CLASSICAL BREEDING TO IMPROVE VEGETABLE VITAMIN AND PROVITAMIN CONTENT

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Summary

Vegetables provide significant dietary vitamin A and C and lesser amounts of B and E vitamins. Progress has been made in applying plant breeding techniques to further increase provitamin A levels, which are derived from carotenoid pigments, with less effort for vitamins B, C, and E.

Most breeding and genetic effort has been directed to crops which already are relatively rich vitamin sources including carrots, sweet potatoes, peppers, tomatoes, squash, pumpkins, and melons. Efforts to continue and expand this research in the future will likely result from greater demands for more nutritious produce by consumers.

1. Introduction

1.1 Vegetables as a Source of Vitamins

Early in the 20th century vitamin A was the first vitamin discovered. Soon after its discovery, animal feeding trials comparing pigmented and unpigmented vegetables demonstrated its ultimate origins in food plants. The fact that all vitamin A is derived from provitamin A carotenoids, those orange, red, and yellow pigments in plants, points to significant role vegetables and fruits play in human health (Table 1). Some dietary B vitamins, most vitamin C, and much vitamin E also come from vegetables, fruits, grains, and staple root and tuber crops. Consequently, vegetable research has focused on improving the quantity, quality, preservation, and availability of vitamin and provitamins (Table 2).

Commodity	Average Provitamin A Carotene Content for the U.S. Crop	High Total Carotenoid Content Reported
Carrot	169	600
Sweet Potato	120	190
Parsley	40	60
Melon	23	120
Pumpkin & Squash	10	213
Broccoli	9	25
Red Pepper	8	248
Tomato	7	95
Green Bean	4	4
Green Pea	3	4
Green Pepper	3	24
Lettuce	2	13
Sweet Corn	2	5
Watermelon	<2	50
Cucumber	<2	6
Cabbage	<2	3
Cassava	0	40
Yams	0	14
Cauliflower	0	3
Potato	0	3

Table 1. Carotenoid content of vegetables containing provitamin A carotenoids, in parts per million, fresh weight basis (revised from Simon, 1992)

Vitamin or Provitamin	Ocurrence in Vegetables?	Breeding Effort in Vegetables?
A	+++	+++

B vitamins:		
B_1 (thiamin)	+	
B_2 (riboflavin)	+	
B_3 (niacin)	+	
B_6	+	
B_{12}	-	
Biotin	+	
Folate	+	+
Pantothenic acid	+	
C	+++	+
E	++	++
D	-	
K	++	

Table 2. Occurrence of vitamins and provitamins in vegetables, and breeding effort

1.2. The Importance of Selection for Vitamin Content in Vegetable Breeding Programs

Commercial production components such as yield, disease resistance, uniformity, postharvest durability, and appearance, are the most important traits receiving the attention of vegetable breeders. In combination with consumer preference factors, such as flavor and convenience, most vegetable production and consumption decisions are made. Yet long-term consumer health, especially in developing regions of the world, also depends on a sustained source of nutrients. This means that the content of vitamins and other nutritional compounds in vegetables such as minerals and phytonutrients are often major breeding goals. Rapid, inexpensive and accurate techniques for estimating vitamin or provitamin are essential for routine evaluation of breeding populations.

2. Breeding Efforts to Improve Vitamin or Provitamin Content in Vegetable Crops

2.1 Provitamin A Carotenoids

Provitamin A carotenoid types and amounts have been evaluated in vegetable crops with yellow, orange, and red edible tissues. Most extensive research including breeding and genetic studies have been in carrots, sweet potatoes, sweet and hot peppers, tomatoes, watermelons, melons, pumpkins and squash, and cucumbers (Table 1).

2.1.1 Breeding Carrots for Carotene Content

Carrot (*Daucus carota* L.) is by far the most important single source of dietary provitamin A in the U.S., estimated to about 14% of the total in 1970's and about 30% in the early 1990's. The overall dietary contribution of carrot as a source of provitamin

A carotenoids was increased in recent years with the advent of a popular class of raw snack products, the so-called 'baby-carrots'. In carrot, deep-orange color of both xylem and phloem has long been a major breeding objective for its visual impact. However, along with the development of 'baby carrots' came a renewed stimulus for breeding programs to improve texture, flavor, and color (and as a consequence provitamin A).

The typical U.S. carrot contains about 169 ppm (mg/kg fresh weight) of provitamin A. Six carotenes are routinely detected in carrot root α-carotene, β-carotene, γ-carotene, ζ-carotene, β-zeacarotene, and lycopene). The total amount of carotenoids can be subdivided into approximately 20% α-carotene, 49-79% β-carotene, 0-20% γ-carotene, 1-3% β-zeacarotene, 0-2% lycopene and 0-10% ζ-carotene. Higher carotene carrots usually have lower relative amounts of β-carotene and tend to accumulate more α-carotene (up to 50%) and ζ-carotene (up to 10%). In comparison, red-rooted cultivars grown in parts of Asia tend to have larger proportions of lycopene than typical orange roots.

Most of the breeding programs for carotene use the traditional inexact selection method that is done by visually rating the carrot roots. Roots with the best exterior color are selected, giving preference to those that exhibit orange color spreading well down to the taproot ('red tails'). Major selection criteria are color intensity, indistinct cambium zone, and perfect color match between phloem and xylem.

Limits of visual selection are around 120ppm of total carotenoids. Difficulties with visual selection differentiating dark-orange from red-orange roots are also commonly reported. Therefore, alternative analytical procedures are necessary to provide more accurate evaluation that will ensure genetic gain by selection. Carotenoids in root have been determined using spectrophotometric analysis and thin-layer chromatography. However, reverse-phase high-performance liquid chromatography (HPLC) has become a progressively more important tool in carrot breeding because less time is needed for carotene analysis and carotene breakdown is less than with other methods.

Genetic studies dealing with carotenoid accumulation in carrot roots are not entirely conclusive since significant environmental and developmental influences have been observed for this trait. For example, low temperature during the growing season can lead to slower maturing carrots that usually contain less carotene. In addition, the total amount and relative proportion of individual carotenoid pigments varies with specific genetic by environment interactions. The cultivar ranking, however, was found to be quite stable in a group of genetic materials evaluated across distinct years and locations.

Large-scale evaluation of carrot germplasm, searching for natural sources of high carotene content, illustrates the phenotypic diversity available for this trait. Evaluation using HPLC of carotenoid and provitamin A activity in European carrot cultivars has indicated that amounts of α- and β-carotene ranged from 22-49 ppm and 46-103 ppm respectively. Lutein and γ-carotene accumulated in all cultivars in the range of 1-6 ppm and 6-27 ppm, respectively. Similar variation was found in Asian germplasm. Expressed as retinol equivalents for 100g FW, the range was from 1200 to 2300 (presuming 0.6 I.U. retinol per microgram β-carotene, and 0.3 I.U. retinol per microgram α-carotene). Cultivar 'Beta III' was a major improvement in terms of

provitamin A (retinol) content with α- and β-carotene levels at 50-120 ppm and 90-200 ppm, respectively, and a retinol equivalent of 2000-5000/100 g FW. However, the highest source of total carotene reported thus far in carrot is 'HCM', which can accumulate up to 600ppm of carotenoid.

Inheritance studies indicated that carotenoid type and amount (with color range from white to orange) are controlled by at least three genes. Degree of orange color intensity seems to be a typical polygenic trait. Gene action studies for β-carotene content indicated additive and dominance effects as well as additive × dominance and dominance × dominance interactions as being significant. No indication of overdominance was obtained so far and only partial degree of dominance was observed for β-carotene content. Progressive and continuous gains observed for total carotenoid concentration in root via recurrent selection is a clear indication that additive genetic variability for this trait is far from be depleted in most carrot populations.

A collection of naturally occurring single-locus mutations affecting carotenoid accumulation has been identified in carrot. They include dominant alleles such as *A* (α-carotene synthesis), *Io* (intense orange xylem, which may be an allelic form of *A*), *L* (lycopene synthesis), *O* (orange xylem, which may also be an allelic form of *A*) as well as one recessive allele *y* (yellow xylem). Three dominant loci named *Y*, *Y1*, and *Y2* control differential distribution of α- and β-carotene (xylem/phloem carotene levels). The mutation *Y2* controls low carotene content of the storage root xylem ('core') in high carotene orange backgrounds. AFLP molecular markers for *Y2* in carrot have been successfully converted to a PCR-based, codominant marker and can now be employed as a tool in marker-assisted selection programs.

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