Capsaicinoid and Carotenoid Composition and Genetic Diversity of Kas I and Ccs in New Mexican Capsicum annuum L. Landraces

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Abstract. Two key fruit qualities in Capsicum annuum are fruit pungency and color. We characterize 13 New Mexican landraces for fruit quality traits at both the chemical level measuring the capsaicinoids, dihydrocapsaicin, and capsain as well as five carotenoids, β-carotene, β-cryptoxanthin, zeaxanthin, violaxanthin, and capsanthin, and at the genetic level sequencing two genes in these landraces, Kas I, a capsainid pathway gene, and Ccs, a carotenoid pathway gene. All of the landraces had unusually high levels of dihydrocapsaicin in comparison with capsainic levels. The levels of the most abundant red pigment, capsanthin, ranged between 468 and 1007 µg·g⁻¹ dry weight fruit in field-grown fruit, whereas levels of β-carotene were more similar in the landraces (13 to 22 µg·g⁻¹ dry weight fruit). Twelve different Kas I alleles were found in the landraces, which predicted six novel Kas proteins in these landraces. Seven alleles of Ccs were found, which predicted three novel CCS proteins. These results demonstrate that the landraces under cultivation in small farms and pueblos in northern New Mexico are sources of important genetic diversity for Capsicum crops.
San Juan, Santo Domingo, Velarde, and Zia Pueblo, were purchased from Native Seed/SEARCH (Tucson, AZ). Seed was sown in greenhouses on the New Mexico State University (NMSU) campus and at nearby Fabian Garcia Research Center (FGRC), Las Cruces NM. Seedlings from the FGRC were transplanted in a field at the NMSU Los Lunas Agricultural Experimental Station. After transplanting in Los Lunas, field-grown plants were watered and fertilized as needed and according to standard production practices for the area. Plants were also grown to maturity on the NMSU campus in a greenhouse in 13.5-L pots with SunGro Metro-Mix 360 (Agawam, MA), soilless growing medium, fertilized with Osmocote (14N–14P–14K) (Scotts Miracle-Gro, Marysville, OH), and watered daily. Mature red fruit from at least three independent plants were harvested from both growing areas and processed for pigment and capsaicinoid extractions.

**Carotenoid and capsaicinoid extractions.**

Fruit from the plants grown in the greenhouse were dried in an oven at 50 °C for 2 d, and fruit from the field-grown plants were dried at 40 °C for 1 week. The seeds were removed and five to six dry peppers per plant were pooled and ground to fine powder in a coffee grinder. For each sample, 1 g dry ground fruit was extracted with chloroform (25 mL). An aliquot (≥1 mL) was retained for capsaicinoid composition analysis by gas chromatography–flame ionization detection (GC-FID), whereas the remainder was processed for carotenoids. Capsaicinoid separation by GC-FID was achieved on a 30 m x 0.25-mm I.D. Rxi-5 Sil MS column (Restek, <http://www.restek.com/>); injection volume, 5 μL, split 20:1; chromatographic conditions: 1 min at 80 °C; temperature ramp of 20 °C/min up to 200 °C, then 10 °C/min up to 320 °C, hold for 5 min at 320 °C; and FID detector at 250 °C. Calibration curves (50 to 10,000 mg/L) were generated for capsaicin and dihydrocapsaicin using reference standards (Sigma, <http://www.sigmaaldrich.com/united-states.html>); technical replicates were less than 5.0% CV. Representative chromatograms for the GC-FID detection of capsaicin and dihydrocapsaicin are presented (Fig. 1).

For detection of carotenoids, the chloroform extract was concentrated under a stream of nitrogen, resuspended in 2-propanol, and an aliquot saponified before separation by high-performance liquid chromatography (HPLC) as described earlier (Rodriguez-Uribe et al., 2012). Calibration curves for the carotenoids on the HPLC were generated at 450 nm using the reference standards β-carotene (Sigma, <http://www.sigmaaldrich.com/united-states.html>) and capsanthin, β-cryptoxanthin, violaxanthin, and zeaxanthin (CaroteNature GmbH, <http://www.carotenature.com/>). Representative chromatograms for the HPLC detection of carotenoids are presented (Fig. 2).

Total carotenoids were estimated as the sum of the total peak area in the HPLC chromatogram with absorbance at 450 nm. The calibration curve for β-carotene was used to convert this absorbance to mass units for total carotenoids because there were peaks for unknown carotenoids without calibration standards.

**DNA extraction and PCR analysis.**

Genomic DNA was isolated from pooled leaves collected from three plants from each landrace grown in the greenhouse using a cetyl trimethyl ammonium bromide protocol (Rogers and Bendich, 1985). Polymerase chain reaction (PCR)-based amplification was carried out with Platinum PCR SuperMix High Fidelity (Invitrogen Life Technology, Grand Island, NY). The primers for Ccs and Kas I were designed to amplify the entire gene. Ccs primer sequences were: forward AGCCAT ATGTATAGTTTCAAACACA and reverse GTGCCTCGAGTCAAAGGCTCT CATTGCTAGATTC. The cycle conditions for Ccs amplification were: one cycle 95 °C 5 min; 35 cycles 94 °C 30 s, 58 °C 40 s; and a final extension at 68 °C for 2 min. Kas I primer sequences were: forward GATTA CAAATGCCAG CAAAGCTCTGTTGC and reverse GTCAAGGTTTGTAGGGTGC. The cycle conditions for Kas I amplification were: 95 °C 3 min; 35 cycles 94 °C 30 s, 57.5 °C 50 s; and a final extension at 68 °C for 4.5 min.

Amplicons from both genes were cloned into pGEM-T Easy Vector (Promega, Madison, WI) as described by the manufacturer. Plasmid DNA from at least six independent clones of Ccs and Kas I from each landrace was isolated.

![Fig. 1. Gas chromatography–flame ionization detection (GC-FID) of capsaicinoids.](image-url)
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sequence contigs, protein translation predictions, and multiple sequence alignments were performed using the DNAStar software suite (<http://www.dnastar.com/>). Phylogenetic analysis was performed using software provided by Phylogeny.fr (Dereeper et al., 2008; <http://www.phylogeny.fr/>). The C. annuum reference genes for Ccs was GenBank HQ229922.

Statistical analysis. The mean concentration for each specific metabolite was calculated using the General Linear Model (GLM) procedure (SAS 9.4; SAS Institute Inc., Cary, NC) based on independent chromatographic analysis of at least triplicate individuals for each landrace. Duncan’s multiple range test using the GLM procedure determined whether there were significant differences between the means for these metabolite concentrations among the landraces. Unpaired Student’s t-tests were conducted to determine if the metabolite concentration in fruit in a specific landrace was different when grown in the field compared with the greenhouse.

Results and Discussion

Carotenoid composition in New Mexican landraces. The fruit in all 13 New Mexican chile landraces are pungent and mature to a red color. Tables 1 and 2 report the mature fruit carotenoid content in field-grown and greenhouse-grown plants, respectively; the entries in the tables are ordered alphabetically by landrace name. Across all landraces and in both environments, capsanthin was the most abundant carotenoid, representing close to one-third of the total carotenoid content, whereas β-carotene was usually the least abundant carotenoid. Between landraces within an environment, there was a great deal of overlap in the abundance levels of the carotenoids. For example, in the field (Table 1) there were no differences between landraces for the accumulation of β-carotene or violaxanthin. In the greenhouse (Table 2), there were no differences between landraces for the accumulation of either total carotenoids or zeaxanthin. For the red pigment capsanthin, fruit from field-grown Alcalde and San Juan landraces had higher levels than Chiyamo or Escondida. In the greenhouse environment, capsanthin levels were higher in Jemez landrace than in Cochiti or Jarales.

To investigate the effect of environment on accumulation of carotenoids, unpaired, one-tailed Student’s t-tests were run on the specific carotenoids accumulated by specific landraces in the field vs. the greenhouse. The levels for β-cryptoxanthin in 12 landraces (in all but San Felipe) and capsanthin (San Juan, Casados and Zia Pueblo), violaxanthin (Chiyamo, Casados, and Santo Domingo), or β-carotene in three landraces (Isleta, Jarales, and Escondida) were higher in field-grown fruit than greenhouse-grown fruit.

The carotenoid levels reported here are similar to those found for cultivars of C. annuum. The cultivar, NuMex Garnet, which was bred for low pungency and high pigment content (Walker et al., 2004), accumulated 116 μg·g⁻¹ fresh weight of capsanthin and 36 μg·g⁻¹ fresh weight of β-carotene (Rodriguez-Uribe et al., 2012). These values would be 10-fold higher if expressed on a fruit dry weight basis as is presented in Tables 1 and 2. Other reports of carotenoid levels in red-fruited Capsicum sp. determined capsanthin levels at 180 to 210 μg·g⁻¹ dry weight.

Table 1. Carotenoids in field-grown mature fruit of New Mexican Capsicum annuum landraces.†

| Landrace | β-carotene | β-cryptoxanthin | Zeaxanthin | Violaxanthin | Capsanthin | Total |
|----------|------------|-----------------|------------|--------------|------------|-------|
| Alcalde  | 18.4 a      | 67.6 abcde      | 160.9 ab   | 117.0 a      | 1,006.8 a  | 3,481.6 a |
| Casados  | 14.4 a      | 53.3 bcde       | 168.0 a    | 105.8 a      | 918.2 ab   | 2,860.1 abc |
| Chiyamo  | 13.8 a      | 39.1 cde        | 82.8 bc    | 62.5 a       | 467.8 b    | 1,808.2 bc |
| Cochiti  | 17.6 a      | 56.1 bcde       | 77.3 bc    | 78.4 a       | 771.1 ab   | 2,502.9 abc |
| Escondida| 21.6 a      | 37.4 cde        | 73.8 c     | 72.4 a       | 465.2 b    | 2,183.4 abc |
| Isleta   | 22.0 a      | 97.3 a          | 115.2 abc  | 96.0 a       | 872.5 ab   | 2,910.1 abc |
| Jarales  | 15.5 a      | 56.2 bcde       | 127.2 abc  | 66.7 a       | 673.0 ab   | 2,216.9 abc |
| Jemez    | 13.0 a      | 39.8 cde        | 143.7 abc  | 109.8 a      | 869.2 ab   | 2,736.2 abc |
| San Felipe| 12.5 a   | 92.8 e          | 81.8 bc    | 61.8 a       | 518.5 ab   | 1,697.7 c  |
| San Juan | 18.5 a      | 87.8 ab         | 169.0 a    | 125.8 a      | 977.0 a    | 3,186.0 ab |
| Santo Domingo| 13.9 a | 38.7 cde       | 116.2 abc  | 63.2 a       | 761.0 ab   | 2,168.6 abc |
| Velarde  | 16.8 a      | 72.6 abc        | 116.4 abc  | 125.2 a      | 882.0 ab   | 2,876.8 abc |
| Zia Pueblo| 14.2 a | 29.9 de         | 150.6 abc  | 88.8 a       | 897.0 a    | 2,511.9 abc |

†High-performance liquid chromatography was used to quantify pigments expressed as μg·g⁻¹ dry weight fruit.

Table 2. Carotenoids in greenhouse-grown mature fruit of New Mexican Capsicum annuum landraces.‡

| Landrace  | β-carotene | β-cryptoxanthin | Zeaxanthin | Violaxanthin | Capsanthin | Total |
|-----------|------------|-----------------|------------|--------------|------------|-------|
| Alcalde   | 17.3 b     | 9.6 ab          | 96.6 a     | 33.6 b       | 530.5 ab   | 2,215.5 a |
| Casados   | 13.3 b     | 7.4 abc         | 121.1 a    | 48.5 ab      | 651.1 ab   | 1,870.4 a |
| Chiyamo   | 10.2 b     | 6.3 b           | 84.3 a     | 50.8 ab      | 635.4 ab   | 2,012.1 abc |
| Cochiti   | 14.5 b     | 7.8 ab          | 131.2 a    | 46.2 ab      | 330.4 b    | 2,348.2 abc |
| Escondida | 15.6 b     | 12.8 a          | 112.6 a    | 35.7 b       | 627.1 ab   | 2,186.3 abc |
| Isleta    | 5.0 b      | 6.1 b           | 89.9 a     | 34.3 b       | 444.8 b    | 1,347.5 a |
| Jemez     | 13.5 b     | 6.8 b           | 154.8 a    | 59.9 ab      | 811.6 a    | 2,295.5 a |
| San Felipe| 8.2 b      | 10.2 a          | 115.3 a    | 63.7 a       | 681.2 ab   | 2,376.5 a |
| San Juan  | 16.1 b     | 9.8 ab          | 109.9 a    | 35.2 b       | 673.5 ab   | 2,392.6 abc |
| Santo Domingo| 12.6 b  | 9.1 a           | 112.3 a    | 33.3 b       | 670.4 ab   | 2,108.9 a |
| Velarde   | 34.9 a     | 11.6 ab         | 132.9 a    | 47.2 ab      | 687.5 ab   | 2,285.6 a |
| Zia Pueblo| 12.3 b     | 6.2 b           | 126.3 a    | 35.5 b       | 672.9 ab   | 2,295.5 a |

‡High-performance liquid chromatography was used to quantify pigments expressed as μg·g⁻¹ dry weight fruit.

Mean values within a column followed by different letters are significantly different Duncan’s multiple range test (P = 0.05).
1013 µg·g⁻¹ dry weight depending on variety (Ha et al., 2007); several New Mexican landraces had capsanthin levels close to 1000 µg·g⁻¹ dry weight (Table 1).

Capsaicinoid composition in New Mexican landraces. Table 3 reports the mature fruit capsaicin and dihydrocapsaicin content in field-grown and greenhouse-grown plants; the entries in the table are rank-ordered by the total capsaicinoid content in field-grown fruit. The capsaicinoid levels of the landrace fruit were variable and the levels were higher in fruit grown in field conditions as compared with the greenhouse. Under field conditions, the fruit of the landrace San Felipe had the highest level of capsaicinoids; in contrast, this landrace grown in the greenhouse had very low levels of capsaicinoids. Under greenhouse conditions, fruit of the landrace San Juan had the highest levels of capsaicin. Landraces Casados, Alcalde, and Isleta consistently had mild or low capsaicinoid levels in their fruit grown in either the field or the greenhouse. To investigate the effect of environment on accumulation of capsaicinoids, unpaired, one-tailed Student’s t tests were run on the capsaicin and dihydrocapsaicin amounts accumulated by specific landraces in the field vs. the greenhouse. Four landraces, Zia Pueblo, Jemez, Casados, and Isleta, all accumulated higher levels of both capsaicinoids in the field as compared with the greenhouse. Three landraces, Escondida, Santo Domingo, and Chimayo, accumulated higher levels of dihydrocapsaicin in the field compared with the greenhouse.

One novel feature of the capsaicinoid content of the landrace fruit was the very high dihydrocapsaicin concentration. Typically capsaicin is twice as abundant as dihydrocapsaicin. The cDNA version of Kas I (Bosland, 1996; Kozukue et al., 2005; Wahyuni et al., 2011). Ratios of the capsaicin to dihydrocapsaicin were calculated and were compared with three C. annuum cultivars: Jalapeno PX206, Jalapeno PX211 (Keyhaninejad et al., 2014), and Santa Fe Grande (Fig. 3). Under field conditions, all of the landraces had higher concentrations of dihydrocapsaicin than capsaicin. Under greenhouse conditions, these compounds were present at similar relative abundances, resulting in ratios close to 1.0. One landrace, Jarales, when cultivated in the greenhouse had a 2-fold ratio of capsaicin to dihydrocapsaicin.

The capsaicinoid content for the New Mexican landraces was similar to the level reported for cultivars of the “New Mexican type.” The total capsaicinoid content in these landraces ranged between 250 and 1750 µg·g⁻¹ dry weight fruit. A mild cultivar grown in the area, ‘NuMex Heritage 6-4’, has total capsaicinoids at 1000 µg·g⁻¹ dry weight fruit (Bosland, 2012), whereas a slightly hotter type, ‘NuMex Heritage Big Jim’, has total capsaicinoids at 600 µg·g⁻¹ dry weight fruit (Bosland and Coon, 2013). However, the high levels of dihydrocapsaicin in the landraces were unusual for C. annuum lines. High ratios of dihydrocapsaicin to capsaicin, as observed in these landraces, have been reported in C. pubescens Ruiz and Pavon (Collins et al., 1995; Zewdie and Bosland, 2001).

Genetic variability among the New Mexico landraces capsaicinoid biosynthetic gene Kas I. Genomic DNA, isolated from the 13 landraces, was amplified using the primers for Kas I. The amplicon products were 3.4 kb and contained all of the exons and seven introns in this gene. A total of 12 distinct Kas I alleles (a, c to m) were detected within the 13 landraces (GenBank accessions: KM037707 to KM037726). Table 4 lists the alleles observed within each landrace sample. Half of the DNA polymorphisms that produced these alleles were located in the intronic regions. DNA polymorphisms were detected in introns 1, 2, 5, and 7. Some alleles, like Kas I allele a, were detected in five different landraces, but most of the other alleles were detected in only one landrace. Six independent clones were sequenced for each population, but for an allele to be scored as present, that unique sequence variant needed to be detected in two or more clones. This filter was applied to rule out technical sources of sequence variability. Because our biological sample size was limited to only three individuals for each landrace, these results likely underestimate the Kas I allelic diversity within these populations.

The cDNA sequences for the Kas I genes were assembled from the genomic sequences and a multiple sequence alignment of those sequences was obtained, which included the cDNA version of Kas I from C. annuum cv. Yidu-Red (GeneBank HQ229922). A phylogenetic tree was generated using this multiple sequence alignment (Fig. 4). The exons for alleles a and j were identical as were the exons for alleles d and e and alleles f, i, k, and m.

Table 3. Capsaicinoids in mature fruit of New Mexican Capsicum annuum landraces. *

| Field | Capsaicin | Dihydrocapsaicin | Greenhouse | Capsaicin | Dihydrocapsaicin |
|-------|-----------|------------------|------------|-----------|------------------|
| San Felipe | 719.5 a** | 1,024.9 a | 13.4 b | 13.3 b |
| Zia Pueblo | 410.4 ab*** | 921.4 ab** | 159.9 b | 253.1 b |
| Escondida | 399.6 ab | 508.6 abc** | 93.3 b | 125.6 b |
| Jemez | 332.6 ab** | 477.0 abc** | 149.3 b | 148.4 b |
| Cochiti | 284.9 b | 405.0 bc | 60.8 b | 71.3 b |
| Velarde | 280.6 b | 436.0 abc | 520.3 ab | 684.0 a |
| Santo Domingo | 252.5 b | 409.4 abc** | 289.4 ab | 272.2 b |
| San Juan | 236.4 b | 323.6 bc | 710.0 a | 369.4 ab |
| Jarales | 216.5 b | 329.2 bc | 169.1 b | 84.1 b |
| Chimayo | 142.4 b | 293.7 c** | 215.2 ab | 206.4 b |
| Casados | 126.6 b** | 167.6 c** | 80.3 b | 49.2 b |
| Alcalde | 118.7 b | 158.5 c | 128.9 b | 145.0 b |
| Isleta | 103.1 b** | 146.7 c** | 10.6 b | 12.1 b |

*Gas chromatography–flame ionization detection was used to quantify capsaicinoids expressed as µg·g⁻¹ dry weight fruit.

Mean values within a column followed by different letters are significantly different Duncan’s multiple range test (P = 0.05).

Mean values followed by ** are higher in field- vs. greenhouse-grown fruit; Student’s t test, unpaired, one-tailed (P ≥ 0.05).

Fig. 3. Ratio of capsaicin to dihydrocapsaicin in C. annuum fruit. Capsaicinoid content was determined in fruit collected from field- (dark gray) or greenhouse- (light gray) grown plants. Student’s t test (unpaired, two-tailed) on the means of the ratio of capsaicin to dihydrocapsaicin were calculated on each landrace grown in the two environments; significantly different means are indicated (****).
The alleles c, g, h, and l were unique. None of the landraces were identical to the Kas I sequence published in GenBank for C. annuum; the reference sequence was the most distant sequence on the phenogram.

From a functional perspective, the DNA sequence polymorphisms detected in the Kas I alleles resulted in only a few amino acid changes; altogether there were six distinct predicted Kas I protein products (KAS I A to F). Kas I encodes a protein 489 amino acids long; all of the landraces had a Kas I with amino acid changes as a result of DNA sequence variability. The positions of amino acid changes in Kas I and the landraces with these variants are presented in Table 5. None of these changes altered the amino acids at the active site motifs TACAT (aa 237 to 241) and the histidines at positions 351 and 317 (Aluru et al., 2003; Siggaard-Andersen et al., 1991).

Table 5. Distribution of Kas I and Ces alleles in the New Mexican landraces

| Landrace       | Kas I alleles | Ces alleles |
|----------------|---------------|-------------|
| Alcalde        | h             | f           |
| Casados        | a             | f           |
| Chimayo        | a             | f, a        |
| Cochiti        | d             | f           |
| Escondida      | j, k          | e, f        |
| Isleta         | i             | f           |
| Jarales        | k, l          | f, g        |
| Jemez          | c             | a, b        |
| San Felipe     | a, g          | f           |
| San Juan       | a             | f           |
| Santo Domingo  | f, m          | f           |
| Velarde        | a             | a, c        |
| Zia Pueblo     | e             | f           |

Table 4. Distribution of Kas I and Ces alleles in the New Mexican landraces

Fig. 4. Phylogenetic relationship of Kas I in C. annuum New Mexican landraces. The analysis was performed using the Phylogeny.fr platform (MUSCLE, PHYLIP, and Drawgram). The exonic sequences were assembled from the genomic DNA sequences of the 12 Kas I alleles (a to m) and the sequences were aligned with MUSCLE; the phylogenetic tree was constructed using the PHYLIP neighbor joining method, and a phenogram was generated using Drawgram. The landrace source of the allele is indicated in the figure.

Table 5. Amino acid differences in Kas I proteins in New Mexican landraces.

| Amino acid position | 55 | 105 | 140 | 220 | 327 | 361 | 434 | Landraces |
|---------------------|----|-----|-----|-----|-----|-----|-----|-----------|
| wt Kas I            | G  | R   | H   | A   | M   | A   | A   | Alcalde   |
| KAS IA              | E  | S   | H   | A   | S   | K   | T   | Casados, Escondida, Isleta, Jarales, Casados, Escondida, Isleta, Jarales, Santo Domingo |
| KAS IB              | G  | S   | H   | A   | S   | K   | T   | Casados, Escondida, Isleta, Jarales, Casados, Escondida, Isleta, Jarales, Santo Domingo |
| KAS IC              | V  | S   | R   | A   | S   | K   | T   | Jemez     |
| KAS ID              | G  | S   | R   | A   | S   | K   | T   | San Juan, Velarde, Casados, Escondida, Isleta, Jarales, Casados, Escondida, Isleta, Jarales, Santo Domingo |
| KAS IE              | G  | S   | R   | A   | A   | K   | T   | Casados, Escondida, Isleta, Jarales, Casados, Escondida, Isleta, Jarales, Santo Domingo |
| KAS IF              | G  | S   | R   | T   | S   | K   | T   | San Felipe |

*Single letter codes for amino acids are indicated: A = alanine; E = glutamic acid; G = glycine; H = histidine; K = lysine; M = methionine; R = arginine; S = serine; T = threonine; V = valine.

The amino acid sequences of CCS-A and -B both had the conserved domains for function identified earlier (Jeknic et al., 2012; Mialoundama et al., 2010). Specifically, the putative cleavage site of the N-terminal plastid transit peptide (FLDLA aa 54 to 58), the domain essential for B- and K-cyclase activity (FLEET motif aa 293 to 297), the conserved amino acids with important catalytic activity (MDW aa 258 to 260, E 332, and VHPS 359 to 362), and the potential dinucleotide binding site (aa 88 to 103 GTGPAGPRLAEQVKY) were found in all the CCS proteins except in CCS-4. The amino acid substitutions that were detected were conservative replacements at three of the four sites with changes in CCS-A and CCS-B: V126I, Y270H, and T483A.

Fig. 5. Amino acid sequence alignments of Kas I alleles from the New Mexican landraces. The positions of amino acid changes and thus variant Kas I alleles are shown by the letter above the corresponding residue.

Conclusions

The recurrent selection for red pungent fruit among the New Mexican chile landraces has generated a set of germplasm that appears to be suited to the environment of New Mexico. The fruit quality of these landraces measured in terms of pigment accumulation was better in fruit cultivated in fields in New Mexico than under greenhouse conditions, and the carotenoid content approached the levels reported for in cultivars bred for high pigment. The content of one of the capsaiacinoids, dihydrocapsaicin, was also higher in fruit of landraces cultivated in fields in New Mexico in contrast to greenhouses. The genetic variability between landraces was very low, measured in terms of DNA sequence differences in the Kas I and Ces genes. All of the amino acid sequences identified as important in CCS function were conserved among the two functional CCS variants detected in the landrace populations. Similarly, among the more polymorphic alleles for Kas I, there were very few changes in gene sequence that resulted in amino acid differences in the predicted protein; again, none of these changes involved amino acids identified as important in enzyme activity. The polymorphisms for Kas I and Ces were independently observed in the populations; in some landraces, for example Velarde, only one allele for Kas I was detected, but this
Table 6. Amino acid differences in CCS proteins in New Mexican landraces.a

| Amino acid position | Landraces |
|---------------------|-----------|
| 79 126 203 270 483  |           |
| wt CCS G V C Y T   | Alcalde, Casados, Chimayo, Cochiti, Escondida, Isleta, Jarales, San Felipe, San Juan, Santo Domingo, Zia Pueblo |
| CCS-A R V C Y T    |                       |
| CCS-B R I C H A     | Jarales               |
| CCS-4 R V stop      | Chimayo               |

*aSingle letter codes for amino acids are indicated: A = alanine; C = cysteine; G = glycine; H = histidine; I = isoleucine; R = arginine; T = threonine; V = valine; Y = tyrosine.

sample had two alleles for CCS. A full analysis of the genetic diversity at the DNA sequence level within these landrace populations will need larger sample sizes and more loci screened.

The New Mexican landrace population does present a novel trait, high dihydrocapsaicin content, which will be interesting to investigate in the future. The biosynthetic pathway for capsaicin is partially described, but the regulation of this pathway needs further study. Comparisons between these landraces and selected cultivars may reveal important aspects about the control of the capsaicin and dihydrocapsaicin content.

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