DETERMINATION OF CAROTENE CONTENT IN YELLOW-SEEDED COMMON BEAN VARIETIES

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Introduction

Yellow-seeded beans are a minor commercial grain type from the Andean gene pool that are eaten in a subset of Latin American bean-producing countries, notably in Peru, Mexico and Brazil. Recently, they have become embroiled in a controversy in the United States where a patent was claimed on the marketing of yellow beans. In the meantime, the scientific basis for the yellow color is not known. In this study we decided to analyze whether the yellow color was derived from carotenoids or not. To do this we selected a group of accessions representing all the major types of yellow beans that are found in primary and secondary centers of diversity for common beans and analyzed them for seed carotenoid content to determine if there is genetic variability for the trait.

Materials and Methods

A total of 10 common bean genotypes with various tones of yellow-colored grain (from canary to sulphur yellow) were selected for this study (Table 1). Three parts of the grain were analyzed: 1) whole seed, 2) peeled seed (embryo) and 3) seed coat. In the case of seed coat tissue, we were interested in studying the effect of seed storage and oxidation on carotenoid content of the seed coat and therefore extracted the seed coat of both fresh and stored dry grain samples. The tissues were ground into a fine powder with a coffee mill and a tungsten ball bearing mill. Extraction began by resuspending 0.3 g (for the stored seed coat and embryo) or 1.0 g (for the whole seed) of ground tissue in 10 ml of petroleum ether and 5 ml of water at 35-60 °C. The mixture was homogenized before centrifugation at 3000 rpm for 5 min at 10 °C. The ether phase was removed to a new tube while the aqueous phase was re-extracted with an additional 10 ml of petroleum ether and combined with the previous ether phase. Both of these aliquots were dried down with sodium sulfate in a vacuum centrifuge. The extracts were then resuspended in 1 ml of petroleum ether and used immediately for the analysis. The samples were analyzed in a UV-visible spectrophotometer at 455 nm, using petroleum ether at 30-60 °C as a blank. A standardization curve was made from β-carotene in a petroleum ether solvent and used for the quantification of β-carotene in the sample. The linear regression of the calibration curve was \( C(\text{ppm}) = K \times \text{Abs} + B \), where \( K = 6.6707 \) and \( B = 0.2084 \). To confirm the presence of β-carotene in each group of samples, readings were made for the UV-visible spectra in the range from 200-600 nm and compared to the commercial standard (Sigma).

Results and Discussion

Table 1 and 2 summarize the amount of carotenoid detected in the different tissues analyzed. The amount of β-carotene found in the stored and freshly-ground seed coats was similar and relatively low, averaging 0.131 mg per 100 g of tissue. Although the seeds ranged from light to dark yellow and had different tones ranging from canary to sulphur yellow, the amount of carotenoid was not associated with the intensity of the yellow seed coat color. Whole seed contained lower amounts of β-carotene (averaging 0.042 mg per 100 g of tissue) than the embryo tissue alone (averaging 0.147 mg per 100 g of tissue) indicating that most of the carotenoid is in the embryo tissue not in the yellow seed coat. This carotenoid concentration was within the same range as that found for the dissected seed coat in the previous experiment, however these would be comparisons across experimental conditions given that 1 g of embryo and whole seed tissue was used versus only 0.3 g of seed coat tissue, an amount that is at the lower limit of detection. In these experiments absorbance levels at 455 nm were very low and no additional readings were taken at other wavelengths. Therefore to confirm that the measurements were detecting carotenoid and not some other substance at this absorbance spectra, the spectra of a larger sample of 10 g of fresh whole bean seed tissue, processed as described above, was measured at
wavelengths from 200 to 600 nm and was compared to that of the \( \beta \)-carotene standard. In this analysis, there generally was a good fit between the data for the sample and the standard, indicating that \( \beta \)-carotene was indeed the pigment being measured at 455 nm absorbance and that this carotenoid is present in the fresh bean seeds. These results suggest various avenues for future research. First, given that carotenoid levels are very low in both the dry bean embryo, the optimal amount of tissue to analyze for future \( \beta \)-carotene experiments in beans, should be increased to 10g. Second, since the yellow color in yellow seed coats is unlikely to be due to carotenoids given their low concentrations, but rather is probably due to some other pigment, it will be important to read the absorbance wavelength of 490 nm as well as at 455 nm. Also, to confirm the presence of \( \beta \)-carotene, more sensitive tests using MS/HPLC can be undertaken. Mass spectrophotometry especially should be able to determine the identity of xanthophylls that have been suggested as the major pigment in seed coat of yellow beans. The amount of \( \beta \)-carotene in various stages of bean seed maturation will be another area of interest. Especially in the Andes and certain areas of the Caribbean, bean seeds are commonly consumed at physiological maturity but before they dry down. To do this beans are shelled while the pods are still green or just starting to turn yellow. These beans are often referred to as green-shelled beans and command a premium price because of their fast cooking time. In future experiments, we will look at carotenoid levels during grain development, their stability upon cooking and whether this is different in mature dry grain versus green-shelled beans.

**Table 1.** Analysis of carotene concentration (expressed in mg carotene/100g tissue) in stored and fresh seed coat (SC) tissue from 10 common bean genotypes with yellow grain color.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Name</th>
<th>Origin</th>
<th>Weight (g)</th>
<th>Fresh SC mg carotene /100g tissue</th>
<th>Stored SC mg carotene /100g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-5703</td>
<td>Canario Corriente (LM2-57)</td>
<td>Peru</td>
<td>0.30</td>
<td>0.03</td>
<td>0.19</td>
</tr>
<tr>
<td>G-57</td>
<td>Swedish Brown</td>
<td>USA</td>
<td>0.30</td>
<td>0.10</td>
<td>0.19</td>
</tr>
<tr>
<td>G-2288</td>
<td>Maragwe Oga</td>
<td>Kenya</td>
<td>0.30</td>
<td>0.10</td>
<td>0.18</td>
</tr>
<tr>
<td>G-4547</td>
<td>Liborino de Mata</td>
<td>Colombia</td>
<td>0.30</td>
<td>0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>G-11035</td>
<td>Bayo Regional</td>
<td>Mexico</td>
<td>0.30</td>
<td>0.12</td>
<td>0.20</td>
</tr>
<tr>
<td>G-13094</td>
<td>Mayocoba</td>
<td>Peru/Mexico</td>
<td>0.30</td>
<td>0.08</td>
<td>0.18</td>
</tr>
<tr>
<td>G-14253</td>
<td>Peru 13</td>
<td>Peru</td>
<td>0.30</td>
<td>0.09</td>
<td>0.20</td>
</tr>
<tr>
<td>G-19833</td>
<td>Caucha Chuga</td>
<td>Peru</td>
<td>0.30</td>
<td>0.07</td>
<td>0.20</td>
</tr>
<tr>
<td>G-21715</td>
<td>Dore de Kirundo</td>
<td>Burundi</td>
<td>0.30</td>
<td>0.09</td>
<td>0.31</td>
</tr>
<tr>
<td>G-22041</td>
<td>Garbancillo Zarco</td>
<td>Mexico</td>
<td>0.30</td>
<td>0.09</td>
<td>0.10</td>
</tr>
</tbody>
</table>

**Table 2.** Analysis of carotene content of whole seed and fresh embryo tissues in 10 common bean genotypes.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Whole seed</th>
<th>Embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight (g)</td>
<td>Absorbance (( \lambda ) 455nm)</td>
</tr>
<tr>
<td>G-5703</td>
<td>1.0</td>
<td>0.013</td>
</tr>
<tr>
<td>G-57</td>
<td>1.0</td>
<td>0.019</td>
</tr>
<tr>
<td>G-2888</td>
<td>1.0</td>
<td>0.017</td>
</tr>
<tr>
<td>G-4547</td>
<td>1.0</td>
<td>0.024</td>
</tr>
<tr>
<td>G-11035</td>
<td>1.0</td>
<td>0.085</td>
</tr>
<tr>
<td>G-13094</td>
<td>1.0</td>
<td>0.029</td>
</tr>
<tr>
<td>G-14253</td>
<td>1.0</td>
<td>0.027</td>
</tr>
<tr>
<td>G-19833</td>
<td>1.0</td>
<td>0.041</td>
</tr>
<tr>
<td>G-21715</td>
<td>1.0</td>
<td>0.022</td>
</tr>
<tr>
<td>G-22041</td>
<td>1.0</td>
<td>0.022</td>
</tr>
</tbody>
</table>

1/ carotene extracted from 0.3 g and 1.0 g of tissue for tissues described in table 1 and 2, respectively