in glutamic acid and leucine but low in lysine and tryptophan. Carbohydrates (65-70%) come mainly from starch, mostly amylopectin, stored in the endosperm. Lipids (3.9-5.5%) are concentrated in the germ and high in unsaturated fatty acids. Kernels also contain minerals (1.3-1.8%), cellulose (1.9-2.4%), and carotenoids, which give color and provide provitamin A. Most maize hybrids naturally have low levels of vitamin E, vitamin A, lysine, and tryptophan—nutrients essential for human health. Numerous studies further indicate that over 20% of the global population is deficient in α -tocopherol.

Enhancing vitamin E content through molecular breeding - over the past decade, considerable research has focused on increasing vitamin E in various crops, including *Arabidopsis* (Mène-Saffrané and Pellaud, 2017), soybean (Arun et al., 2014) and lettuce (Cho et al., 2005). In maize, extensive mapping studies have identified numerous QTLs linked to important quality traits such as protein, starch, lipid, carotenoid, and tocopherol content. Maize exhibits a wide genetic variation in tocopherol composition. Measuring tocopherol fractions (α -, β -, γ -, and δ -) using HPLC is straightforward and fast (~15 min/sample), but the high cost (25-30 USD/sample) limits its routine use. This highlights the value of targeting key genes to increase tocopherol content in a cost-effective manner (~0.5 USD/PCR assay). Several studies (Lipka et al., 2013; Diepenbrock et al., 2017) have identified QTLs controlling α -tocopherol, γ -tocopherol, and total tocopherol levels. Among them, *ZmVTE4* (α -tocopherol methyltransferase) stands out as a major gene, converting γ -tocopherol into α -tocopherol (Li et al., 2012). Two key insertion-deletion polymorphisms (InDel7 and InDel118) significantly affect α -tocopherol levels: InDel118, located just upstream of the transcription start site, regulates *VTE4* transcription, while InDel7 influences translation efficiency. Subsequent work identified a favorable *VTE4* haplotype that distinguishes maize lines with high versus low α -tocopherol content (Das et al., 2019). Plants carrying these favorable alleles can accumulate 2-3 times more vitamin E (Das et al., 2019). Other natural variants, such as crtRB1 (β -carotene hydroxylase) and lcyE (lycopene ϵ -cyclase), can increase vitamin A content 2-10 fold, while the recessive opaque2 (o2) allele doubles lysine and tryptophan content (Hossain et al., 2018). These advances have already been applied in breeding: several QPM hybrids carrying o2 and proA hybrids with *crtRB1* and *lcyE* are now in cultivation (Hossain et al., 2018; Prasanna et al., 2020). Recent studies confirm the development of maize hybrids combining higher vitamin E, vitamin A, lysine, and tryptophan content (Das et al., 2021).

Materials and methods

The study focused on the molecular characterization of a set of old maize populations at the *ZmVTE4* locus, which plays a role in tocopherol accumulation. Plant material - a total of 89 samples, representing local populations, inbred lines, breeding lines and maize hybrids were analyzed. DNA isolation - seeds were sown in seedling trays. Leaves were collected and stored at -25° C. Each sample was ground with liquid nitrogen until a fine powder was obtained and then transferred into sterile 2 ml tubes. DNA extraction - was performed using the NucleoSpin Plant II kit. For old maize populations, 2-3 extractions per genotype were performed to validate the purity/impurity of the materials. DNA amplification - PCR reactions were carried out using the DreamTaq Green enzyme in a 15 μ l reaction volume, containing 1 \times master mix, 0.5 μ M primers and 50-60 ng of DNA. PCR product electrophoresis - was performed on 2% agarose gel (Routine Use) for the *Vte4* - InDel118 marker and on 3% agarose gel (High Resolution) for the *Vte4* - InDel7 marker. Data analysis - allowed the identification of favorable alleles in the analyzed maize germplasm.



Table 1 - Results on the genetic variability present in old and modern maize germplasm at the *ZmVTE4* gene level

Results

PCR Analysis with marker InDel118 allowed us to clearly identify favorable alleles in the maize germplasm under study. The favorable allele corresponds to a 373 bp PCR product, while the unfavorable allele produces a 491 bp fragment (fig.1). In some samples, both products were observed, reflecting either heterogeneity/impurity/ heterozygosity. PCR analysis with marker InDel7 - the favorable allele is characterized by a PCR product of 160 bp, whereas the unfavorable allele corresponds to a product of 167 bp (fig.1). In the case of this marker, a PCR product of approximately 180 bp, indicated with “*”, was detected in the local population Orange 1. Further chemical analyses focusing on the tocopherol content of this population will enable a more accurate interpretation of this finding.

The screening of the analyzed germplasm using the InDel118 and InDel7 markers (table 1) highlighted the presence of favorable alleles in both traditional local populations and modern germplasm. The InDel118 marker generated clearly distinguishable alleles across the evaluated materials, in contrast to InDel7, where the difference between alleles was only 7 bp. Notably, the InDel7 marker also revealed a previously unreported polymorphism, a PCR product of approximately 180 bp in a single traditional population, Portocaliu 1. Within the older genotypes, the population Românesc de Studina proved particularly noteworthy. It displayed both favorable alleles in two out of the three analyzed DNA extractions, suggesting that the population is not fully homogeneous. This finding indicates the need for further molecular analyses at the level of individual plants. Other traditional populations carried the favorable allele associated with InDel118 but were heterozygous for InDel7 (Scorummic de Olimpești, Peretu 283, Dintele oii de Sohatu, Românesc de Butimanu and Bănățean). In contrast, Moldovenesc de Pechea and Răzvani exhibited only the favorable allele associated with InDel7. Among the modern genotypes, six inbred lines and three hybrids were found to possess both favorable alleles. The modern hybrids Felix, Magnus, Amurg and Miraj also carried both favorable alleles; however, for InDel118, the favorable allele was identified in the heterozygous state.

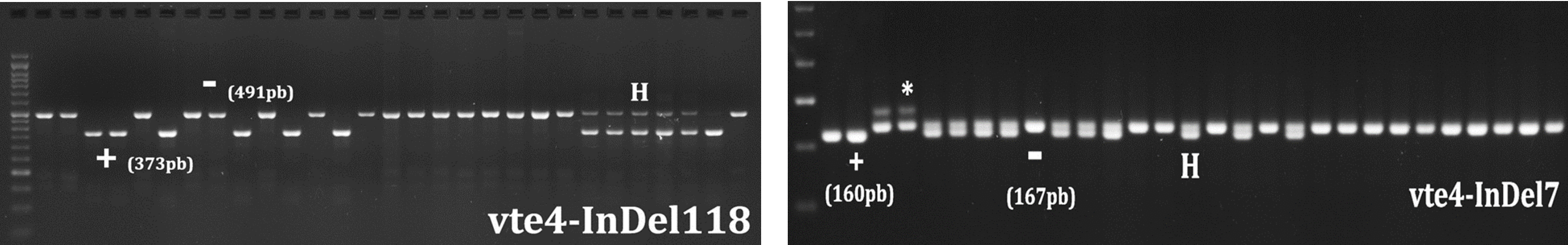


Fig. 1 - Electrophoretic profile obtained with InDel118 and InDel7 (“+” indicates the favorable allele; “-” unfavorable allele; H denotes heterogeneous or heterozygous)

PERSPECTIVES

Molecular markers can accelerate the identification and selection of genotypes carrying favorable alleles for improved nutritional quality.

Due to its rich nutrient profile, maize has strong potential to contribute to human nutritional needs.

Integrating molecular data with detailed phenotypic evaluations and HPLC results can make the selection process more efficient and guide the development of higher-quality maize hybrids.

CODE	Name	Markers associated with ZmVTE4		CODE	Name	Markers associated with ZmVTE4		CODE	Name	Markers associated with ZmVTE4	
		InDel118	InDel7			InDel118	InDel7			InDel118	InDel7
Pb 1-1	Românesc de Studina	+	+	Pb 15-1	Bănățean	+	H	Pb 48-1	Felix	H	+
Pb 1-2		+	+	Pb 15-2		-	-	Pb 48-2		H	+
Pb 1-3		-	-	Pb 17-1		-	H	Pb 49-1		H	+
Pb 2-1	Portocaliu 1	-	+	Pb 17-2	Dobrogeean	-	-	Pb 50-1	Amurg	H	+
Pb 2-2		-	+	Pb 18-1		-	-	Pb 50-2		H	+
Pb 2-3		-	-	Pb 18-2		-	-	Pb 51-1		H	+
Pb 3-1	Portocaliu	-	-	Pb 19-1	ICAR-54-1	-	-	Pb 54-1	HSF 1033	-	-
Pb 3-2		-	-	Pb 19-2		-	-	Pb 55-1		HSF 1034	-
Pb 4-1		-	-	Pb 20-1		-	-	Pb 56-1		HSF 3877	+
Pb 4-2	Sincantiv	-	-	Pb 20-2	Lest Pfister	-	-	Pb 57-1	HSF 4075	-	+
P4 4-3		-	-	Pb 21-1		-	-	Pb 58-1		HSF 1032	-
Pb 5-1		+	H	Pb 21-2		-	-	Pb 59-1		HSF 1214	-
Pb 5-2	Scorummic Olimp	-	-	Pb 22-1	Arieșan	-	-	Pb 60-1	HSF 1370	-	+
Pb 5-3		+	H	Pb 23-1		-	+	Pb 61-1		HSF 1142	-
Pb 6-1		-	-	Pb 23-2		-	H	Pb 62-1		HSF 7417	+
Pb 6-2	Pop Peretu	+	H	Pb 24-1	LC 403	+	+	Pb 63-1	HSF 4687	-	-
Pb 6-3		+	H	Pb 25-1		+	+	Pb 64-1		HSF 1089	+
Pb 7-1		-	-	Pb 26-1		-	+	Pb 65-1		HSF 10879	+
Pb 7-2	Galben timpuriu	-	-	Pb 27-1	LC 508	+	+	Pb 66-1	HSF 10901	+	+
Pb 7-3		-	-	Pb 28-1		-	+	Pb 67-1		HSF 1094	+
Pb 8-1		-	-	Pb 29-1		-	-	Pb 68-1		HSF 10959	H
Pb 9-1	Mold Pechea	-	+	Pb 30-1	LC 740	+	+	Pb 69-1	HSF 10935	H	+
Pb 10-1		-	-	Pb 31-1		-	+	Pb 70-1		HSF 11035	-
Pb 10-2		-	+	Pb 32-1		+	+	Pb 71-1		HSF 11397	+
Pb 10-3	Pop Răzvani	-	-	Pb 34-1	LC 810	-	-	Pb 72-1	HSF 11423	-	-
Pb 11-1		-	-	Pb 35-1		+	+	Pb 74-1		HSF 11513	+
Pb 11-2		-	-	Pb 36-1		-	-	Pb 75-1		HSF 11936	-
Pb 12-1	Bătrân Progresu	-	-	Pb 37-1	LC 813	-	-	Pb 76-1	HSF 11990	-	-
Pb 12-2		-	H	Pb 38-1		-	-	Pb 77-1		HSF 6479	-
Pb 12-3		-	H	Pb 39-1		-	-	Pb 78-1		HSF 6383	-
Pb 13-1	Dintele oii Sohatu	+	H	Pb 40-1	LC 817	-	-	Pb 79-1	HSF 6641	-	H
Pb 13-2		-	-	Pb 42-1		-	+	Pb 80-1		HSF 6785	+
Pb 13-3		-	-	Pb 43-1		-	-	Pb 87-1		HSF 7612	+
Pb 14-1	Românesc Butimanu	+	H	Pb 44-1	Desert	-	+	Pb 88-1	HSF 7621	+	H
Pb 14-2		+	-	Pb 47-1		-	H	Pb 89-1		HSF 7720	+

CONCLUSIONS

The optimized protocols provided a reliable and detailed characterization of the maize germplasm analyzed.

The InDel7 marker revealed a previously unreported polymorphism in the Portocaliu 1 population, confirming the high genetic variation still present in traditional germplasm.

The traditional population Românesc de Studina showed notably high α -tocopherol levels, while ten modern inbred lines also displayed favorable profiles.

Marker-assisted selection remains an effective approach for improving nutritional traits, particularly when phenotyping is limited by high costs and labor demands.