Kiwiberries, a better version of Kiwifruit (Kiwifruit 2.0): Understanding the carotenoid biosynthesis pathway in orange-fleshed Kiwiberries

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CAROTENOIDS AND WHY STUDY THEM?

BENEFITS IN PLANTS

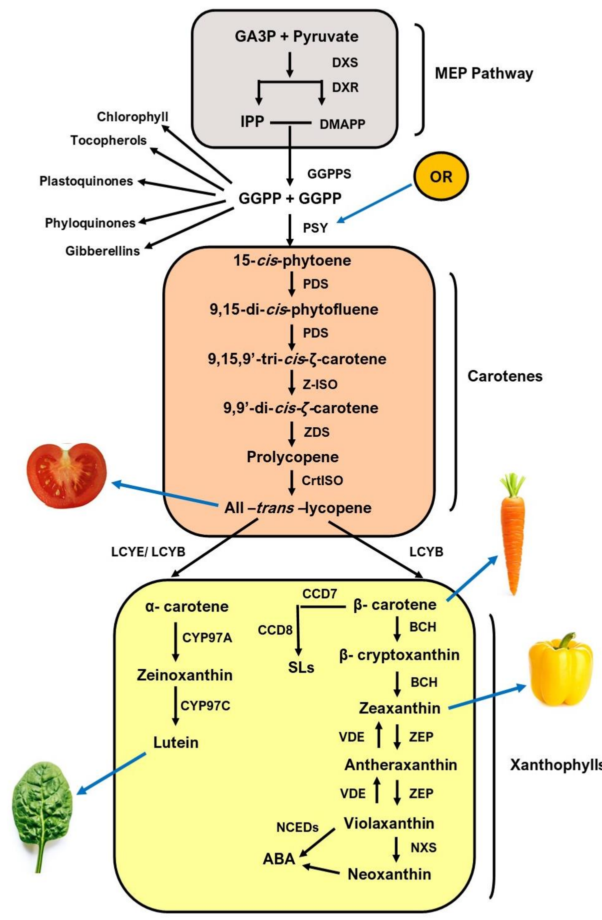
- Carotenoids are colourful pigments (secondary metabolites) synthesized in plants and other photosynthetic organisms to conduct several biological functions.
- Their uniqueness is defined by their C40 polyene backbone along with the conjugated double bonds that lead to their orange, yellow and red colour. Therefore, they act as colour pigments in flowers and fruits playing an important role pollination and seed dispersal, respectively.
- Carotenoids also act as precursors to abscisic acid (ABA) and strigolactones (SL)~ plant hormones responsible for plant growth and development
- They also protect the photosynthetic unit form photo-oxidative damage due to excess light. Therefore, promoting plant adaptability in changing abiotic conditions.

BENEFITS TO HUMAN HEALTH

- Provitamin A (β-carotene) provides yellow and orange colours to fruits and vegetables and is responsible for maintaining vision and overall eye health as provitamin A is a precursor retinoic acid and 11-cis-retinal (both are essential for vision).
- Lutein and zeaxanthin, both yellow-coloured pigments, are known as macular pigments due to their availability in macular part of retina and are responsible for retinal protection from blue light. Moreover, they reduce the risk of age-related macular degeneration (AMD).
- Some studies suggests the role of lycopene in reducing the risk of prostate cancer and in improving cardiovascular health.
- Carotenoids primarily β-carotene, lutein, lycopene are associated with better immune response, thereby, enhancing immunity.

Due to their numerous health benefits, understanding the carotenoid biosynthesis pathway in different plant species has been gaining momentum with the goal to enhance their bioavailability in food crops.

CAROTENOID BIOSYNTHESIS IN PLANTS



- The pathway is well conserved in all plant species
- The biosynthesis pathway genes have been identified in model plant species.
- The first substrate of the pathway i.e., isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are derived from MEP (methylerythritol-4-phosphate) pathway.
- Geranylgeranyl diphosphate (GGPP) is a substrate for other isoprenoids; therefore, Phytoene synthase (PSY) activity is critical in diverting the flux towards carotenoid biosynthesis.
- PSY catalyzes the rate limiting step of the pathway in most plants and is therefore targeted to produce crops with higher carotenoid concentration. For example, golden rice (Datta et al., 2003).
- Lycopene cyclization bifurcates the pathway giving rise to coloured xanthophylls.

Carotenoid concentration depends on 3 factors:

1. **Their synthesis**
2. **Their degradation**
3. **Their localization (localized and synthesized in plastids called chromoplast)**

WHY KIWIBERRIES?

Species belonging to the genus Actinidia exhibit range of fruit flesh and skin colours (green, red, orange, yellow and purple) (see figure) and have the potential to be commercialized or used as a genetic resource for breeding new cultivars with novel characteristics (fruit size, shape, flavour, storage, flesh colour and high health benefits).

- Kiwifruit (belonging to genus Actinidia) dominates the New Zealand horticultural export market and has consistently been the most exported crop since 2016 with the exports of 2020 valued at $2.53 billion (FreshFacts, 2020)
- The most popular Kiwifruit cultivar are *A. chinensis var deliciosa* (green-fleshed) and *A. chinensis var. chinensis* (golden-fleshed) due to their high anti-oxidant content and Vitamin C content.
- Kiwiberries belong to the genus Actinidia along with Kiwifruit. However, both the species have numerous morphological differences.
- Kiwiberries are small grape sized fruit with hairless edible skin. 'Hortgem Tahi' is one cultivar that has been commercialized recently.
- Kiwiberries with high carotenoid concentration can be used to expand our understanding of the biosynthesis pathway with an aim to breed Kiwifruit varieties with novel colours and enhanced health benefits.

OBJECTIVE 1

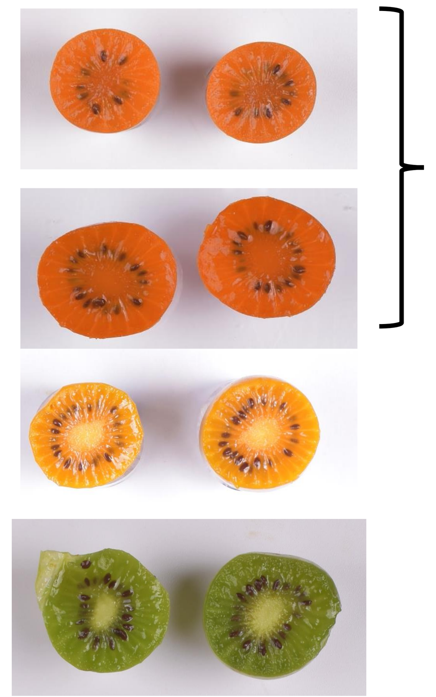
To identify the carotenoid biosynthesis pathway genes that may be regulating the high carotenoid concentration in yellow, and orange fleshed samples

METHODOLOGY

Nucleotide and predicted protein sequence analysis of the pathway and carotenoid candidate genes between the 3 Actinidia spp. **AND** their expression analysis

Quantification of the flesh colour of the three species

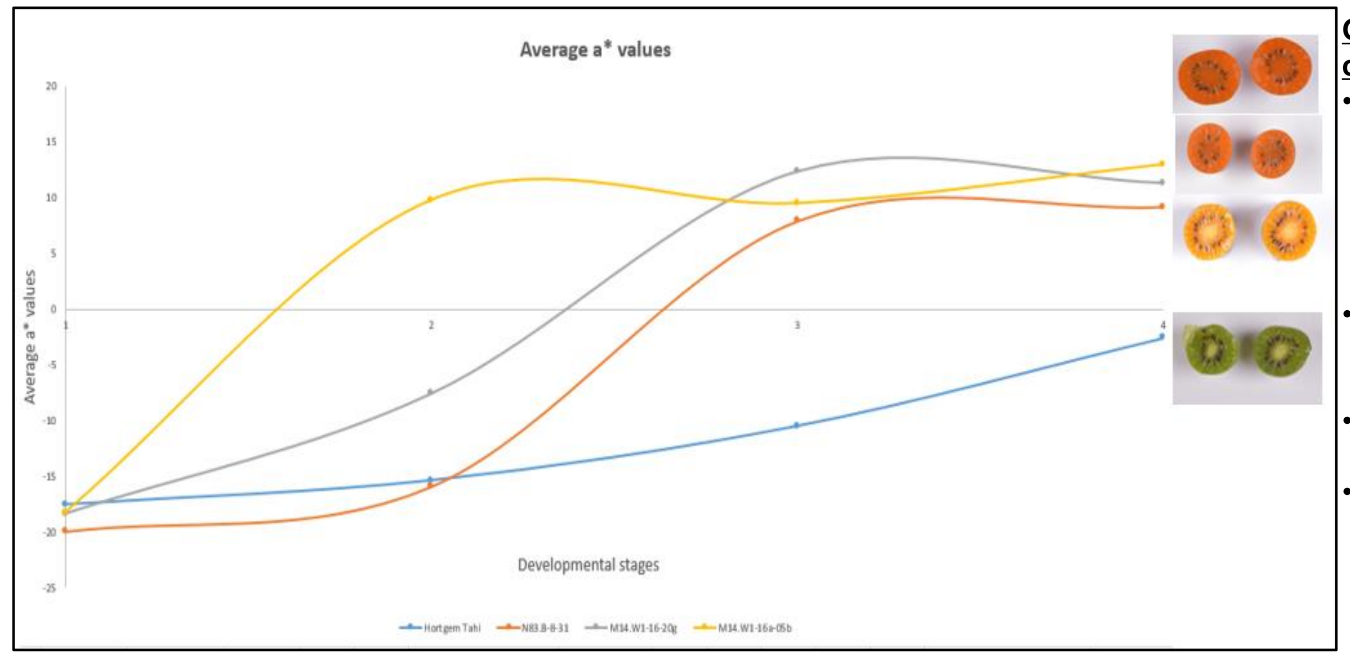
Metabolic analysis (quantification of the total carotenoids in the fruit skin and flesh; identify the predominant carotenoid present between the species)



Actinidia valvata
- Orange fleshed
- Tetraploid

Actinidia polygama
- Yellow fleshed
- Diploid

Actinidia arguta (Hortgem Tahi)
- Green fleshed
- Tetraploid



Quantification of fruit flesh colour

- Difference in the flesh was observed between the 3 species across the four developmental stages (70DAFB, 95DAFB, 120DAFB, and 150DAFB)
- The data consists of L*a*b* colour space and L*C*H* colour space
- a* values indicate red colour (+) and green colour (-).
- b* values indicate yellow (+) and blue (-) and were positive throughout the fruit development for all the species

AIM

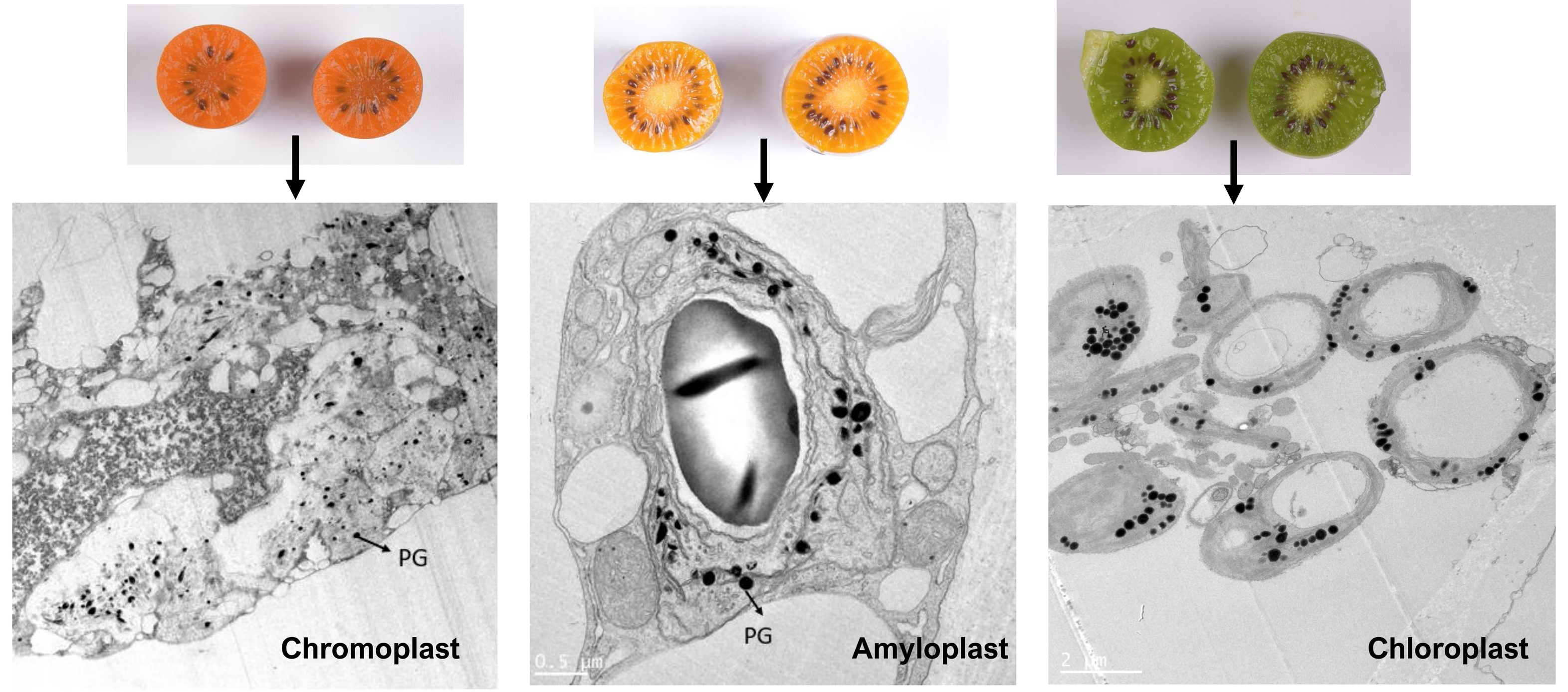
To understand the genetic mechanism that controls the carotenoid derived orange flesh phenotype in Actinidia spp.

OBJECTIVE 2

To understand the difference between the plastid type between the three Actinidia spp.

METHODOLOGY

Transmission electron microscopy (TEM) was used to visualize the ultrastructure and type of plastid present in the mature fruit flesh



OBJECTIVE 3

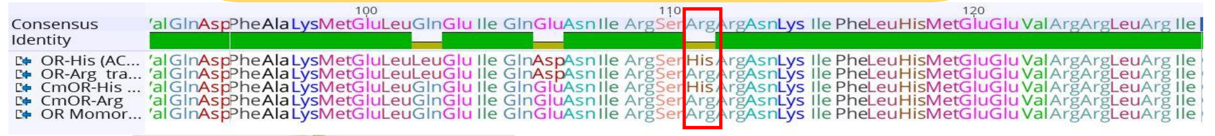
To determine the role of OR gene in regulating the carotenoid biosynthesis pathway and in chromoplast differentiation

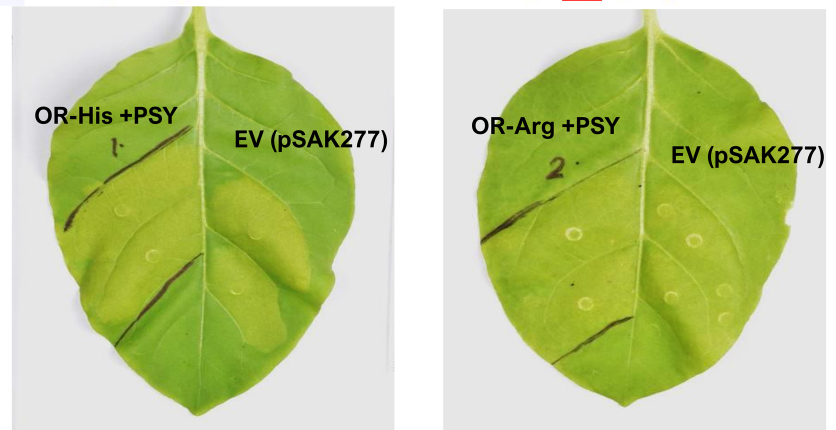
WHY ORANGE GENE?

- Orange gene stabilizes PSY gene via holdase chaperone activity thereby increasing carotenoid concentration.
- 'Golden SNP', found in melon (Arg[108]→His) leads to β-carotene accumulation
- OR-His regulates chromoplast biogenesis and number by interacting with ARC3 and interfering with PARC6-ARC3 interaction

METHODOLOGY

- Identification and cloning of OR gene from the three Actinidia species to understand the differences in the encoded protein and its functionality.
- Transient expression of OR-His (*A. chinensis*) and OR-Arg (A. macrosperma) in *Nicotiana tabacum* and stable transformation of *A. chinensis*





The leaves infiltrated with OR-His in combination with PSY and SGR (Stay green) constructs exhibited changes in the leaf pigmentation. Metabolic analysis will reveal the changes in carotenoid concentration

CONCLUSION

- Biosynthesis and accumulation of carotenoids occur in specialized organelles known as plastids. The structural variations in chromoplast have been associated with differential accumulation of carotenoid and their composition in *Capsicum annuum* fruit (Kilcrease et al., 2013).
- Presence of different type of plastid in different Actinidia species i.e., *A. valvata* (chromoplast), *A. polygama* (amyloplast), and *A. arguta* (chloroplast) studied may influence the carotenoid concentration and composition.
- Phytoene synthase catalyzes the rate-limiting step of the biosynthesis pathway and is post transcriptionally regulated by OR gene via physical interaction and is stabilized. Thereby, increasing carotenoid concentration (Park et al., 2016).
- OR gene isolated from *A. chinensis* (containing Histidine residue in the protein sequence instead of Arginine residue) when transiently expressed in *N. tabacum* leaves along with PSY and SGR influenced the leaf pigmentation whereas OR-Arg (cloned from *A. macrosperma*) did not change the phenotype. Metabolic analysis of the infiltrated patches will reveal the changes in the carotenoid concentration.
- OR protein also influences the chromoplast number and chromoplast biogenesis thereby influencing the carotenoid accumulation. The different type of plastids between the orange, yellow and green fleshed species may be influenced the OR-His protein.
- Potential role of SGR (Stay green) gene: SGR2 has been shown to activate chlorophyll degradation in Kiwifruit (Pilkington et al., 2012). It interacts with SlPSY1 and plays a regulatory role in tomato fruit colouration (Luo et al., 2013).
- The results obtained in future can be utilized to breed Kiwifruit varieties with improved quality, enhanced health benefits, and novel colours

WHAT NEXT?

- Nucleotide and Protein sequence analysis of the biosynthesis pathway genes along with OR and SGR between the three Actinidia spp. to identify any variations (indels, SNPs) between the species and cloning the candidates for functional analysis
- Expression analysis of the pathway genes to correlate the changes in the transcript levels to the carotenoid concentration
- Metabolic analysis (HPLC) to identify the pre-dominant carotenoid present in the three genotypes.
- Correlation analysis: to correlate the flesh colour with the carotenoid concentration.
- Stable transformation of A. chinensis lines via overexpression of OR-His and OR-Arg genes.

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