Research Article

Screening Previously Uncharacterized Genotypes of Hot Pepper for Ascorbic Acid, Phenols, β-Carotene, and Soluble Sugars Content

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Abstract

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Surveys of pepper genotypic variation are useful for identification of breeding materials for improved composition and quality of fruits. Hot pepper seeds of *Capsicum* spp. of previously uncharacterized twenty-nine genotypes were planted under field conditions in a randomized complete block design (RCBD). At harvest, mature fruits were collected for extraction and quantification of ascorbic acid (vitamin C), vitamin A (β -carotene), total phenols, and soluble sugars content and measurement of fruit morphological characteristics (fruit length and diameter). Vitamin C content ranged from 217 to 583 µgg⁻¹ fresh fruit. Total phenols and soluble sugars ranged from 203 to 807 and from 4127 to 10474 µg g⁻¹ fresh fruit, respectively.

 β -carotene ranged from 3 to 100 mg g⁻¹ fresh fruits. This variability of Capsicum spp. has important implication in pepper breeding programs. The results also revealed a great variability in fruit morphological characteristics. Fruits of PI485593 (C. chinense), PI 169129 and PI 257048 (C. annuum) were significantly higher in length (13, 10, and 11 cm, respectively). Whereas, fruits of PI 639661 (C. frutescens) and PI 138565 (C. annuum) had the greatest diameter (5.5 and 3.6 cm, respectively). Fruits of PI 485594 and PI 210980 had the greatest weight among the genotypes tested. Screening of Capsicum genotypes revealed that PI 631144 is a candidate genotype for ascorbic acid and phenols content, whereas, PI 127442 and PI 241676 are potential candidates for β -carotene and soluble sugars content for use in fruit quality improvement of pepper breeding programs.

INTRODUCTION

Peppers contain high concentrations of various antioxidants and are widely known for their medicinal properties and food flavor. The consumption and public interest for pepper have been increasing [1]. Food producers have become more interested in growing new crops to meet the growing demands of consumers who are looking for food with better health benefits [2]. Several studies have acknowledged the positive effects of fruit and vegetable consumption on human health and many health benefits have been attributed to their ascorbic acid (vitamin C) and phenols content. According to Naidu [3], the U.S. recommended daily allowance (RDA) for vitamin C varies from 100-120 mg day⁻¹ for adults. A dietary intake of 100 mg day⁻¹ of vitamin C is associated with a reduced risk of heart and cancer diseases [4].

Among the domesticated pepper species, *Capsicum annuum* is the most worldwide cultivated species and is found in tropical America and southeastern Asia. *C. chinense* that includes some of the most-spicy peppers, commonly recognized as habanero peppers, is found in America and commonly grown in the amazon region of South America. *C. baccatum* is found in Bolivia and is distinguished by its characteristic cream-colored flowers, and *C.*

pubescens is found in Mexico and Central America [5]. Capsicum was among the earliest new-world crops. Records showed that chili peppers had a long history in Latin America [6]. Additionally, Perry et al. [7], stated that there is evidence of *C. annuum* being dated back to at least 6000 BP in the Western hemisphere. Antioxidants in Capsicum spp. interact with free radicals and stabilize them by donating hydrogen atoms. Accordingly, antioxidants, such as capsaicin, dihydrocapsaicin, vitamin C, β-carotene and phenols in plants prevent free-radicals, known as reactive oxygen species (ROS) that create from food metabolism, from oxidizing and harming biomolecules [8,9]. Ball [10] stated many potential roles of ascorbic acid as a cofactor for enzymes, absorption of inorganic iron, and regulation of neurotransmitter systems and improvement of immunity against diseases such as the common cold. Low levels of ascorbic acid in individual's diet can lead to anemia, gingival bleeding, capillary hemorrhages, poor wound healing, increases in risk of allergic reactions, decreases in resistance to infectious diseases and inhibition in the immune system [3]. A survey of β -carotene content in 63 accessions of C. chinense revealed variability that could be a useful source of antioxidants [11]. β -carotene, a significant component of ripe pepper fruits, may differ 3 to 15-fold between green and red fruit [12]. Depending on variety, pepper fruits show a range

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of coloration, including green, white, yellow, orange, and red. A survey of red, orange, yellow and green *C. annuum* varieties revealed over 200-fold variation in total carotenoid levels [13], with the lowest levels in green cultivars and the highest in red. Rodriguez-Burruezo et al.[14], found a complete absence of red carotenoids (capsanthin and capsorubin) in *C. pubescens* and *C. baccatum* pepper accessions with yellow or orange fruit.

The study of pepper composition is rapidly expanding as interest in enhancing crop quality rises. Research revealed how levels of plant contents, such as vitamin C depend on fruit development, fruit growing conditions [15] and variability among species and genotypes of the same species due to natural genetic variability [11]. Providing this information, breeders can choose certain accessions (genotypes) that contain beneficial antioxidants such as vitamins C and A for use in breeding programs to promote production of pepper as an industrial crop. Peppers would be one of the better options for a crop that yields high vitamin C content as it is comparable to concentrations present in citrus fruits [16]. Hot peppers are well known within the U.S. and are essential in foods of Mexican, African and Asian cuisines. In Brazil, C. frutescens and C. chinense are exceptional ingredients in several regional dishes [17]. In Mexico, chiltepin pepper (Capsicum annuum L. var. glabriusculum) is a source of phenolic compounds, carotenoids, tocopherols, and vitamin C [18]. In 2016, the Food Chemistry Weekly News reported that total phenolic compounds in Capsicum annuum L. that contains chlorogenic acid, inhibited pancreatic lipase and lipid peroxidation in human [19]. The carotenoids also have been related with numerous health benefits such as the prevention of vitamin A deficiency, anti-ulcer properties, anti-aging effects and general antioxidant activity [20]. The U.S. National Plant inventory holds a large collection of *Capsicum* germplasm at the Southern Plant Introduction Station located in Griffin, Georgia. This collection contains around 5000 Capsicum genotypes, and genetic resources [1].

The main objective of this investigation was to screen and characterize twenty-nine previously uncharacterized *Capsicum* spp. for ascorbic acid (vitamin C), β -carotene (vitamin A), total phenols, soluble sugars content, and fruit quality characteristics (fruit length, diameter, and weight) for potential use in hot pepper breeding programs for improvement in fruit quality.

MATERIALS AND METHODS

Twenty-nine (29) *Capsicum* genotypes were selected from the United States Department of Agriculture (USDA) *Capsicum* Germplasm collection and grown under field conditions in a drained soil, soil pH of 6.8, and 2.2% organic matter. Seedlings, eight weeks old, were planted on June 9, 2016 in a randomized complete block design (RCBD) in raised beds with black plastic mulch and drip irrigation. These genotypes were: twelve *Capsicum annuum* (PI123474, PI127442, PI138565, PI159256, PI164271; PI169129, PI200725, PI210980, PI215743, PI241670, PI246331, PI257048); four *Capsicum baccatum* (GRIF 9213, PI260539, PI260571, PI439409); ten *Capsicum chinense* (PI209028, PI224443, PI238047, PI238051, PI257136, PI439464, PI594139, PI485593, PI439420, PI281443); and three *Capsicum frutescens* (PI241676, PI639661, PI631144). The selection of these genotypes was based on a previous work on 52 genotypes originally acquired from different world-wide locations and planted at University of Kentucky Research Farm for testing early fruit maturity and plant size (data not shown). As a result of our previous work, 29 genotypes were selected for quantification of capsaicin and dihydrocapsaicin [21]. Accordingly, this investigation is a continuation of our previous work on internal composition of hot pepper to test the same 29 genotypes for their fruit composition of other antioxidants (vitamin C, vitamin A, and total phenols).

At harvest (Sept. 21), matured fruits of 140 day-old were collected for extraction and quantification of ascorbic acid, phenols, β -carotene, and soluble sugars. Fruit characteristics (length, diameter, and weight) also were measured. Ascorbic acid was extracted by blending 50 g of fruits with 100 mL of 0.4% oxalic acid solution [11]. The extracts were decanted using filter paper No. 1 and Buchner funnel. The volume of the filtrate was measured and one mL was used for quantification of ascorbic acid using the potassium ferricyanide method [22]. The reaction of ascorbic acid with potassium ferricyanide at pH 3.5 produces ferrocyanide ions that transform to ferrous ions by the catalytic action of mercuric ions (HgCl₂) added to the reacting solutions. The ferrous ions react with 1, 10-phenanthroline to produce a red color complex that was measured at 510 nm. A calibration curve was established using 10 -100 µg mL⁻¹ ascorbic acid standard of 99% purity.

Pepper fruits were also cut into small pieces and 30 g representative subsamples were blended in a household blender at high speed with 100 mL of acetone for 2 min in dim light to extract β-carotene [23].The homogenate was filtered under suction through a Buchner funnel containing Whatman filter paper No. 1 (Fisher Scientific). The resulting thick paste was extracted twice with acetone until the extract was colorless. The filtrates were combined, transferred to separatory funnels containing 50 mL of 4% aqueous NaCl and 100 mL of petroleum ether (BP 40-60°C). After shaking the mixture for one min., the absorption of the petroleum ether layer was measured at 450 nm in dim light. A calibration curve was prepared using 99% pure β -carotene in the range of 10–100 mg mL⁻¹. Representative fruit samples (20 g) were blended with 150 mL of 80% ethanol to extract phenols. Homogenates were filtered through Whatman No. 1 filter paper, and 1 mL aliquot of filtrate was used to determine total phenols [23,24] using a standard calibration curve (1-16 mg mL⁻¹) of chlorogenic acid. To determine the degree of fruit sweetness, soluble sugars were extracted from pepper fruits with 80% ethanol and quantified colorimetrically using methods modified by Antonious et al. [25]. A calibration curve was established for each group of samples using 10, 20, 30, and 50 mg mL⁻¹ glucose standards. Purified standards of β -carotene, ascorbic acid, chlorogenic acid, and glucose were obtained from Sigma-Aldrich Inc. and used to obtain calibration curves.

Concentrations of ascorbic acid, total phenols, soluble sugars, and β -carotene in each genotype were statistically analyzed using ANOVA [26], and the means were compared using Duncan's test for mean comparisons.

RESULTS AND DISCUSSION

The results revealed significant variations in ascorbic acid,

 β -carotene, phenols, and sugars fruit content among the 29 genotypes tested. Ascorbic acid content ranged from 217 to 583 μg g⁻¹ fresh fruits. Previous studies had reported that variability in ascorbic acid content may have been due to differences in fruit maturity [27]. In our investigation, all harvested fruits were collected at maturity. Variability in ascorbic acid content of hot pepper genotypes used in this investigation are in agreement with the study of Jarret et al. [28], that showed a great range of variation in ascorbic acid content among 216 different C. chinense accessions collected from North, South and Central America. Based on our results, it could be concluded that the three genotypes, PI 631144, PI439420, and PI 241676 contained the greatest concentrations of ascorbic acid among the genotypes tested and these three genotypes could be recommended in plant breeding programs to increase hot pepper content of ascorbic acid (Figure 1). Table 1 also contains countries of origin of the twenty-nine genotypes tested. Fruits of PI 631144 from C. frutescens are originated in Guatemala. Fruits of PI 439420 from C. chinense, originated in Colombia, and fruits of PI 241676 are originated in Ecuador.

Morphological characteristics also showed significant variations. Fruits of PI 485593 (*C. chinense*), PI 169129 (*C. annuum*) and PI 257048 (*C. annuum*) were significantly greater in length (13, 10, and 11 cm, respectively) compared to other fruits of the genotypes tested. Whereas, fruits of PI 639661(*C. frutescens*) had the greatest fruit diameter (5.5 cm) and fruits of

PI 485593 (*C. chinense*) had the greatest fruit weight (33.4 g) and length (12.7 cm).

Pungent types of peppers had more phenolic compounds than sweet types, given that pungency is due to capsaicinoids, important phenolic compounds in pepper [29]. PI 631144of *C. frutescens* from Guatemala contains the greatest concentrations of capsaicin and dihydrocapsaicin, the main two capsaicinoids responsible for pungency in hot pepper [30]. PI 631144 also was found to contain significant concentrations of total phenols similar to PI 639661 and PI 241676 (Figure 2). When different pepper species were compared, *C. chinense* and *C. baccatum* had significantly more phenolics than *C. annuum, C. frutescens* and *C. pubescens* accessions [14,31]. Pepper usually ranks first or second in terms of phenolic content (including flavonoids and capsaicinoids) with levels greater than other high-phenolic vegetables including spinach, broccoli and garlic [32,33].

Levels of β -carotene in many varieties of fresh and dried peppers is sufficient to meet 100% of the recommended daily amount (RDA) for vitamin A in adults [34]. The authors [34] assessed 57 varieties across six pepper species (*C. annuum, C. baccatum, C.chacoense, C. chinense, C. eximium,* and *C. frutescens*) and found a direct correlation between β -carotene concentration and total carotenoids. Variability in β -carotene can be ascribed to the influence of light on pigment biosynthesis; it is hypothesized that elevated light levels in the field [14] or greenhouse [35] stimulate carotenoids as a defense mechanism against photo-



Figure 1 Concentrations of ascorbic acid in mature fruits of Capsicum genotypes grown at the University of Kentucky South Farm (Fayette County, KY).

Table 1: Fruit characteristics and origin of twenty-nine previously uncharacterized genotypes of *Capsicum* spp. grown at the University of Kentucky South Farm (Fayette County, KY).

Pepper Species	Genotype	Fruit Length, cm	Fruit Width, cm	Fruit Weight, g	Country of Origin
Capsicum annuum	PI123474	5.88hi	2.37gh	12.23defg	India
	PI127442	2.36mno	2.10hij	4.40jk	Afghanistan
	PI138565	2.87klmn	3.64 b	14.29de	Iran
	PI159256	8.59d	1.97ij	8.77hi	USA
	PI164271	7.52e	2.62fg	13.88de	India
	PI169129	10.32b	2.09hij	12.60def	Turkey
	PI200725	3.08klm	1.59k	4.40 jk	Guatemala
	PI210980	7.42e	3.25 cd	22.34b	USA
	PI215743	5.94hi	2.12hij	10.40gh	Peru
	PI241670	9.32c	0.91mn	4.68jk	Ecuador
	PI246331	7.06efg	1.15 lm	2.80kl	Sri lanka
	PI257048	10.54b	2.80ef	23.41b	Colombia
Capsicum baccatum	GRIF 9213	6.63fgh	1.10m	4.72jk	Costa Rica
	PI260539	7.12ef	1.89j	12.36defg	Argentina
	PI260571	8.69cd	2.26 hi	11.30efgh	Bolivia
	PI439409	3.60jk	3.18cd	15.21d	Uruguay
Capsicum chinense	PI209028	2.55lmn	3.36c	7.11ij	Puerto Rico
	PI224443	2.57lmn	2.80ef	12.94def	Bolivia
	PI238047	1.72op	1.38kl	4.58jk	Guyana
	PI238051	3.31JKL	3.38cb	9.49ghi	Peru
	PI257136	6.31gh	2.99de	18.30c	Ecuador
	PI439464	6.87efg	2.28h	8.48hi	Peru
	PI594139	3.92 ј	3.40 cb	12.95def	Belize
	PI485593	12.69a	3.22cd	33.42a	Jamaica
	PI439420	5.39 i	1.97ij	8.62hi	Colombia
	PI281443	3.02klmn	3.13cd	13.31def	Venezuela
Capsicum frutescens	PI241676	1.26p	0.74n	0.531	Ecuador
	PI639661	2.25n	5.45a	0.331	Vanuatu
	PI631144	6.97efg	2.22hi	8.45hi	Guatemala
1					

Each value in the table is an average of ten replicates. Statistical analyses were carried out among fruits for each characteristic. Values in each column having the same letter(s) are not significantly different [26].



Figure 2 Concentrations of total phenols in mature fruits of Capsicum genotypes grown at the University of Kentucky South Farm (Fayette County, KY).



Figure 3 Concentrations of β -carotene in mature fruits of Capsicum genotypes grown at the University of Kentucky South Farm (Fayette County, KY).KY).



Figure 4 Concentrations of soluble sugars in mature fruits of Capsicum genotypes grown at the University of Kentucky South Farm (Fayette County, KY).



Figure 5 (A-D). Shows plants and fruits morphological characteristics of all the 29 genotypes of *Capsicum* grown at the University of Kentucky South Farm (Fayette County, KY).

oxidation. Marin et al. [35], found that low irrigation frequency increased total carotenoids by 30% and provitamin A carotenoids by 15% in red fruits. Salinity, however, seems to have no effect on pepper carotenoid content [36,37]. Mineral nutrients have variable effects, depending on genotype and production system. Addition of Ca²⁺ and NO₃⁻ to hydroponically grown pepper plants caused increases in carotene concentration; whereas K⁺ treatments had no effect. PI 127442 contained the greatest concentration of β -carotene among all the 29 genotypes tested (Figure 3). Soluble sugars (sucrose, glucose, and fructose) play a substantial role in higher plant development. They are universal

component of living organisms. Soluble sugars concentrations fluctuated among the 29 genotypes analyzed and were greatest (10,474 μ g g⁻¹ fresh fruit) in PI 241676 compared to all genotypes tested (Figure 4). This variability may enable the identification and/or development of pepper germplasm with high levels of health-promoting properties. Accordingly, variability in ascorbic acid, β -carotene, total phenols, and sugars content and fruit quality characteristics could be a factor in selecting parents for hybridizations in breeding and crop improvement programs aimed to produce fruits with elevated levels of nutritional composition. Screening pepper genotypes for variability in

these compounds, is of great importance in promoting food composition and human health. This project could help the USDA to identify pepper genotypes for limited-resource farmers and growers who are interested in producing new *Capsicum* cultivars that yield great fruit quality for use as a cash crop.

The development of new genotypes (varieties) of hot pepper necessitates breeders' access to diversity of germplasm with documentation of their morphological characteristics such as fruit size, fruit color and early maturity. Figure 5 (A-D) shows the plants and fruits morphological characteristics of all the genotypes tested in this investigation. This project could help plant breeders to identify pepper genotypes for limitedresource farmers and growers who are interested in growing and producing new *Capsicum* cultivars of great nutritional and morphological characteristics for use as an industrial crop.

CONCLUSION

The concentrations of ascorbic acid, phenols, β -carotene, and soluble sugars varied among the species tested and even among the genotypes of the same species. This variability may enable the identification and/or development of pepper germplasm with high levels of health-promoting properties. Accordingly, variability in phytochemicals content and fruit morphological characteristics could be a factor in selecting parents for hybridizations in breeding for crop improvement programs aimed to produce pepper fruits with value-added traits. Screening pepper genotypes for variability in antioxidant content (ascorbic acid, phenols, β -carotene) is of great importance in promoting food nutritional composition and human health. Concentrations of ascorbic acid in three genotypes (PI 631144, PI 439420, PI 241676) selected were significantly greater compared to the other genotypes tested. On the contrary, PI 209028 and PI 260571 had the lowest ascorbic acid content among other genotypes tested. In C. frutescens, PI 639661 from Vanuatu, PI 631144 from Guatemala and PI 241676 from Ecuador contained the greatest concentration of total phenols.. PI 209028 contained the lowest concentration of total phenols (203 μ gg⁻¹ fresh fruits). This latter genotype should be excluded when selecting genotypes for phenols content. β-carotene in PI 127442 was found at the greatest concentration (101 mg g⁻¹ fresh fruits) and this genotype could be recommended for plant breeders who are seeking pepper plants with highest levels of β -carotene. On the contrary, PI 485593 from C. chinense should be avoided when selecting candidate genotypes for β -carotene content. Soluble sugars were greatest in PI 241676 compared to all genotypes tested. Accordingly, this genetic natural variability among genotypes in producing vitamin C, vitamin A, total phenols and soluble sugars under natural environmental conditions could be explored in pepper breeding programs.

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