

**ALLELIC POLYMORPHISM OF *crtRB1* AND *LcyE* GENES RELATED  
TO THE  $\beta$ -CAROTENE CONTENT IN VIETNAMESE  
TRADITIONAL MAIZE ACCESSIONS**

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**ABSTRACT**

Maize is the third most important food crop after wheat and rice. Maize is used as food for more than a billion people around the world and is used as animal feed, especially, poultry. The concentration of carotenoids, especially,  $\beta$ -carotene in maize grains, is very low. Therefore, the study of increasing the amount of provitamin A carotenoids including  $\beta$ -carotene is important. In maize, different alleles of *crtRB1* and *LcyE* genes have a significant effect on  $\beta$ -carotene content. In this paper, we present the results of the study of allele polymorphism of these two genes related to the provitamin A carotenoid content in some traditional maize accessions collected from several regions in North and Central Highlands of Vietnam. The results showed that there were polymorphisms at the 3' and 5' ends of the *crtRB1* and *LcyE* genes. Among 22 maize accessions, the proportion of favorable alleles at the 3' end of *crtRB1* gene was relatively high ( $5/22 = 22.73\%$ ). Similar results were obtained for alleles at the 3' end of the *LcyE* gene. Especially, there is an accession (Nep vang trang mien Bac - Northern white gold maize) that carries favorable alleles at the 3' ends of both *crtRB1* and *LcyE* genes. While all investigated maize accessions did not carry favorable alleles at the 5' end of both *crtRB1* and *LcyE* genes. The identification of traditional maize accessions that carry favorable alleles for increasing  $\beta$ -carotene content opens up potential to exploit indigenous genetic resources for genetic research as well as to develop maize varieties with high  $\beta$ - carotene content.

**Keywords:** *Zea mays* L., allelic polymorphism,  $\beta$ -carotene, *crtRB1* gene, *LcyE* gene, maize.

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## INTRODUCTION

Maize is the third most important food crop after wheat and rice and is consumed by more than a billion people worldwide. Besides, is also a food source for livestock, especially for poultry. Carotenoid content in maize seeds is higher than other cereal crops, but low and highly varied in maize lines. According to Harjes et al. (2008), most of the world's cultivated and consumed maize varieties contain only 0.5 to 1.5  $\mu\text{g/g}$   $\beta$ -carotene. Kurilich and Juvik (1999) used HPLC to analyze carotenoids in five sweet maize varieties, indicating that the  $\beta$ -carotene content ranged from 0.14 to 7.97  $\mu\text{g} / \text{g}$  dry weight.  $\beta$ -carotene is a precursor of vitamin A, which helps the body prevent vitamin A deficiency, preventing blindness, strengthening the immune system. Humans cannot synthesize vitamin A, so it takes nutrients from food sources (liver, fish, eggs and milk) containing vitamin A (retinol), and precursors of vitamin A from colored vegetables and fruit (carrot, papaya, pumpkin, red bell pepper, grapefruit) in the form of provitamin A carotenoids. In regions where maize is the main food source, the use of maize will lead to a deficiency of vitamin A. Vitamin A is important for eye health, protection of age-related macular degeneration, adjustment and improve the immune system and increase infection resistance (Ross, 1998; Semba, 2009, Huang et al., 2018). Vitamin A deficiency is a global health problem, making 140 to 250 million people at risk of many health problems (Harjes et al., 2008), which can lead to blindness and increase. illness as well as mortality in preschool children (WHO, 2010).

In maize, there are five genes that play an important role in the final content of provitamin A carotenoids. The first gene, *PSY1*, encodes phytoene synthase with two alleles related to the total carotenoid content (Fu et al., 2013a). The second gene, *LcyE*, encodes lycopene epsilon cyclase with four alleles, involved altering the ratio of different carotenoids in  $\alpha$ - to  $\beta$ - branches in the carotenoid biosynthesis pathway (Harjes et

al., 2008). *crtRB3* is the third gene coding for the enzyme  $\alpha$ -carotene hydroxylase and the fourth gene, *ZEP1*, controls zeaxanthin epoxidase; Both genes have been known to play a role in carotenoid metabolism (Vallabhaneni, Wurtzel, 2009; Zhou et al., 2012). The fifth gene encoding  $\beta$ -carotene hydroxylase enzyme (*crtRB1*) with three alleles has a significant impact on the change of  $\beta$ -carotene content in endosperm (Fu et al., 2013b). The results of Yan et al. (2010) showed the concentration of provitamin A of haplotypes with favorable alleles of *crtRB1*-5'TE and *crtRB1*-3'TE to be 5.2 times higher than all other haplotypes. Babu et al. (2013) reported that *crtRB1* had a much greater effect on provitamin A content than *LcyE*. The *crtRB1* gene is not inherited by Mendel law, while the *LcyE* gene is inherited by Mendel (Zunjare et al., (2017). The study of favorable alleles of the *LcyE* gene in 13 samples of indigenous and imported maize varieties, Zunjare et al. (2018) determined that there were 8 genotypes with favorable and 5 with unfavorable alleles of *LcyE* gene. Identifying indigenous traditional maize genotypes carrying favorable alleles for the increase in  $\beta$ -carotene content is important for varietal selection because in addition to increasing the content of  $\beta$ -carotene, indigenous traditional maize also provides additional tolerance genes and adaptation to native ecological conditions. However, the proportion of favorable alleles of *crtRB1* and *LcyE* genes is quite low and respectively 3.38% and 3.90% (Muthusamy et al (2015). Similar results were also reported in several studies, for example, in 210 investigated maize lines, Selvi et al. (2014) identified only one line had favorable allele of *crtRB1* gene.

In the previous published paper, we examined the frequency of favorable alleles for  $\beta$ -carotene accumulation in some improved and imported maize varieties in Vietnam (Tran Thi Luong, Nguyen Duc Thanh, 2018). In this paper, we present the results of allele polymorphism related to the  $\beta$ -carotene content of *crtRB1* and *LcyE* genes in local traditional maize accessions collected

from several regions in the North and the Central Highlands, with the aim of evaluating these alleles polymorphisms and identifying maize accessions with favorable alleles to exploit indigenous genetic resources as a raw material for selecting maize varieties with high  $\beta$ -carotene content.

## MATERIALS AND METHODS

### Materials

Twenty-two accessions of local traditional maize accessions from Northern and Central Highlands provinces were provided by the

Center for Plant Resources, Vietnam Academy of Science and Technology (table 1).

The alleles of the 3' end of *crtRB1* gene (*crtRB1*-3'TE) were analyzed by *crtRB1*-3'TE-F: 5'-ACACCACATGGACAAGTTCCG-3', *crtRB1*-3'TE-R1: 5'-ACACTCTGGCCCATGAACAC-3' and *crtRB1*-3'TE-R2: 5'-ACAGCAATACAGGGGACCAG-3' primers (Yan et al., 2010). While, the alleles of the 5' end (*crtRB1*-5'TE) were analyzed by *crtRB1*-5'TE-2F: 5'-TTAGAGCCTCGACCCTCTGTG-3' and *crtRB1*-5'TE-2R: 5'-AATCCCTTTCCATGTACGC-3' primers (Liu et al., 2015).

Table 1. Results of allelic polymorphism of *crtRB1* and *LcyE* genes by PCR with corresponding primers

No.	Maize accessions	Origins	<i>crtRB1</i> -3'TE-R1	<i>crtRB1</i> -3'TE-R2	<i>crtRB1</i> -5'TE	<i>LcyE</i> -3'TE	<i>LcyE</i> -5'TE
1	Te vang Lung chang 2	Thai Hoc, Nguyen Binh, Cao Bang	296 bp	<b>543 bp</b>	800 bp	100 bp	280 + 350 bp
2	Te vang Na Lung 1	Ca Thanh, Nguyen Binh, Cao Bang	296 bp	-	-	100 bp	280 bp
3	Bap cham luong	Nam Quang, Bao Lam, Cao Bang	296 bp	-	800 bp	100 bp	280 bp
4	Bap cham deng	Tien Thanh, Phuc Hoa, Cao Bang	296 bp	-	-	100 bp	280 bp
5	Bap cham deng	Nam Quang, Bao Lam, Cao Bang	296 bp	-	800 bp	<b>144 + 100 bp</b>	280 bp
6	Bap nua lai	Nam Quang, Bao Lam, Cao Bang	296 bp	-	800 bp	100 bp	280 + 350 bp
7	Bap cham	Nam Quang, Bao Lam, Cao Bang	296 bp	-	-	<b>144 + 100 bp</b>	280 bp
8	Ta vang Na Leng	Luong Ha, Na Ri, Bac Kan	296 bp	<b>543 bp</b>	800 bp	100 bp	280 + 350 bp
9	Te vang Lung can	Kim Hy, Na Ri, Bac Kan	296 bp	<b>543 bp</b>	-	100 bp	280 bp
10	Nep vang Dong Van	Đông Van, Ha Giang	296 bp	-	800 bp	100 bp	280 bp
11	Da nau vang Hoang Su Phi	Hoang Su Phi, Ha Giang	296 bp	-	800 bp	100 bp	280 + 350 bp
12	Nep vang Mai Chu	Mai Chau, Hoa Binh	296 bp	<b>543 bp</b>	800 bp	100 bp	280 bp
13	Te do Đa Bac	Đa Bac, Hoa Binh	296 bp	-	-	100 bp	-
14	Nep trang Le Loi	Le Loi, Sin Ho, Lai Chau	296 bp	-	800 bp	<b>144 + 100 bp</b>	280 + 350 bp
15	Nep vang trang Mien Bac	Mien Bac	296 bp	<b>543 bp</b>	800 bp	<b>144 + 100 bp</b>	280 + 350 bp
16	Nep vang Pleiku	TX Plei Ku, Gia Lai	296 bp	-	-	100 bp	280 bp
17	Da do chu se	Chu se, Gia Lai	296 bp	-	800 bp	<b>144 + 100 bp</b>	280 bp
18	Nep nau nhat Krong Pach	Krong Pach, Dac Lac	296 bp	-	800 bp	100 bp	280 bp
19	Da tim nau Krong Ana	Krong Ana, Dac Lac	296 bp	-	-	100 bp	280 bp
20	Da vang Krong Ana	Krong Ana, Dac Lac	296 bp	-	800 bp	100 bp	280 bp
21	Ngo vang Lac-Dac Lac	Lac, Dac Lac	296 bp	-	-	100 bp	280 bp
22	Ngo nau vang Lac-Dac Lac	Lac, Dac Lac	296 bp	-	800 bp	100 bp	280 bp

The alleles at the 3' end (LcyE-3'TE) and the 5' end (LcyE-5'TE) of *LcyE* gene were amplified by LcyE-3'TE-F: 5'-ACCCGTACGTCGTTTCATCTC-3', LcyE-3'TE-R: 5'-ACCCTGCGTGGTCTCAAC-3' (Azmach et al., 2013) and LcyE-5'TE-F: 5'-AAGCAGGGAGACATTCCAG-3', LcyE-5'TE-R: 5'-GAGAGGGAGACGACGAGACAC-3' primers (Babu et al., 2013), respectively.

## Methods

### *Amplification of alleles of the crtRB1 and LcyE genes by PCR*

Genome DNA was extracted according to CTAB method of Saghai Maroof et al., (1984). PCR reactions with crtRB1-3'TE-F, crtRB1-3'TE-R1 and crtRB1-3'TE-R2 primers were conducted as previously reported (Tran Thi Luong, Nguyen Duc Thanh, 2018).

PCR reactions with LcyE-3'TE-F, LcyE-3'TE-R and LcyE-5'TE-F, LcyE-5'TE-R primers were performed with a reaction cycle of: 94°C for 10 s, followed by 35 cycles (95°C for 10 s, 58°C for 35 s, and 72°C 10 s (Harjes et al., 2008). PCR products were electrophoresis on 1.5% agarose gel.

## RESULT

### *Allelic polymorphism of β-carotene hydroxylase gene (crtRB1)*

For *crtRB1* gene, allelic polymorphisms at the 3' end (crtRB1-3'TE) and the 5' end (crtRB1-5'TE) were analyzed. The 3'TE polymorphism of *crtRB1* produces 3 alleles related to variation in β-carotene content (Yan

et al., 2010): allele 1 (543 bp without TE insertion), allele 2 (296 bp + 875 bp, with 325 bp TE insertion) and allele 3 (296 bp + 1221 bp + 1880 bp; with the insertion of 1250 bp TE). Allele 1 is known as a favorable allele for the increase in β-carotene by reducing the expression of *crtRB1* gene transcription, while allele 2 and allele 3 are unfavorable for the increase in content of β-carotene. Our results show allelic polymorphism at the 3' end of *crtRB1* gene: out of 22 traditional maize accessions, there are 5 (22.73%) (Te vang Lung chang 2, Te vang Na Leng, Te vang Lung can, Nep vang Mai Chau, Nep vang trang Mien Bac) have favorable allele (543 bp) for the increase in β-carotene (table 1, Fig. 1), for the remaining accessions, no alleles were amplified. Thus, the proportion of investigated accessions that have allele 1 at the 3' end of *crtRB1* genes in traditional maize accessions is quite high compared to the claims of foreign authors (Thirusendura Selvi et al., 2014; Muthusamy et al., 2015; Sagare et al., 2015) and equivalent to those in the imported and improved maize varieties that we previously published (Tran Thi Luong, Nguyen Duc Thanh, 2018).

With the crtRB1-3'TE-F / R1 primer pair (Fig. 2), no favorable alleles were recorded in all investigated maize. There were 3 accessions (2, 13, 17) without allele amplification, 19 accessions with unfavorable allele 2 (296 bp), of which 2 accessions (3 and 8) have an insertion of 325 bp.

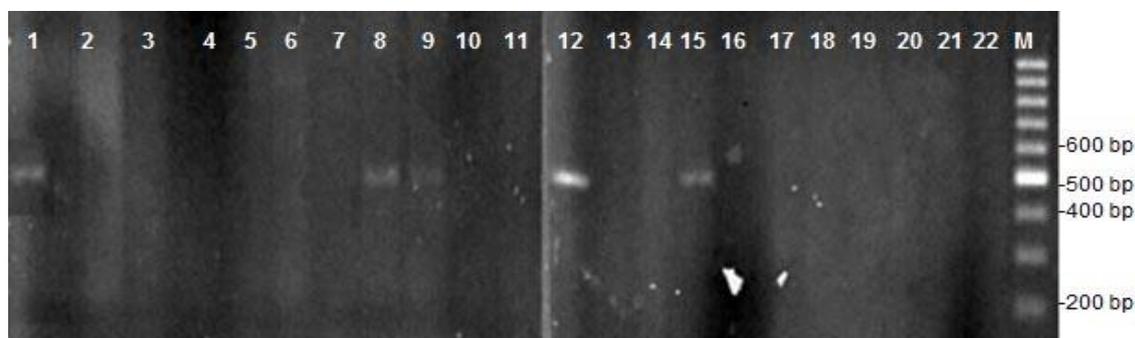


Figure 1. PCR results for alleles at the 3' end of *crtRB1* gene with crtRB1-3'TE-F/R2 primers. M. Marker 100 bp; 1–22 accession numbers as shown in table 1

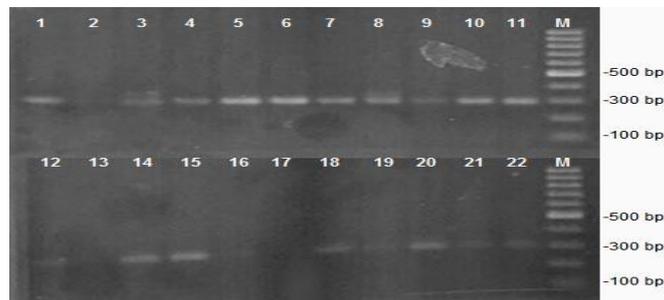


Figure 2. PCR results for alleles at the 3' end of *crtRB1* gene with *crtRB1*-3'TE-F/R1 primers. M. Marker 100 bp; 1–22 accession numbers as shown in table 1

Allelic polymorphism at the 5' end of *CrtRB1* gene is due to the change of 397/206 bp indel (Yan et al., 2010). Allele 2 (600 bp) is favorable allele. The analyses of 22 maize accessions showed that there was polymorphism among the accessions.

However, there were no allele-specific bands for favorable alleles. Fourteen accessions have allele 1 (800 bp) that is unfavorable (Fig. 3). The remaining accessions do not have specific allele.

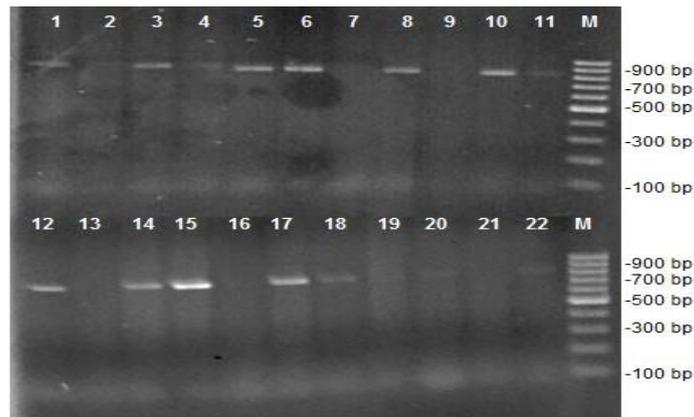


Figure 3. PCR results for alleles at the 5' end of *crtRB1* gene with *crtRB1*-5'TE-F/R1. M. Marker 100 bp; 1–22 accession numbers as shown in table 1

#### Allelic polymorphism of *Lycopene E* gene (*LcyE*)

According to Harjes et al. (2008), the 3' end of *LcyE* gene has 2 alleles: Allele1 (399 + 502 bp) and allele 2 that has 8 bp deletion (144 + 502 bp) affecting the content of  $\beta$ -carotene. When analyzing 22 maize accessions using *LcyE*-3'TE-F / R primers, 5 accessions (22.73%), including Bap cham deng, Bap cham, Nep trang Le Loi, Nep vang trang Mien Bac and Da do chu se possessed allele 2 (144 bp) affecting the content of  $\beta$ -carotene (Fig. 4). The remaining 17

accessions have a band of about 100 bp, this may be the altered allele 2 that lost 44 bp.

Allele polymorphism at the 5' end *LcyE*-5'TE was analyzed by *LcyE*-5'TE-F / R primers. With this pair of primers, 4 alleles can be amplified, in which allele1 (150 bp + 280 bp) and allele 4 (933 bp) are favorable for the accumulation of  $\beta$ -carotene, and allele 2 (250 bp) and allele 3 (250 bp + 380 bp) are unfavorable (Harjes et al., 2008).

The results in tables 1 and figure 5 show that in the 22 traditional maize accessions, there were polymorphisms among the

accessions, but there are no accessions that carry favorable alleles. There were 21 accessions having the band of about 280 bp, including 6 accessions that have the bands of 280 bp and 350 bp, this may be a variation in

allele 2 (250 to 280 bp) and allele 3 (250 + 380 bp to 280 + 350 bp). In one accession (13- Te Do, Da Bac), no alleles were amplified.

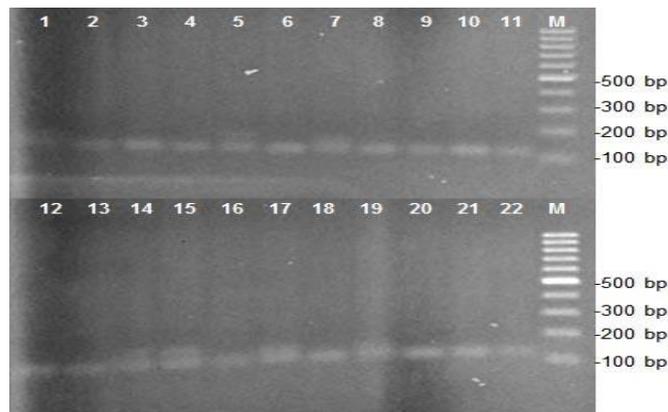


Figure 4. PCR results for alleles at the 3' end of *LcyE* with *LcyE*-3'TE-F/R primers. M: Marker 100 bp; 1–22 accession numbers as shown in table 1

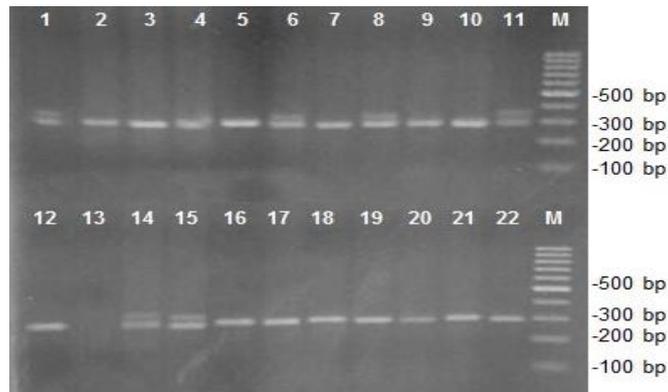


Figure 5. PCR results for alleles at the 5' end of *LcyE* with *LcyE*-5'TE-F/R primers. M: Marker 100 bp; accession numbers as shown in table 1

Thus, there were no accessions among investigated maize accessions that have favorable alleles for increasing the  $\beta$ -carotene at the 5' end of the *LcyE* gene, while there were 5 accessions have the favorable alleles at the 3' end of *LcyE*.

## CONCLUSION

The results of the study on allelic polymorphism related to the  $\beta$ -carotene content of *crtRB1* and *LcyE* genes in the group of 22 Vietnamese traditional maize

accessions show that there are alleles polymorphisms at the 3' and 5' ends of *crtRB1* and *LcyE* genes. The proportion of favorable alleles related to  $\beta$ -carotene levels at the 3' end of *crtRB1* is quite high (5/22 = 22.73%). Similar results were obtained for alleles at the 3' end (*LcyE*-3'TE) of the *LcyE* gene. The five accessions have favorable allele at the 3' end of *crtRB1* genes, including: Te vang Lung chang 2, Te vang Na Leng, Te vang Lung can, Nep vang Mai Chau, Nep vang trang Mien Bac, and the five

accessions: Bap cham deng, Bap cham, Nep trang Le Loi, Nep vang trang Mien Bac and Da do chu se possessed the favorable alleles at the 3' end of *LcyE5* gene. Interestingly, accession Nep vang trang mien Bac has favorable alleles at the 3' end of both *crtRB1* and *LcyE* genes. While all investigated accessions did not carry any favorable alleles at the 5' end of *crtRB1* and *LcyE* genes. The identification of local traditional maize accessions that carry favorable alleles related to  $\beta$ -carotene content opens up the potential of exploiting indigenous genetic resources for genetic research as well as the creation of maize varieties with high  $\beta$ -carotene content.

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