Characterization of Carotenoid Composition of Carrots Affected by "Light Root Syndrome"

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Introduction

Uniform, bright orange color is a major quality attribute for fresh market and 'fresh-cut' carrots in both retail consumer markets and foodservice outlets and institutions. Overall poor root color or irregular color development have been identified as a production defect that impacts postharvest marketability. California carrot production is affected by a variety of incompletely understood disorders that impact root color development or intensity. Divergent factors are believed to be responsible for roots with poor color (carotenoid) development as compared to irregular carotenoid development. The severity of the problem is most acute during the production season that transitions from late summer seeding to mid-winter harvest. Roots symptoms are characterized as having a streaked or blotchy appearance due to the variability in pigment development within groups of plant cells. Originally reported as "white root" and later "light-root syndrome"(LRS), predominantly in the Imperial Valley, this disorder appears to have been grouped with a broad range of sub-optimal production conditions and practices that effect carotenoid content.

Research is in progress to distinguish between low color roots and the malady "light root syndrome" (LRS). Currently, we consider the term LRS to be restricted to roots with irregular, patchy, or streaked carotenoid development that extends from external to internal tissues. The cause of this disorder remains unclear but appears distinct from roots with only internal carotenoid deficiencies or overall poor color. From a marketing and consumer satisfaction perspective, both conditions are undesirable. Progress in developing management strategies, in parallel with opportunities for variety screening, is hampered by the unpredictable occurrence of LRS and the likely temporal separation of inducing factors and symptoms.

Carotenoids and carrot root development

To review, β-carotene is the primary contributor to orange color intensity. Total carotenoid content and distribution of carotenoids at harvest is influenced by genetic and environmental factors including;

- Physiologic maturity; peak carotenoid content is an index of maturity See Fig. 1
- Climatic conditions during the period 3-6 weeks preceding harvest
- Temperatures below or above the optimal range of 10-16˚C
- Micronutrient levels; preliminary research identified excess Mo, Zn , and Se as significantly depressing total carotenoid or relative β-carotene content (Biacs et. al., 1995)
- Soil oxygen content, soil water potential, and stand density are commonly held as a strong contributory factors to carotene synthesis and orange color intensity. Specific data or references to support this observation have not been obtained, at this time.

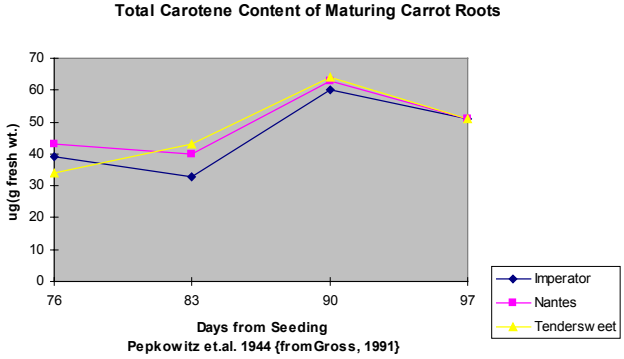

Figure 1. *Carotenoid content of carrots increases with maturity.*

Prior Research

Early efforts to determine the cause and possible solutions to LRS have focused on the possibilities that LRS is associated with whitefly feeding, a transmissible virus or ds-RNA elements, or a methyl bromide sensitive, soil-borne agent (Creamer et. al. 1993). Experimental results were negative and largely inconclusive. A corrective program using ethephon (Ethrel®) has been conducted to determine whether orange color intensity of roots could be improved by

foliar applications (McGiffen, 1995, 1997). Varietal variability in orange root color intensity was also addressed, but the opportunities to distinguish between low root color and the originally described LRS were not available.

Recent efforts to identify a major or sole contributing factor to LRS have not, thus far, been successful. Research progress has been hampered by the unpredictable incidence of the disorder in production fields. The identification of presumptive contributing factors may have been misdirected by the misidentification of low color roots as LRS.

Research Approach

We have taken two approaches to resolve this situation that will hopefully determine whether low color roots (a common situation) can be differentiated from LRS roots (a more specific disorder). The first approach seeks to determine whether low orange root color and LRS are chromatically and biochemically distinct. The second approach, described below, takes an applied "biotechnology" approach by developing novel tools to evaluate production and environmental impacts on carotenoid development and timing in roots.

It is popularly held that carrot roots with a high β-carotene: α- carotene ratio will result in a more intense orange color. The absolute β-carotene content is believed to be less important than a high β: α ratio in reaching the color potential of a given genetic background. This is not apparent from published analytical comparisons of carotenoid content (Simon and Wolff 1987) See Fig. 2. A β: α -carotene ratio of only 1.4 is characteristic of the dark orange color line HCM whereas an intermediate color line had a ratio of 2.3. The distinction between total and proportional β-carotene content may prove useful in resolving the basis for LRS.

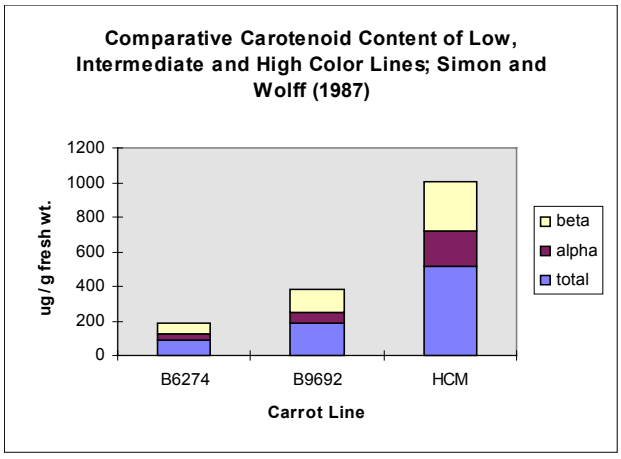

Figure 2. *A comparatively low β: α -carotene ratio* but high absolute content was associated with intense orange color.

Carotenoid Content

HPLC analysis during the initial phase of this project showed that the total carotenoids are depressed in low color root tissue and LRS tissue. A significant shift in the ratio of key biosynthetic products is not detectable. A preliminary conclusion would be that a variety of factors that influence root color quality and, more specifically, LRS results in an overall reduction in carotenoid biosynthesis and not a block in one of the biosynthetic precursor steps between phytoene and β - carotene. No accumulation of compounds leading to β - carotene have been detected thus far. Although the total concentrations are variable for variety and sample date, the ratio of the compound phytoene to α and β-carotene remain proportional and not significantly different between light and dark roots. Disruption of carotenoid production may occur at earlier steps in the pathway or at some other point of cellular disfunction.

Small replicated trials demonstrated that low color deficiencies and poor color development that is restricted to internal tissue are variety dependent (Fig. 3 and 4). Despite limited field trial results to date, it is apparent that variety selection within periods of moderate to severe low color development would be beneficial to avoiding the problem. From observations and review of past literature, reaching physiologic maturity prior to low air and soil temperatures will likely result in greater color development.

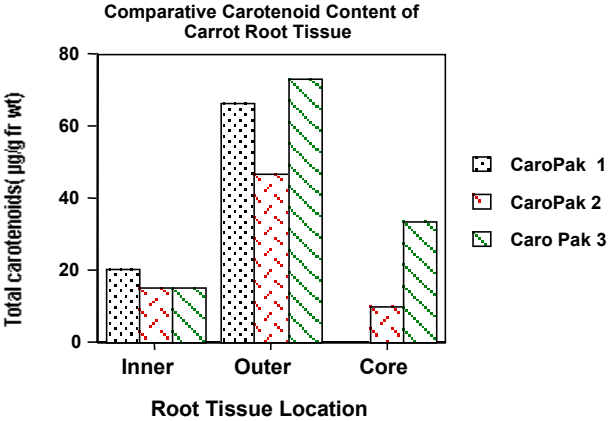

Figure 3. *Comparative carotenoid content of carrot root tissue.*

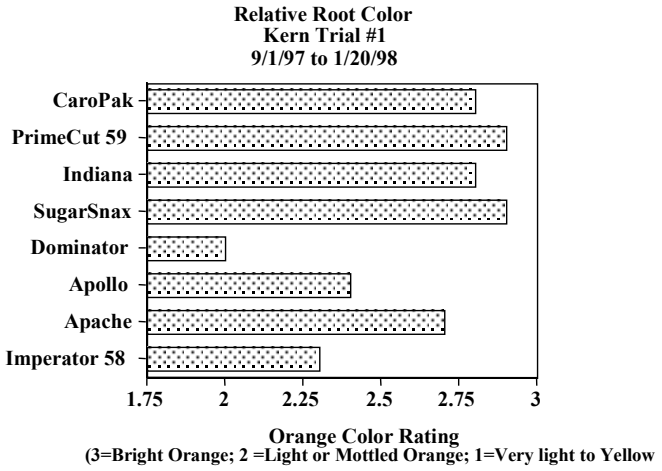

Figure 4. *An example of research in progress, this figure represents the mean Orange Color rating of 15-20 roots from four replicated plots of each variety. Data analysis of other subjective and objective color comparisons is in progress.*

Molecular Research Tools

Novel research tools are desperately needed to facilitate the investigation of potentially complex environmental, crop management and physiologic interactions that result in diminished carotenoid biosynthesis. In conjunction with continuing to explore the basis for LRS in the field, it is essential to create methods to make the evaluation of multiple variables that impact the production of carotenoids in carrot roots more feasible. The approach we feel has significant merit would result in the development of a genetic reporter system for factors that delay or promote the expression of key enzymes in the carotenoid production pathway.

Carotenoid biosynthesis is a widely studied area of great interest for photosynthesis, stress tolerance, and as nutrients with cancer-reducing potential. This prior art is much to our advantage as the enzyme pathway is well characterized and several key genes have been described at the molecular sequence level. One key gene, phytoene synthase has been cloned and sequenced from several plants, including tomato, pepper and melon. The phytoene synthase genes in bacteria, algae, and higher plants are highly similar. Surprisingly, phytoene synthase has not been evaluated in carrot. The conserved nature of the gene, at the DNA level, has been an advantage to expedite its isolation for our applied objectives.

Once cloned and characterized, tools developed with this carrot specific gene will make feasible the evaluation of the impacts of

1. Maturity
2. Stand density
3. Soil and air temperature
4. Daylength/solar irradiance
5. Soil type/texture
6. Soil oxygen
7. Water status
8. Micronutrients
9. Pesticide applications
10. Many other variables

Preliminary Data

Applying molecular strategies to cloning the phytoene synthase gene from carrot (PS-Dc), we have obtained a PCR fragment (section of DNA) of the correct predicted size by a RT-PCR technique. Applying this technique, four PCR primers were designed based on the deduced amino acid sequences of ten PS genes including tomato, pepper, melon, maize, and others. As potential guidance in our effort, the expression of phytoene synthase in melon is strongly related to the stage of fruit maturation. The peak is during the color change from green to orange followed by a gradual decrease in the level of the gene message for enzyme production but an increase in orange color intensity (Karvouni et.al.,1995).

A PCR product of the correct molecular size has been obtained and verified by DNA sequencing to correspond to a phytoene synthase gene (Fig. 5). Much

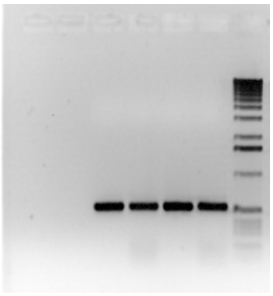

Figure 5*. Lane 1 and 3 visualize the PCR products of PS-primers applied to carrot root cDNA. Lane 2 and 4 visualize the PCR products of PS-primers applied to tomato fruit cDNA. Each pair is the product of DNA binding temperatures that were set 2°C apart.*

work lays ahead to fully develop and implement this research tool but experiments evaluating the timing of natural gene expression in carrot roots have already been initiated.

Selected Background Literature

Carrots and Carotenoids

Biacs, P., Daood, H., and Kadar, I.. 1995. Effect of Mo, Se, Zn, and Cr treatments on the yield, element concentration, and carotenoid content of carrot. J. Agric. Food Chem.. 43: 589-591

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Simon, P.W. , and X.Y. Wolff. 1987. Carotenes in Typical and Dark Orange Carrots. J. Agric. Food Chem. 35: 1017-1022

Phytoene Molecular Function and Gene Cloning

Karvouni, Z., I. John, J.E. Taylor, C.F. Watson, A.J.Turner et al., (1995) Isolation and characterization of a melon cDNAclone encoding phytoene synthase. Plant Mol.Biol. 27: 1153-1162.