Sequence Analysis of the Carotenoid Isomerase Gene in Potato (Solanum tuberosum)

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Abstract. Carotenoid Isomerase (CRTISO) is an important enzyme in carotenoid biosynthesis. Here, the Solanum tuberosum CRTISO (StCRTISO) gene sequences were obtained from Spud DB database, and preformed for sequence analysis. The StCRTISO gene mapped to chromosomes 10, and contains an open reading frame of 1,848 bp that encodes a 615-amino acid protein with a calculated molecular mass of 67.52 kD and an isoelectric point (pI) of 6.92. Subcellular localization predicted the StCRTISO gene was in the cytoplasm. The conserved domain of the StCRTISO protein is Rossmann-fold NAD(P)H/NAD(P)(+) binding (NADB) domain. The StCRTISO protein is most closely related to Solanum lycopersicum. The findings of the present study provide a molecular basis for the elucidation of CRTISO gene function in potato.

1. Introduction
Potato (Solanum tuberosum) is ranked as the third most important food crop in the world. Potato is not only of importance as a food crop, and also one of the major crops grown for starch production [1]. It yields a high-energy output per hectare, and is a rich source of nutrients, including carbohydrates, and carotenoids [1-2].

The enzymes involved in the carotenoid biosynthetic pathway have been extensively studied in various plants, including Arabidopsis [3], tomato [4], and citrus [5]. The first key step in carotenoid biosynthesis involves the production of a 40-carbon phytoene from two geranylgeranyl pyrophosphate (GGPP) molecules, which is catalyzed by phytoene synthase (PSY) [6-7]. Then, lycopene (colored carotenoid) is converted from phytoene (non-color carotenoid) by desaturases and isomerases, including phytoene desaturases (PDS) [8], ζ-carotene desaturase (ZDS) [9], 15-cis-ζ-carotene isomerase (Z-ISO) [10], and carotenoid isomerase (CRTISO) [3]. Hereafter, bifurcation of the carotenoid biosynthetic pathway occurs, and the production of β-carotene and α-carotene is catalyzed by lycopene β-cyclase (β-LCY) and lycopene ε-cyclase (ε-LCY) [11-12].

CRTISO is an important enzyme in carotenoid biosynthesis, catalyzing the prolycopene into lycopene [13]. The genes encoding the CRTISO protein have been isolated in various plant species, including Arabidopsis [3], tomato [4], tobacco [14], and Brassica rapa [15]. To date, research studies on CRTISO in potato are limited. In the present study, the CRTISO gene sequence of potato was obtained from web database, and then sequence analysis of the CRTISO gene was analyzed. The
The present study aimed to establish the foundation for further studies on the molecular mechanism of CRTISO in potato.

2. Materials and methods

2.1. Sequence Obtain of the StCRTISO Gene
The genomic DNA and mRNA sequences of CRTISO gene of potato were downloaded and obtained from The Spud DB database (http://solanaceae.plantbiology.msu.edu), and then used to subsequent bioinformatic analysis.

2.2. Sequence Analysis of the StCRTISO Gene
The amino acid sequence, protein molecular weight, isoelectric point, stability index, and hydrophobicity of the StCRTISO gene were analyzed and predicted by ExPASy (http://web.expasy.org) and NCBI (https://www.ncbi.nlm.nih.gov/). Subcellular localization was predicted by WoLF PSORT (http://www.genscript.com/wolf-psort.html). The conserved domain were predicted by NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Homology analysis of the CRTISO proteins was executed in DNAMAN.

3. Results

3.1. Analysis on genomic organization
The Spud DB database was used to analyze the chromosomal localization and genomic organization of StCRTISO. The gene ID in Spud DB database is PGSC0003DMT400072543. The StCRTISO gene was mapped to chromosomes 10 and has 13 exons and 12 introns (Fig. 1).

![Chromosomal location and genomic structure of StCRTISO.](image)

3.2. Protein physical and chemical properties analysis
Sequence analysis indicated that the StCRTISO gene contained a 1,848-bp open reading frame (ORF), which encoded a 615-amino acids protein with a calculated molecular mass of 67.52 kD and an isoelectric point (pI) of 6.92. The amino acid types and proportions of the StCRTISO gene was shown in Figure 2, the highest number of amino acid is Leucine (Leu), whereas the lowest number is Tryptophan (Trp). Its predicted formula was C_{3039}H_{4769}N_{803}O_{891}S_{22}. Its total average hydrophilicity index was -0.094, liposoluble index was 91.14. There is no transmembrane structure in StCRTISO.
3.3. Subcellular localization and conserved domain analysis
Subcellular localization of the StCRTISO gene was predicted by WoLF PSORT to be in the cytoplasm. The analysis using Conserved Domain Database (CDD) demonstrated that the amino acid sequence of the StPCRTISO protein has one NADB Rossmann superfamily that share a Rossmann-fold NAD(P)H/NAD(P)(+) binding (NADB) domain (Fig. 3).

3.4. Homology analysis
Homology analysis demonstrated that the amino acid sequence of the StCRTISO protein shared high homology with those of 17 other higher plant species (Table 1). Table 1 shows that the StCRTISO had the highest identities (> 90%) with several CRTISO proteins of Solanaceae such as Solanum lycopersicum, Capsicum chinense, Lycium barbarum, and all of the levels of identity were > 75% with other species cited in our study, indicating that the CRTISO protein is highly conserved among different species.
Table 1. The homology comparison among amino acid sequences of CRTISO from plant species

| Plant species            | Protein name | GenBank accession No. | Identity with StCRTISO (%) |
|--------------------------|--------------|-----------------------|----------------------------|
| Solanum lycopersicum     | SlCRTISO     | NP_001296161.1        | 98                         |
| Capsicum chinense        | CcCRTISO     | PHU05741.1            | 94                         |
| Capsicum baccatum        | CbCRTISO     | PHT36936.1            | 94                         |
| Lycium barbarum          | LbCRTISO     | AIX87497.1            | 93                         |
| Lycium ruthenicum        | LrCRTISO     | AIX87521.1            | 93                         |
| Lycium chinense          | LcCRTISO     | AIZ50714.1            | 93                         |
| Prunus persica           | PpCRTISO     | XP_020423714.1        | 85                         |
| Hevea brasiliensis       | HbCRTISO     | XP_021667983.1        | 85                         |
| Citrus maxima            | CmCRTISO     | AJT59423.1            | 85                         |
| Citrus clementine        | CcCRTISO     | XP_006430007.1        | 85                         |
| Vitis vinifera           | VvCRTISO     | XP_002269554.1        | 84                         |
| Citrus unshiu            | CuCRTISO     | GAY32839.1            | 84                         |
| Theobroma cacao          | TcCRTISO     | EOY08564.1            | 83                         |
| Prunus avium             | PaCRTISO     | XP_021804866.1        | 82                         |
| Populus trichocarpa      | PtCRTISO     | PNS98232.1            | 79                         |
| Populus tomentosa        | PtCRTISO     | APR64120.1            | 79                         |
| Spinacia oleracea        | SoCRTISO     | XP_021858982.1        | 77                         |

4. Conclusion
The present study analyzed the StCRTISO gene of potato. It is reported the identification of a CRTISO as the candidate gene for orange head by high-resolution genetic mapping using F2S4 population [15]. Loss of BrCRTISO function, upregulation of the upstream genes, and downregulation of downstream genes lead to the accumulation of prolycopene and confer an orange color to the inner head leaves in Chinese cabbage [16]. Previous studies have shown that the CRTISO protein is relatively conserved in plants. The CRTISO protein of N. tabacum is similar to the CRTISO protein of tomato and potato, showing 93% and 93% homology [14]. The findings of the present study show that CRTISO from potato is highly conserved in plants, similar to that observed in earlier reports. The findings of the present study may serve as a foundation for future studies on the functions of StCRTISO in carotenoid metabolism in potato.

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