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Quantitative evaluation of cucumber fruit texture and shape traits reveals extensive diversity and differentiation

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ABSTRACT

For many years, cucumber (*Cucumis sativus* L.) has been genetically improved by breeders to meet consumer demands. A wide variety of cucumber cultivars, differing in fruit qualities such as texture and shape, are grown around the world, and several major cucumber types have been developed in different regions. We used several computer-aided evaluation methods to assess the diversity in fruit texture and shape traits among a wide variety of world cucumber cultivars. Firmness of fruit skin, placenta and flesh and crispness of flesh were evaluated quantitatively and objectively by analysis of puncture-test data (force-deformation curves). Fruit shape was also evaluated quantitatively and objectively by using elliptic Fourier descriptors (EFDs) and principal component analysis (PCA). Fruit firmness and crispness clearly differed among cultivars and growth stages. Flesh crispness scores were significantly higher in Japanese F_1 hybrid cultivars, which maintained relatively high crispness even at maturity; in contrast, the crispness of the other type cultivars remained low at all stages. PCA of EFDs detected three major components of fruit shape variation: the ratio of length to diameter, the amount of swelling at the fruit ends and the shape of the stem end. Significant differences were detected in these shape components among cultivars and cucumber types. These results indicate that quantitative differentiation has occurred in the fruit texture and shape traits of cucumber during domestication and subsequent breeding.

1. Introduction

Fruit quality traits, including external appearance (shape and color) and texture (described by characteristics such as firmness and crispness), are the most important traits in cucumber (*Cucumis sativus* L.) because they are directly related to the product's commercial value. Cucumber has been genetically improved by breeders over many years to meet consumer demands. Consequently, a wide variety of cucumber cultivars with various fruit qualities are grown around the world, and several major cucumber types have been created. These include Beit Alpha (a Middle-Eastern slicer), European greenhouse (a Dutch greenhouse slicer), pickling (an American pickling), slicer (an American slicer), and oriental trellis slicer (Shetty and Wehner, 1998). The oriental cucumbers comprise at least three types: North Chinese, South Chinese and Japanese (Sakata and Sugiyama, 2002). The primary differences among these major cucumber types are the appearance of the fruit, which can vary in shape and color (Shetty and Wehner, 1998) and in fruit texture (Sakata et al., 2011). In addition, the marketable fruit size differs greatly among types. Together, these differences result in an extremely wide variation of fruit quality in the market.

In general, fruit quality is evaluated on the basis of appearance traits, taste and texture traits, nutrient and functional compounds, freshness, microbiological and chemical safety, and so on. However, very little progress has been made on clarifying the genetic diversity and differentiation of fruit quality among genetic resources and identifying promising breeding materials for fruit quality improvement, because of the lack of sufficiently accurate and efficient methods for the evaluation of fruit quality, especially fruit texture traits. For example, fruit texture traits other than firmness have been difficult to quantify objectively. The firmness of cucumber fruit has long been quantified by the use of instruments (Breene et al., 1972; Jeon et al., 1973; Kanno and Kamimura, 1978;

Abbreviations: CI, crispness index; EFDs, elliptic Fourier descriptors; FDK, apparent fractal dimensions based on the box-counting method (Kolmogorov's dimension); FDR, apparent fractal dimensions based on the Richardson plot method; PC, principal component; PCA, principal component analysis.

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Thompson et al., 1982). However, firmness is only one of the texture components, and other components such as crispness and juiciness are widely known to be important factors that affect the deliciousness of cucumber fruits. Recent improvements in computer and instrument performance have allowed researchers to develop new quantitative methods of evaluating fruit texture traits on the basis of mechanical (Horie et al., 2004; Yoshioka et al., 2009, 2010), acoustic (Sakurai et al., 2005) and bio-rheological (Dan et al., 2003; Kohyama et al., 2009, 2013) measurements. In particular, Yoshioka et al. (2009) applied several methods based on analysis of puncture-test data (force-deformation curves) to quantify cucumber fruit texture. Since these new methods allow us to quantify texture traits related to the degree of crispness more accurately than earlier methods, they can be used in texture analysis of genetic resources in cucumber as a replacement for subjective sensory evaluation.

Cucumber fruit shape is characterized mainly by length, diameter, the ratio of length to diameter (L/D ratio), and the curvature of the stem and blossom ends. These are also important fruit quality traits and determinants of the commercial grade of fruit in the market. In spite of its importance, fruit shape (with the exception of length and diameter) has been mainly evaluated qualitatively. For example, in the guidelines of UPOV (the International Union for the Protection of New Varieties of Plants; UPOV, 2007), the shapes of the stem and blossom ends are categorized into three classes (necked, acute and obtuse) and four classes (acute, obtuse, rounded and truncate), respectively. These classifications are based on rough estimates by visual judgments, which are subject to human error. Recent improvements in computer performance, combined with reductions in the cost of digital imaging hardware and software, have contributed to the increasingly widespread use of digital image processing in morphological analysis in biological and agricultural research.

Several approaches have been used to quantify the variation in shape of biological organs such as seeds, leaves, fruits, and roots. One approach uses simple measurements such as length, diameter, area, and perimeter. These measurements have the advantage of simplicity, but do not capture shape features in sufficient detail for more complex morphologies. Another approach captures the underlying factors that define a shape by using a set of parameters such as coordinate values or descriptors obtained by means of Fourier analysis. One of the most effective methods in this category involves a combination of elliptic Fourier descriptors (EFDs) and principal component analysis (PCA) (Kuhl and Giardina, 1982; Rohlf and Archie, 1984). This method describes the overall shape mathematically by transforming coordinate information on the image contours into EFDs, then summarizing the EFDs by means of PCA. The combination of EFDs and PCA has been successfully used in genetic and evolutionary studies of the shapes of plant organs such as *Betula* leaves (White et al., 1988), Japanese radish roots (Iwata et al., 1998, 2000; Tanaka et al., 2012), common buckwheat kernels (Ohsawa et al., 1998), *Rhus* leaves (Hiraoka and Kuramoto, 2004), *Primula* petals (Yoshioka et al., 2004, 2005, 2007), chicory roots (Lootens et al., 2007), rice grains (Iwata et al., 2009), olive leaves (D'Imperio et al., 2011), tomato leaflets (Chitwood et al., 2012a), *Alstroemeria* leaves (Chitwood et al., 2012b), *Epimedium* leaflets (Horie, 2012), azalea leaves (Keyser et al., 2013), carnation petals (Chacon et al., 2013) and grape leaves (Chitwood et al., 2014). Moreover, advances in recent molecular techniques have allowed researchers to analyze plant organs in genetic (Chacon et al., 2013; Iwata et al., 2009; Keyser et al., 2013) and physiological studies (Chitwood et al., 2012a,b, 2014).

In this study, we clarified the differentiation in fruit texture and shape traits among cultivars and among cucumber types by using computer-aided quantitative evaluation methods. Specifically, we evaluated fruit texture traits related to fruit firmness and crispness by using analyses of mechanical measurement data (Horie et al., 2004; Yoshioka et al., 2009), and fruit shape by using EFDs and PCA. Using the quantitative scores of these fruit quality traits, we analyzed differentiation of these traits in cucumber, and we discuss the prospects for future breeding and research.

Table 1
Cultivars and lines used in this study.

No.	Cultivar or line name	Type
B1	Atar	Beit alpha
B2	Khassib RZ	
B3	Media RZ	
B4	Beit Alpha x	
B5	Beit Alpha y	
B6	Beit Alpha z	
E1	Marianna RZ	European greenhouse
E2	Aviance RZ	
E3	Bologna RZ	
E4	Burgos RZ	
E5	Codiz RZ	
E6	Cumlaude RZ	
E7	DC-008	
E8	DC-011	
E9	Proloog RZ	
J1	Eterno	Japanese F_1 hybrid
J2	Excellent Fushinari 2	
J3	Freedom House 3	
J4	Kiseki	
J5	Mondor	
J6	Project X	
J7	Syun-ei	
J8	Top Run	
J9	V Arch	
J10	ZQ7	
J11	Encore 10	
J12	Fresco 100	
J13	High Green 21	
J14	Hisaki	
J15	Sharp 1	
J16	Freedom House 2	
J17	Magical 2	
O1	Ao Fushinari	Old Japanese
O2	Kaga Aonaga Fushinari	
O3	Natsu Fushinari	
O4	Sagami Hanjiro	
O5	Shimo Shirazu jibai	
O6	Santon	
O7	Shinsyo Shirakawa	
O8	Su Yo	
O9	Tokiwa	
P1	Patton	Pickling
S1	Poinsett 76	Slicer
W1	CS-PMR1	Wild (derived from India)

2. Materials and methods

2.1. Plant materials

A total of 44 cucumber cultivars or fixed lines (Table 1) were used for morphological and texture analyses. These cultivars comprised 6 Beit Alpha cultivars or lines, 9 European greenhouse cultivars, 17 Japanese F_1 hybrid cultivars, 9 old Japanese cultivars, 1 pickling cultivar, 1 slicer cultivar and 1 wild cucumber line.

For morphological and textural analyses of immature fruits (ca. 110–140 g), 36 cultivars (B1–B6, E1–E9, J1–J15, O1–O5, S1; Table 1) were grown in the autumn of 2010 and again in the spring of 2011. Both plantings used a completely randomized design with four replications with one plant per replication. Seeds were sown in a greenhouse in 9-cm-diameter plastic pots filled with a 1:1 mixture of Rakusaku soil (Mikado Kyowa Seed Co., Tokyo, Japan) and Engei Baido soil (Kureha Chemical Industry Co., Tokyo, Japan) on 22 September 2010 and 17 March 2011, and then seedlings were