NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Preparatory Reading for
To Pick a Peck of Orange Peppers:
From Molecular Variation to Macroscopic Change

by
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An Introduction to the Many Colors of Bell Peppers

Bell peppers *(Capsicum annuum)* are commonly found in supermarkets, grocery stores and farmers' markets across North America. Also known as sweet peppers, capsicum, or simply "peppers," these popular vegetables come in many cultivated varieties (or cultivars), all derived from the wild chili pepper *Capsicum annuum* glaubriusculum (Kraft et al., 2014).

In botany, a variety is a group of individuals of a given species that have some superficial distinguishing characteristics in common, not unlike an animal breed. Bell pepper varieties may differ from each other in their productivity, ability to withstand particular environmental conditions, morphology, and of course in fruit shape, size, flavor, texture, or color.

The wild-type color for ripe peppers is bright red. However, there are numerous varieties with different fruit colors such as white, purple, brown, green, and several shades of yellow and orange (Figure 1). In this reading, we are going to take a closer look at what determines fruit color in bell peppers.

Fruit Ripening Process

Although they are considered vegetables, bell peppers are actually the fruits of the bell pepper plant. Initially, bell peppers are small and green in color; as they develop and mature, they grow larger, but remain green. Then, through the ripening process, the fruits build up carotenoid pigments while degrading the chlorophyll that was responsible for their green ("unripe") color.

Figure 1. Bell peppers of different colors, ©Laurie Hayball | Dreamstime.com ID 10582183.

The degradation of chlorophyll and the biosynthesis of carotenoids are complex multi-step processes that involve many biochemical reactions. Each of these biochemical reactions is catalyzed by enzymes, and lack or malfunction of any of these enzymes has the potential to affect the final fruit color. Indeed, several pepper varieties with non-red fruits have mutations in genes that encode proteins involved in chlorophyll degradation (Barry et al., 2008; Borovsky & Paran, 2008) or carotenoid biosynthesis (reviewed in Yuan et al., 2015). Let us look in more detail at these two processes: chlorophyll degradation and carotenoid biosynthesis.

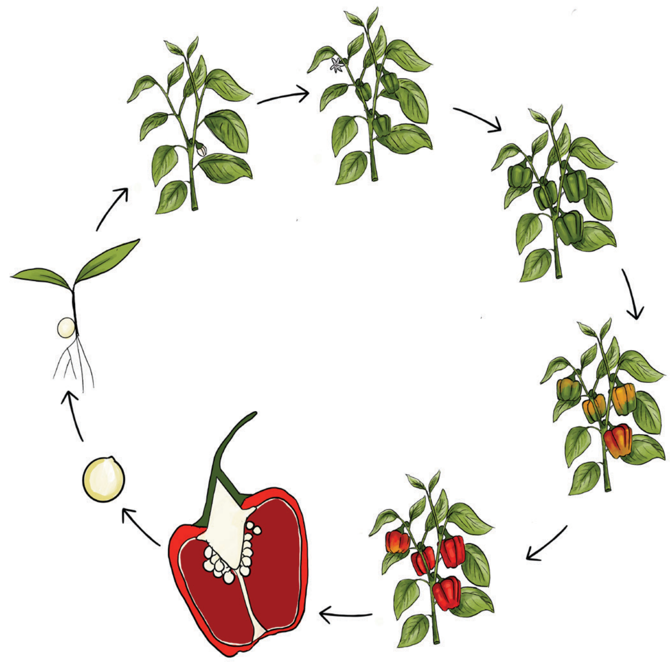

Figure 2. A schematic of the growth and ripening process in red bell peppers.

Degradation of Chlorophyll

A fruit's color is determined by the mixture of pigments present in its cells. If the majority of the pigments in a ripe bell pepper are red, then the fruit appears red; if we have a mixture of red and green the fruit will appear somewhat purple or brown, depending on the relative proportions of the red and green pigments. Interestingly, a mixture of yellow and green pigments generally results in a green, or light green, appearance. With this in mind, when we see a bright red pepper, we can infer that red pigments have been synthesized, and that the chlorophyll (green pigment) once present in its cells has all been broken down and turned into colorless compounds. How does this happen?

The SGR (for STAY-GREEN) protein is one of many players involved in chlorophyll degradation. Although researchers have not yet been able to pinpoint its precise role, we do know that its action is essential for yellowing of leaves and ripening of fruits. Plants with defects in the gene encoding the SGR protein display phenotypes characterized by leaves that do not turn yellow with age, fruits that retain their chlorophyll during ripening, or other plant parts that remain green when they normally should not. Two twenty-first century papers, by Amsted and colleagues (2007) and by Sato and colleagues (2007) respectively, found that Gregor Mendel's green mutant peas have a mutation in the gene encoding the peas' SGR protein!

The bell pepper version of SGR is known as CaSGR (for *Capsicum annuum* STAY-GREEN), and its function is essential to wild-type fruit color. Peppers with defective alleles of *CaSGR* cannot degrade chlorophyll.

Biosynthesis of Carotenoids

The carotenoid biosynthesis pathway in *C. annuum* has been a topic of research for several decades and we now have a relatively complete picture of the enzymes involved and of the reactions that they each catalyze. Figure 3 (see next page) summarizes the (known) steps in the synthesis of carotenoids in peppers. For each reaction (represented by an arrow), the enzyme responsible for catalyzing it is indicated next to it, and the respective colors of the compounds are listed in the box.

If an enzyme is missing, the corresponding step of the pathway cannot take place, resulting in a build up of the compound upstream of the step and a lack of the compound downstream. For example, if phytoene desaturase is completely missing, the pepper will accumulate phytoene and not have any Z-carotene. As a result, all the compounds made from Z-carotene will also be absent.

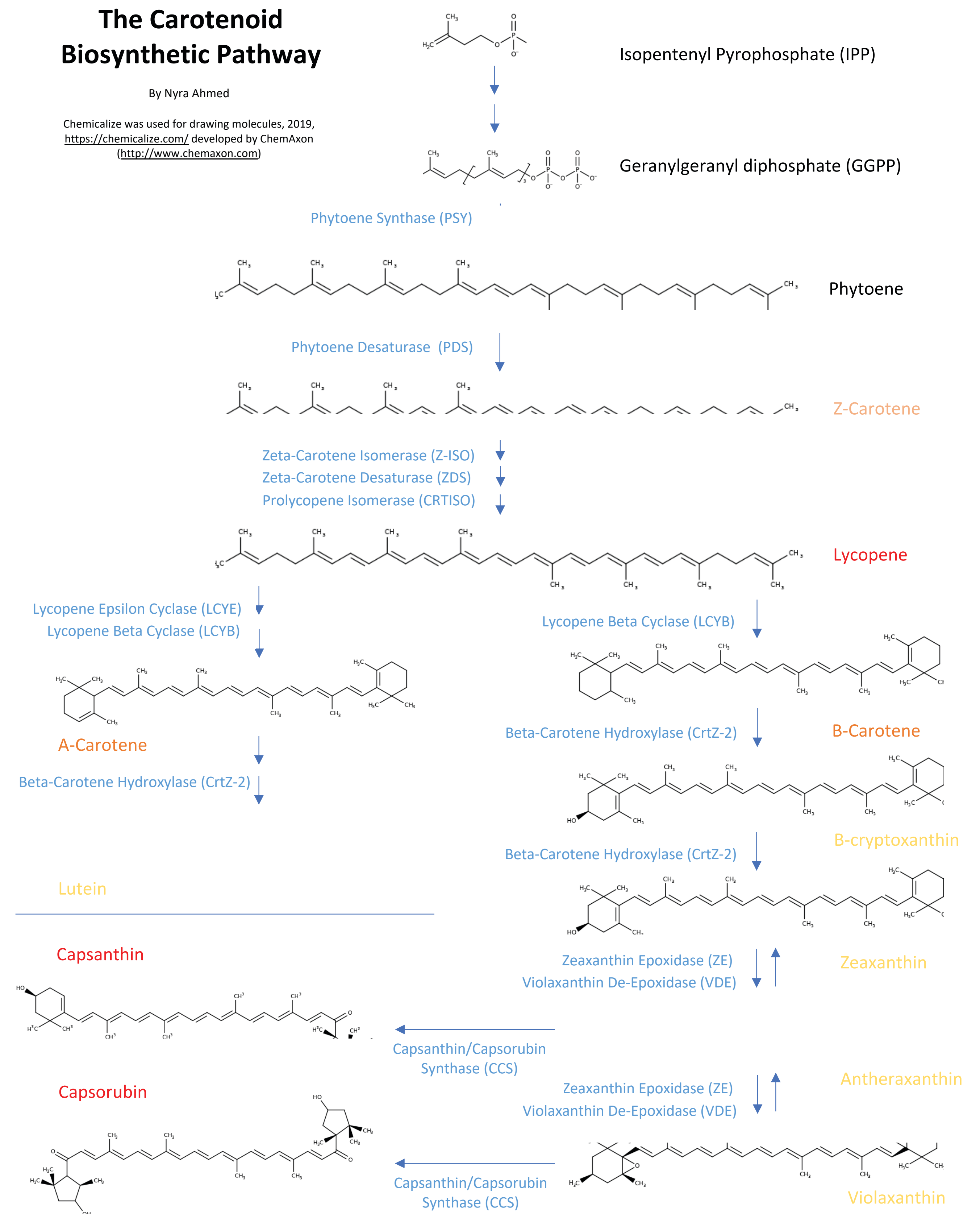

Figure 3. Known steps of the carotenoid biosynthesis pathway in bell peppers *(Capsicum annuum).* Ripe, wild-type peppers are rich in capsanthin and capsorubin. (Figure available from https://oercommons.s3.amazonaws.com/media/courseware/relatedresource/file/Carotenoid_Biosynthetic_Pathway-2_U8trfou.pdf.)

References

Armstead, I., I. Donnison, S. Aubry, J. Harper, S. Hortensteiner, C. James, J. Mani, M. Moffet, H. Ougham, L. Roberts, et al. (2007). Cross-species identification of Mendel's *I* locus. *Science* 315(5808): 73. <https://doi.org/10.1126/science.1132912>

Barry, C.S., R.P. McQuinn, M.-Y. Chung, A. Besuden, & J.J. Giovannoni. (2008). Amino acid substitutions in homologs of the STAY-GREEN protein are responsible for the green-flesh and chlorophyll retainer mutations of tomato and pepper. *Plant Physiology* 147(1): 179–87. <https://doi.org/10.1104/pp.108.118430>

Borovsky, Y. & I. Paran. (2008). Chlorophyll breakdown during pepper fruit ripening in the chlorophyll retainer mutation is impaired at the homolog of the senescence-inducible stay-green gene. *Theoretical and Applied Genetics* 117: 235–40. <https://doi.org/10.1007/s00122-008-0768-5>

Kraft, K.H., C.H. Brown, G.P. Nabhan, E. Luedeling, J. de Jesús Luna Ruiz, G. Coppens d'Eeckenbrugge, R.J. Hijmans, & P. Gepts. (2014). Multiple lines of evidence for the origin of domesticated chili pepper, *Capsicum annuum*, in Mexico. *Proceedings of the National Academy of Sciences* 111(17): 6165–70. <https://doi.org/10.1073/pnas.1308933111>

Sato, Y., R. Morita, M. Nishimura, H. Yamaguchi, & M. Kusaba. (2007). Mendel's green cotyledon gene encodes a positive regulator of the chlorophyll-degrading pathway. *Proceedings of the National Academy of Sciences* 104 (35): 14169–74. <https://doi.org/10.1073/pnas.0705521104>

Yuan, H., J. Zhang, D. Nageswaran, & L. Li. (2015). Carotenoid metabolism and regulation in horticultural crops. *Horticulture Research* 2: 15036. <https://doi.org/10.1038/hortres.2015.36>

Further Reading

The following articles and excerpts from the Nature Education Scitable website <https://www.nature.com/scitable> provide adequate background on the fundamental concepts in transcription, translation, mutation, their relationships to phenotype, as well as some key genetic terminology that, combined with the information in this document, should be sufficient background to complete the Preparatory Quiz.

Clancy, S. (2008). DNA transcription. *Nature Education* 1(1): 41. <https://www.nature.com/scitable/topicpage/dna-transcription-426>

Clancy, S. & Brown, W. (2008). Translation: DNA to mRNA to protein. *Nature Education* 1(1): 101. <https://www.nature.com/scitable/topicpage/translation-dna-to-mrna-to-protein-393/>

Clancy, S. (2008). Genetic mutation. *Nature Education* 1(1): 187. <https://www.nature.com/scitable/topicpage/genetic-mutation-441>

"Frameshift mutation/frame-shift mutation; frameshift." (Definition—Scitable Glossary). <https://www.nature.com/scitable/definition/frameshift-mutation-frame-shift-mutation-frameshift-203>

"Nonsense mutation." (Definition—Scitable Glossary). <https://www.nature.com/scitable/definition/nonsense-mutation-228>

"The information in DNA determines cellular function via translation." (Concept—Scitable Library). <https://www.nature.com/scitable/topicpage/the-information-in-dna-determines-cellular-function-6523228>

The following resources on northern blots and qRT-PCR are recommended in order to better understand the data presented in Part III of the case study:

"Northern blot." (Definition—Scitable Glossary). <https://www.nature.com/scitable/definition/northern-blot-287/>

Northern blotting [video]. (2019). Frank Lectures. Running time: 5:10 min. YouTube. <https://youtu.be/G2Z1wuEOZUU>

Ghannam, M.G., & M. Varacallo. (2022). Biochemistry, polymerase chain reaction. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. <https://www.ncbi.nlm.nih.gov/books/NBK535453/>