ALLELIC POLYMORPHISM OF \textit{crtRB1} AND \textit{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 \textit{crtRB1} and \textit{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 \textit{crtRB1} and \textit{LcyE} genes. Among 22 maize accessions, the proportion of favorable alleles at the 3' end of \textit{crtRB1} gene was relatively high (5/22 = 22.73\%). Similar results were obtained for alleles at the 3' end of the \textit{LcyE} gene. Especially, there is an accession (Nep vang tranh mien Bac - Northern white gold maize) that carries favorable alleles at the 3' ends of both \textit{crtRB1} and \textit{LcyE} genes. While all investigated maize accessions did not carry favorable alleles at the 5' end of both \textit{crtRB1} and \textit{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: \textit{Zea mays} L., allelic polymorphism, \(\beta\)-carotene, \textit{crtRB1} gene, \textit{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, it 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 μg/g β-carotene. Kurilich and Juvik (1999) used HPLC to analyze carotenoids in five sweet maize varieties, indicating that the β-carotene content ranged from 0.14 to 7.97 μg/g dry weight. β-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 α- to β-branches in the carotenoid biosynthesis pathway (Harjes et al., 2008). *crtRB3* is the third gene coding for the enzyme α-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 β-carotene hydroxylase enzyme (*crtRB1*) with three alleles has a significant impact on the change of β-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 β-carotene content is important for varietal selection because in addition to increasing the content of β-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 β-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 β-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 β-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’-ACACCACATGGCAAGTTCG-3’, *crtRB1*-3’TE-R1: 5’-ACACTCTGGCCCATGAACAC-3’ and *crtRB1*-3’TE-R2: 5’-ACAGCAATACGGGACCAG-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                  | *crtRB1*-3’TE-R1 | *crtRB1*-3’TE-R2 | *crtRB1*-5’TE | *LcyE-3’TE | *LcyE-5’TE |
|-----|------------------------|--------------------------|------------------|------------------|--------------|------------|------------|
| 1   | Te vang Lung chang 2   | Thai Hoc, Nguyen Binh, Cao Bang | 296 bp           | 543 bp           | 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           | -                | 100 bp      | 280 bp     |
| 4   | Bap cham deng          | Tien Thanh, Phuc Hou, Cao Bang | 296 bp           | -                | 100 bp      | 280 bp     |
| 5   | Bap cham deng          | Nam Quang, Bao Lam, Cao Bang | 296 bp           | -                | 100 bp      | 280 bp     |
| 6   | Bap nau lai            | Nam Quang, Bao Lam, Cao Bang | 296 bp           | -                | 100 bp      | 280 + 350 bp |
| 7   | Bap cham               | Nam Quang, Bao Lam, Cao Bang | 296 bp           | -                | 100 bp      | 280 + 350 bp |
| 8   | Ta vang Na Leng        | Luong Ha, Na Ri, Bac Kan  | 296 bp           | 543 bp           | 800 bp      | 100 bp     | 280 + 350 bp |
| 9   | Te vang Lung can       | Kim Hy, Na Ri, Bac Kan   | 296 bp           | 543 bp           | -           | 100 bp     | 280 bp     |
| 10  | Nep vang Dong Van      | Dong Van, Ha Giang       | 296 bp           | 543 bp           | -           | 100 bp     | 280 bp     |
| 11  | Da nau vang Hoang Su Phi | Hoang Su Phi, Ha Giang | 296 bp           | 543 bp           | -           | 100 bp     | 280 + 350 bp |
| 12  | Nep vang Mat Chu       | Mac Chau, Hoa Binh      | 296 bp           | 543 bp           | -           | 100 bp     | 280 + 350 bp |
| 13  | Te do Da Bac           | Da Bac, Hoa Binh        | 296 bp           | -                | 100 bp      | 280 + 350 bp |
| 14  | Nep trang Le Lai       | Le Loi, Sin Ho, Lai Chau | 296 bp           | -                | 100 bp      | 280 + 350 bp |
| 15  | Nep vang trang Mien Bac | Mien Bac               | 296 bp           | 543 bp           | 800 bp      | 144 + 100 bp | 280 + 350 bp |
| 16  | Nep vang Pleiku        | TX Ploi Ku, Gia Lai     | 296 bp           | -                | 100 bp      | 280 bp     |
| 17  | Da do chu se           | Chu se, Gia Lai         | 296 bp           | -                | 100 bp      | 280 + 350 bp |
| 18  | Nep nau nhat Krong Pach| Krong Pach, Dac Lac      | 296 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           | -                | 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           | -                | 100 bp      | 280 bp     |
The alleles at the 3’ end (LcyE-TE) and the 5’ end (LcyE-TE) of LcyE gene were amplified by LcyE-TE-F: 5’-ACCCGTACGCTGTTCATCTC-3’ (Azmach et al., 2013) and LcyE-TE-F: 5’-AAGCAGGGAGACATTCCAG-3’, LcyE-TE-R: 5’-GAGAGGGAGACGACGACAC-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-TE-F, crtRB1-TE-R1 and crtRB1-TE-R2 primers were conducted as previously reported (Tran Thi Luong, Nguyen Duc Thanh, 2018).

PCR reactions with LcyE-TE-F, LcyE-TE-R and LcyE-TE 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-TE) and the 5’ end (crtRB1-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-TE-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.
Allelic polymorphism of **crtRB1** and **LcyE** genes

**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 β-carotene. When analyzing 22 maize accessions using **LcyE-3’TE-F / R** primers, 5 accessions (22.73%), including Bap cham dung, Bap cham, Nep trang Le Lòi, Nep vang trang Mien Bac and Da do chu se possessed allele 2 (144 bp) affecting the content of β-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 β-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.

Thus, there were no accessions among investigated maize accessions that have favorable alleles for increasing the β-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 β-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 β-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 β-carotene content opens up the potential of exploiting indigenous genetic resources for genetic research as well as the creation of maize varieties with high β-carotene content.

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