Capsaicinoid profiles are not good chemotaxonomic indicators for Capsicum species

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Abstract

Capsaicinoids have been suggested as an aid in identifying Capsicum species. The distribution of seven capsaicinoids and their chemotaxonomic significance were examined within nearly 200 accessions of six Capsicum species. The seven capsaicinoids were separated and quantified using high-performance liquid chromatography. The capsaicinoid profiles were not consistent when examined within a species, therefore they have limited use as a chemotaxonomic indicator. In addition, the generalization that capsaicin and dihydrocapsaicin are always the major capsaicinoids was not true, exceptions were found for some of the accessions studied. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Based on classical morphological taxonomy, the Capsicum genus consists of 22 wild and five domesticated species (Bosland, 1994). The capsaicinoids, which produce the organoleptic sensation of heat, are unique to this genus (Bosland, 1996). Recent report shows that more than 20 capsaicinoids are found in chiles (Capsicum spp.) (Bosland and Votava, 1999). A chile sample may contain some or all of these capsaicinoids.

There are two classes of capsaicinoids (capsaicin and dihydrocapsaicin) and they differ in the presence or absence of double bond in the fatty acid side chain. Individual capsaicinoids within a class vary in the length of the chain and the branching point (Krajewska and Powers, 1988).

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Several researchers studied synthesis of capsaicinoids during the development of the chile fruit. Ohta (1962) studied the time when capsaicinoid synthesis began in five *Capsicum* species. The results showed that in *C. chacoense* Hunz, the synthesis of capsaicinoids could be detected one week after flowering. In *C. annuum* L., *C. frutescens* L., *C. baccatum* Willd., and *C. pubescens* Ruiz and Pav. the synthesis of capsaicinoids was detected two weeks after flowering. The maximum capsaicinoid concentration was detected two weeks after flowering in *C. chacoense*, three weeks after flowering in *C. annuum*, four weeks after flowering in *C. frutescens* and *C. baccatum*, and 11 weeks after flowering in *C. pubescens*. The results of a similar study by Iwai et al. (1979) found that in *C. annuum* capsaicinoids were first detected 20 days after flowering. Then the accumulation increased gradually and reached maximum level approximately 40 days after flowering. The capsaicinoid concentration decreased significantly 50 days after flowering. Further studies by Contreras-Padilla and Yahia (1998) showed that capsaicinoids concentration decreased after reaching the maximum level. The decrease of capsaicinoid concentration is related to the increase of peroxidase activity, indicating that capsaicinoids may be degraded by peroxidase. Unlike the previous experiments (Iwai et al., 1979; Contreras-Padilla and Yahia, 1998), Sukrasno and Yeoman (1993) reported that the level of capsaicinoids remained constant in the fruit after reaching the maximum concentration in *C. annuum*. The complete biosynthetic pathway for capsaicinoids is not yet known. The array and relative quantity of individual capsaicinoids in a given chile sample is defined as its “capsaicinoid profile.”

In early studies, Bennett and Kirby (1968) demonstrated the presence of at least five capsaicinoids in *C. annuum* L. samples at proportions of 69% capsaicin, 22% dihydrocapsaicin, 7% nordihydrocapsaicin, 1% homocapsaicin, and 1% homodihydrocapsaicin. Because of their abundance, capsaicin and dihydrocapsaicin were considered the major capsaicinoids, while the others were considered the minor capsaicinoids. In later studies, Collins et al. (1995) reported a capsaicinoid profile in *C. pubescens* accessions, where dihydrocapsaicin is the largest proportion, i.e., 35% dihydrocapsaicin, 29% capsaicin, 21% nortihydrocapsaicin, 8% unidentified capsaicinoid, 4% isomer of dihydrocapsaicin, 2% unidentified capsaicinoid and 1% homodihydrocapsaicin. Recently, Zewdie et al. (1998) reported an unusual capsaicinoid profile in two *C. pubescens* accessions, where the isomer of dihydrocapsaicin is the largest proportion, i.e., 39% isomer of dihydrocapsaicin, 17% homodihydrocapsaicin, 13% capsaicin, 13% dihydrocapsaicin, 13% nordihydrocapsaicin, and 5% nornordihydrocapsaicin. Because the proportion of capsaicinoids did not change during fruit growth stages, Iwai et al. (1979) reported that interconversion among capsaicinoids within the class or between classes (e.g., capsaicin to dihydrocapsaicin or vice versa) does not occur. Observations made at New Mexico State University found no significant difference for capsaicinoid profiles among the mature green and red ripened fruit stages within eight *C. pubescens* accessions (Personal observation).

The above findings demonstrate that there is variation in capsaicinoid profiles within and between *Capsicum* species. The capsaicinoid variation might have phylogenetic implications and might be useful for taxonomic classification. Based on their genetic relationship, Eshbaugh (1979, 1993) grouped *Capsicum* species into three
species complexes. *C. pubescens* forms a distinct genetic lineage and the wild species, *C. eximium* Hunz. and *C. cardenasii* Heiser and Smith, are closely related to it. The second genetic lineage is the *C. baccatum* complex and the third one is the *C. annuum*, *C. chinense* Jacq., and *C. frutescens* L. complex.

A number of researchers have investigated the use of chemical variation in plants for taxonomic and phylogenetic relationships (Smith, 1976). Jurenitsch et al. (1979) reported that the capsaicinoid composition of a chile accession may help in identifying its species designation. Similarly, Suzuki and Iwai (1984) suggested the usefulness of capsaicinoid profiles for taxonomic purposes in *Capsicum*. The objective of this study was to examine the capsaicinoid profiles for their usefulness in identifying *Capsicum* species.

2. Materials and methods

2.1. Plant material

Capsaicinoid profiles of 191 accessions, where 58 accessions of *C. baccatum*, 57 accessions of *C. chinense*, 51 accessions of *C. annuum*, 10 accessions of *C. frutescens*, eight accessions of *C. chacoense*, and seven accessions of *C. pubescens*, were examined. We obtained 165 accessions of the total from United States Department of Agriculture Plant Introduction Station, Griffin, GA, USA and the other 26 accessions, where 15 of them are open-pollinated cultivars, from the Chile Breeding and Genetics Program at New Mexico State University, Las Cruces, NM, USA. The open-pollinated cultivars were maintained under insect-proof caging for more than three generations. The complete list of accessions can be obtained from the authors. All accessions were characterized by morphological traits and were assigned to a species. None of the genotypes studied were hybrids. The *C. baccatum*, *C. chinense*, and *C. annuum* accessions were evaluated in 1994, *C. chacoense* accessions in 1996, and *C. pubescens* and *C. frutescens* in 1997. Plants of *C. pubescens* and *C. chacoense* were grown in the greenhouse and plants of the other species were grown in the field, at Las Cruces, New Mexico, USA. Mature red succulent fruits were harvested from the first four node positions and then bulked by accession for analysis.

2.2. Laboratory analysis

Capsaicinoids were extracted, separated, and quantified using high-performance liquid chromatography (HPLC) following the ‘long-run’ method described by Collins et al. (1995). Each accession was examined for seven capsaicinoids, i.e., nornornordihydrocapsaicin, nornordihydrocapsaicin, nordihydrocapsaicin, capsaicin, dihydrocapsaicin, an isomer of dihydrocapsaicin, and homodihydrocapsaicin. These capsaicinoids are described in previous research (Bennett and Kirby, 1968; Collins et al., 1995; Torabi, 1997) and identified based on retention time in comparison to standards.
2.3. Statistical analysis

Because the main objective of the study was to examine the usefulness of capsaicinoid profiles for taxonomic purposes, the concentrations of the individual capsaicinoids in an accession were transformed to percentage in relation to the sum of the capsaicinoids. Because this is an exploratory type of study, different statistical methods were attempted to find an association with capsaicinoid profiles and taxonomic classification. Both discriminant and cluster analyses were performed using SAS program (SAS Institute Inc., 1996). A discriminant analysis was performed to separate and allocate the accessions to the different species. A dendogram was constructed by “Wards method” (SAS Institute Inc., 1996) of the cluster analysis. A scatter diagram was established by using the first three canonical variates (from canonical discriminant analysis), which accounted for 98% of the total variation, to visualize the relationship of the accessions on a three-dimensional graph (Fig. 1).

Even though there is no definite way of determining the number of clusters, prior knowledge of the genetic material helps in interpretation of the cluster analysis (Brown, 1991). If the null hypothesis (i.e., capsaicinoids are good indicators of species) is true, then it was expected that the six species will cluster separately. Therefore, the

![Fig. 1. The scatter diagram showing the relationship of the accessions based on capsaicinoid profiles (Cluster I = heart; Cluster II = cross; Cluster III = pyramid; Cluster IV = star; Cluster V = flag; and Cluster VI = diamond).](image)
A wide range of variation among the accessions within each species was observed for capsaicinoid profiles. Capsaicin and dihydrocapsaicin were present in all the accessions studied. In 13 accessions; four C. annuum accessions, four C. baccatum accessions, four C. chinense accessions, and one C. chacoense accession, capsaicin and dihydrocapsaicin were the only capsaicinoids present.

When the accessions were examined for the highest relative amount of a given capsaicinoid, it was found that 3% nornornordihydrocapsaicin in C. pubescens accession, 7% nornordihydrocapsaicin in C. pubescens accession, 30% nordihydrocapsaicin in C. chacoense accession, 78% capsaicin in C. chinense accession, 66% dihydrocapsaicin in C. chacoense accession, 42% isomer of dihydrocapsaicin in C. pubescens accession, and 23% homodihydrocapsaicin in C. pubescens accession were the highest.

The classification of accessions to species based on capsaicinoid profiles using linear discriminant function is presented in Table 2. For all species examined, some accessions were categorized into a different species than their true species classification. For example, with C. baccatum, only 34 out of the total 58 accessions were identified as belonging to C. baccatum. With C. chinense, 47 out of the 57 accessions examined were identified as C. chinense, while the rest were classified as other species. When C. annuum was examined, about half (29 out of 51 accessions) were classified as C. annuum. The remaining C. annuum accessions were classified as either C. baccatum or C. chinense (Table 2).

When the $R^2$ value of 70% was used, six clusters were observed (data not presented). The clusters were not specific to species. Cluster I consisted of 83 accessions, which contained accessions belonging to C. chinense, C. annuum, C. baccatum, and C. frutescens. The accessions within this cluster were characterized by having capsaicin and dihydrocapsaicin in relatively high percentages (Table 3).

Cluster II consisted of 80 accessions belonging to C. baccatum, C. annuum, C. chinense, C. frutescens, and C. chacoense. This cluster had a capsaicinoid profile where capsaicin and dihydrocapsaicin were in relatively high percentages, but the difference between the capsaicin percentage and the dihydrocapsaicin percentage was lower in comparison to Cluster I (Table 3).

Cluster III contained 19 accessions belonging to C. baccatum, C. chacoense, and C. annuum. This cluster had a capsaicinoid profile where the dihydrocapsaicin percentage was greatest. Capsaicin was the second largest and nordihydrocapsaicin was the third largest (Table 3).
Table 1
The capsaicinoid profiles for representative genotypes and the range of the relative distribution of the nornorthern capsiacin (3-ND), nornorthern capsaicin (2-ND), nornorthern capsaicin (NDH), capsaicin (CAP), dihydrocapsaicin (DH), isomer of dihydrocapsaicin (ISO), and homodihydrocapsaicin (HD) for each species in percent

<table>
<thead>
<tr>
<th>Capsicum species</th>
<th>Genotype</th>
<th>3-ND</th>
<th>2-ND</th>
<th>ND</th>
<th>CAP</th>
<th>DH</th>
<th>ISO</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>baccatum</td>
<td>PI 281340</td>
<td>0.00</td>
<td>0.00</td>
<td>6.88</td>
<td>60.46</td>
<td>30.80</td>
<td>1.02</td>
<td>0.85</td>
</tr>
<tr>
<td>Range</td>
<td>0.00–0.51</td>
<td>0.00–1.27</td>
<td>0.00–20.15</td>
<td>29.83–75.56</td>
<td>22.71–52.11</td>
<td>0.00–7.96</td>
<td>0.00–3.36</td>
<td></td>
</tr>
<tr>
<td>chinense</td>
<td>Habanero yellow</td>
<td>0.00</td>
<td>0.22</td>
<td>4.05</td>
<td>58.14</td>
<td>33.86</td>
<td>3.09</td>
<td>0.64</td>
</tr>
<tr>
<td>Range</td>
<td>0.00–0.50</td>
<td>0.00–0.96</td>
<td>0.00–10.53</td>
<td>44.40–78.45</td>
<td>20.84–46.22</td>
<td>0.00–6.15</td>
<td>0.00–2.62</td>
<td></td>
</tr>
<tr>
<td>annuum</td>
<td>Thick cayenne</td>
<td>0.00</td>
<td>0.00</td>
<td>8.06</td>
<td>55.27</td>
<td>28.09</td>
<td>5.72</td>
<td>2.86</td>
</tr>
<tr>
<td>Range</td>
<td>0.00–0.13</td>
<td>0.00–0.93</td>
<td>0.00–11.65</td>
<td>23.53–67.58</td>
<td>24.72–60.23</td>
<td>0.00–8.70</td>
<td>0.00–3.84</td>
<td></td>
</tr>
<tr>
<td>frutescens</td>
<td>Tabasco</td>
<td>0.20</td>
<td>0.33</td>
<td>3.27</td>
<td>64.83</td>
<td>30.99</td>
<td>0.20</td>
<td>0.18</td>
</tr>
<tr>
<td>Range</td>
<td>0.00–0.76</td>
<td>0.00–0.63</td>
<td>0.22–6.23</td>
<td>53.08–73.44</td>
<td>20.34–38.59</td>
<td>0.00–1.96</td>
<td>0.00–1.47</td>
<td></td>
</tr>
<tr>
<td>chacoense</td>
<td>PI 439414</td>
<td>0.00</td>
<td>0.00</td>
<td>4.94</td>
<td>33.68</td>
<td>58.71</td>
<td>0.73</td>
<td>1.94</td>
</tr>
<tr>
<td>Range</td>
<td>0.00</td>
<td>0.00–3.83</td>
<td>0.00–29.94</td>
<td>24.46–44.04</td>
<td>35.83–65.62</td>
<td>0.00–7.48</td>
<td>0.00–3.16</td>
<td></td>
</tr>
<tr>
<td>pubescens</td>
<td>PI 593632</td>
<td>1.50</td>
<td>2.70</td>
<td>20.36</td>
<td>27.88</td>
<td>45.27</td>
<td>1.40</td>
<td>0.90</td>
</tr>
<tr>
<td>Range</td>
<td>0.00–2.84</td>
<td>0.00–6.59</td>
<td>12.62–24.38</td>
<td>12.11–39.80</td>
<td>8.76–49.28</td>
<td>1.40–42.37</td>
<td>0.90–22.90</td>
<td></td>
</tr>
</tbody>
</table>
Table 2
The number of accessions classified from their true species classification to other species based on capsaicinoid profile using linear discriminant function

<table>
<thead>
<tr>
<th>True Capsicum species</th>
<th>baccatum</th>
<th>chinense</th>
<th>annuum</th>
<th>frutescens</th>
<th>chacoense</th>
<th>pubescens</th>
<th>Total</th>
<th>% Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>baccatum</td>
<td>34b</td>
<td>14</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td>chinense</td>
<td>6</td>
<td>47</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>57</td>
<td>82</td>
</tr>
<tr>
<td>annuum</td>
<td>12</td>
<td>9</td>
<td>29</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>51</td>
<td>57</td>
</tr>
<tr>
<td>frutescens</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>chacoense</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>8</td>
<td>38</td>
</tr>
<tr>
<td>pubescens</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>7</td>
<td>86</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>78</td>
<td>46</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>191</td>
<td>63</td>
</tr>
</tbody>
</table>

*a Number of accessions within each species.
*b The bold diagonal is the number of accessions matching with the true species designation.

Table 3
Mean distribution of normornordihydrocapsaicin (3-ND), normordihydrocapsaicin (2-ND), nordihydrocapsaicin (NDH), capsaicin (CAP), dihydrocapsaicin (DH), isomer of dihydrocapsaicin (ISO), and homodihydrocapsaicin (HD) within groups in percent

<table>
<thead>
<tr>
<th>Cluster</th>
<th>3-ND</th>
<th>2-ND</th>
<th>NDH</th>
<th>CAP</th>
<th>DH</th>
<th>ISO</th>
<th>HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (83)</td>
<td>0.004</td>
<td>0.048</td>
<td>3.394</td>
<td>63.360</td>
<td>31.436</td>
<td>1.102</td>
<td>0.657</td>
</tr>
<tr>
<td>II (80)</td>
<td>0.050</td>
<td>0.086</td>
<td>6.539</td>
<td>48.459</td>
<td>42.646</td>
<td>1.232</td>
<td>0.988</td>
</tr>
<tr>
<td>III (19)</td>
<td>0.000</td>
<td>0.291</td>
<td>13.848</td>
<td>35.302</td>
<td>46.502</td>
<td>2.659</td>
<td>1.398</td>
</tr>
<tr>
<td>IV (5)</td>
<td>0.706</td>
<td>3.222</td>
<td>15.419</td>
<td>34.419</td>
<td>42.024</td>
<td>2.172</td>
<td>1.697</td>
</tr>
<tr>
<td>V (3)</td>
<td>0.000</td>
<td>3.012</td>
<td>12.841</td>
<td>16.753</td>
<td>11.885</td>
<td>36.751</td>
<td>18.758</td>
</tr>
<tr>
<td>VI (1)</td>
<td>2.836</td>
<td>6.587</td>
<td>24.379</td>
<td>17.534</td>
<td>44.679</td>
<td>2.907</td>
<td>1.078</td>
</tr>
</tbody>
</table>

*a ( ) = Number of accessions within the cluster.

Cluster IV consisted of five accessions, two belonging to C. chacoense and the other three belonging to C. pubescens. This cluster had a capsaicinoid profile where the dihydrocapsaicin percentage was the greatest. Capsaicin was the second largest and nordihydrocapsaicin was the third, but the difference between the capsaicin percentage and the dihydrocapsaicin percentage was lower in comparison to Cluster III (Table 3).

Cluster V consisted of three accessions and all belonging to C. pubescens. This cluster was distinguished by having the highest percentage of the isomer of dihydrocapsaicin and homodihydrocapsaicin (Table 3).

Cluster VI consisted of one accession belonging to C. pubescens. This cluster also had a capsaicinoid profile where the dihydrocapsaicin percentage was greatest as in Clusters III and IV. It was distinguished from Clusters III and IV by having the nordihydrocapsaicin as the second largest and capsaicin as the third largest (Table 3).
The first three axes of the canonical variate accounted for 81, 14, and 3% of the total variance, respectively. Fig. 1 represents the relationship of the accessions on a three-dimensional graph. Clusters I–III were overlapping and hard to distinguish on the scatter diagram. Clusters V and VI were distinct, with all accessions within the cluster belong to \textit{C. pubescens}, and were separated from the other clusters. The two accessions of \textit{C. chacoense} in Cluster IV were more widely spaced than the rest of the members, showing variability within this cluster (Fig. 1).

4. Discussion

Though Bennett and Kirby (1968) considered capsaicin and dihydrocapsaicin as the major capsaicinoids, this generalization was not true for several accessions in this study. For example, Cluster V, containing the \textit{C. pubescens} accessions, had the isomer of dihydrocapsaicin and homodihydrocapsaicin as major capsaicinoids.

The various aspects of using secondary plant constituents, chemicals, to classify higher plants is discussed by Harborne (1968). In tea, \textit{Camellia} spp., different forms are easily separated on the basis of phenolic pattern (Smith, 1976). Williams et al. (1999) classified accessions into different species in the genus \textit{Tanacetum} by using flavonoids, i.e., in terms of presence/absence or variation in distribution in the different organ of the plant. However, in this study using capsaicinoid profiles, a chemotaxonomic classification was not conclusive in placing an accession in the known taxonomic species. The distribution and the percentage of the capsaicinoids were inconsistent within a species, therefore, it was not possible to identify a species by a capsaicinoid profile. For example, accessions belonging to \textit{C. pubescens} were split among clusters, Clusters IV–VI. Conversely, Clusters I–IV contained more than one species. In addition, \textit{C. baccatum} was clustered within the \textit{C. annuum}, \textit{C. chinense}, and \textit{C. frutescens} complex. It should be noted that within \textit{C. pubescens} there are unique capsaicinoid profiles. Even though these capsaicinoid profiles are specific and useful to identify some \textit{C. pubescens} accessions, a general statement that capsaicinoid profiles are good chemotaxonomic indicators cannot be made.

It has been suggested (Jurenitsch et al., 1979; Suzuki and Iwai, 1984) that capsaicinoid profiles may aid in classifying \textit{Capsicum} species. If capsaicinoid profiles were dependable chemotaxonomic indicators, the accessions should have clustered by species. However, results of this study, by using several different statistical methods, clearly indicate that the capsaicinoid profile is not a flawless taxonomical criterion to distinguish \textit{Capsicum} species. The accuracy of using capsaicinoids for taxonomic identification was only 63%. Therefore, for taxonomic purposes other criteria must be used to correctly identify \textit{Capsicum} species.

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