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Seed and Oil Pumpkin: A Potential Specialty Crop for the Southeast

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Pumpkin seeds are a popular snack in many parts of the world and provide an important source of vegetable oil, particularly in Eastern Europe. Although currently of minor value, the use of pumpkin in the snacking and vegetable oil industry is expected to increase in the United States as the market for healthy foods and vegetable oil increases. The goal of the current study was to determine the genotypic and phenotypic (nutrition) diversity among 35 pumpkin cultivars, and horticultural performance of two "naked" seed pumpkin ('Beppo' and 'Naked Bear') cultivars in South Florida. Variation was observed in seed oil (29.33 to 48.41%) and seed protein (19.48% to 31.35%). Linoleic acid ($\bar{x}$ = 51.19%) was the major fatty acid in the seed, followed by oleic ($\bar{x}$ = 30.77%), palmitic ($\bar{x}$ = 9.84%), and stearic ($\bar{x}$ = 5.63%) acids. Genetic diversity among the pumpkin accessions using 39 SSR markers revealed a total of 102 alleles averaging 3.92 alleles per loci. The weighted neighbor joining dendrogram grouped the genotypes into three major clusters. Cluster I consisted primarily of hulled seed accessions of low oil but high protein that are grown for edible flesh. On the other hand, clusters II and III consisted primarily of accessions with reduced hulls that had high oil, but low protein. 'Beppo' cultivar exhibited a vining habit with a low fruit yield (1.3 fruits/plant) and moderate seed yield/fruit (313.5). On the other hand, 'Naked Bear' had short vines with a higher fruit yield (2.3 fruits/plant) and moderate seed yield/fruit (274.1). Collectively, these data suggest wide phenotypic and genotypic variation within *C. pepo* and provides information on horticultural performance of seed-oil pumpkins in South Florida.

Naked seeds derived from "naked-seed" pumpkin (*Cucurbita pepo* L.) are a popular ingredient in many snacks, breads, breakfast cereal, soups and other edible goods (Baxter et al., 2012; Loy, 2004). Vegetable oil derived from the seed can be purchased by the bottle for culinary / condiment use or as formulated capsules in health food stores (Stevenson et al., 2007). Unlike conventional hulled pumpkin seeds, naked seeds lack a complete seed coat, thus are preferred for snacking and oil production because they eliminate the need for manual de-hulling prior to use. The popularity of naked seed products is expected to increase in the U.S. as the market for healthy foods increases.

Pumpkin seed is rich in oil (50% w/w), protein (35%), unsaturated fatty acids (86%) (Meru et al., 2018), and antioxidants that have many health benefits, including a reduced risk of certain cancers (Lelley et al., 2009; Nesaretnam et al., 2007; Stevenson et al., 2007), treatment of enlarged prostate, and lowering cholesterol levels (Fruhwirth and Hermetter, 2007; Thompson and Grundy, 2005). Despite growing popularity of pumpkin seeds in the United States, most naked seeds consumed in the United States are imported. Hence, the need to identify/develop cultivars adapted to local growing conditions. Moreover, "naked" seed-oil pumpkin has poor flesh quality and thus is not commonly grown in the United States, where flesh quality is emphasized. The overarching goal of our breeding program is to develop "dual purpose" pumpkins that can be used for production of nutritious "naked" seeds, but with excellent flesh quality.

The goal of the current study was to determine the genotypic and phenotypic (nutrition) diversity among 35 pumpkin cultivars, and determine the horticultural performance of two 'naked' seed pumpkin cultivars in South Florida.

Materials & Methods

Nutrition analysis. Thirty-five *C. pepo* plant introductions and cultivars were sourced from the United States Department of Agriculture–Agricultural Research Service germplasm collection and commercial seed companies, respectively. Determination of seed oil and protein percentage was carried out using a nuclear magnetic resonance instrument [NMR (MiniSpec MQ20 NMR analyzer; Bruker Optics, Billerica, MA)] as described by Burke et al. (2005) and Wills et al. (2010). For fatty acid analysis, the standard method (Ce 1-62) for analyses of fatty acid composition in fats and oils recommended by the American Oil Chemists' Society was used to prepare fatty acid methyl esters (American Oil Chemists' Society, 2005), following protocols developed for watermelon seed (Meru and McGregor, 2014).

Genetic diversity analysis. For each genotype, five seeds were germinated in cells (5.98 × 3.68 × 4.69 cm) filled with

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Fafard 3B soil amended with Osmocote Classic in a greenhouse (22° to 32°C). At the two true-leaf stage, three leaf punches from three individuals of each accession were collected, bulked, and immediately frozen in liquid nitrogen. DNA was extracted using the E.N.Z.A kit (Omega Biotek, Norcross, GA) according to the manufacturer's instructions.

Thirty-nine previously published SSR primer pairs distributed across 19 linkage groups of *C. pepo* were used for genetic diversity analysis. For each primer pair, PCR was performed in a 15 μL reaction containing 40 ng of template DNA, 0.32 μm of a fluorescently (either 6-FAM, VIC or PET; Supplementary Table 2) labeled M13 forward primer (GCCTCCCTCGCGCCA; Blacket et al., 2012), 0.04 μm of M13-tagged forward primer, 0.4 μm unlabeled reverse primer, and 1× PROMEGA Colorless GoTaq mastermix (Promega, Madison, WI). Amplification products for three primer pairs, each labeled with a different fluorescent dye, were multiplexed and combined with a GeneScan-500 ROX internal-lane size standard and Hi-Di™ Formamide before analysis on a 3730 96-capillary DNA Analyzer (Applied Biosystems) at the Gene Expression and Genotyping Core facility, Interdisciplinary Center for Biotechnology Research, University of Florida. GeneMapper v.4.0 software was used for allele calling and size estimation.

Darwin software (v6.0) was used to calculate a pairwise dissimilarity matrix by Simple Matching coefficients with minimal proportion of valid data for each unit pair set to 90% (Perrier and Jacquemoud-Collet, 2006). A weighted neighbor joining tree was derived from the dissimilarity matrix with bootstrapping value set to 1000.

Field trial. Two hybrid 'naked-seed' cultivars, 'Beppo' and 'Naked Bear', were sourced from Territorial Seed Company (Cottage Grove, OR). Seeds for both cultivars were sowed on 5 Mar. 2018 in cells filled with Fafard 3B soil amended with Osmocote Classic in a greenhouse (22 to 32 °C). After three weeks, the seedlings were transplanted into plastic mulched beds with a 4 ft in-row spacing in a field at the UF/IFAS Tropical Research and Education Center, Homestead, Florida. The plants were in a randomized complete block design consisting of three plots, each plot with 10 plants. Fertilizer (3–0–10, N–P–K) was delivered weekly for the duration of the experiment. Weekly pest management was conducted following recommendation for cucurbits in the 2017–18 Vegetable Production Handbook of Florida <http://edis.ifas.ufl.edu/cv292>. Data were collected on growth habit (bush or vining), flowering time (days to male or female flowering), fruit quality (weight), and yield (fruit and seed). Fruits were harvested at 91 days after planting. For statistical analysis, trait means were calculated and separated using Fisher's protected least significant (P < 0.05) difference test using PROC GLM procedure of SAS (SAS Institute Inc., Cary, NC).

Results and Discussion

Nutrition profile. Seed oil percentage ranged from 29.33% to 48.41% (Fig. 1A) while seed protein percentage ranged from 19.48% to 31.35% (Fig. 1B). Seed oil percentage observed in the current study was similar to that reported in several cucurbit species including *C. pepo* (31.2 to 51.0%) (Applequist et al., 2006; El-Adawy and Taha, 2001), *C. argyrosperma* (36 to 40.1%) (Applequist et al., 2006), *C. ficifolia* (43.5%) (Bernardo-Gil et al., 2004), *C. maxima* (10.9 to 42.3%) (Stevenson et al., 2007), *C. moschata* (29.1 to 43.3%) (Tsuyuki et al., 1985), and *Citrullus lanatus* (20.1 to 40.6%) (Prothro et al., 2012). Seed protein

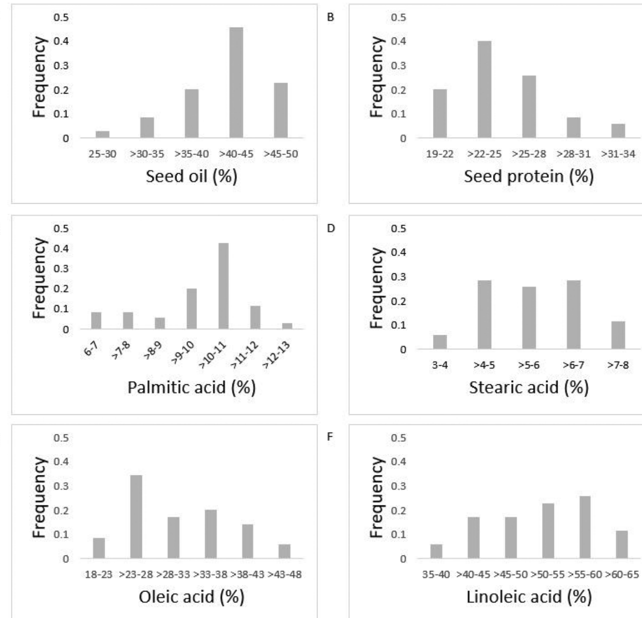

Fig. 1. Phenotypic distribution for (**A**) seed oil percentage, (**B**) seed protein percentage, (**C**) palmitic acid, (**D**) stearic acid, (**E**) oleic acid, and (**F**) linoleic acid among 35 pumpkin accessions.

percentage observed in the current study (19.48 to 31.35%) was within the range reported for other *Cucurbita* species (17.3 to 44.4%) (Achu et al., 2005).

Palmitic acid [$C_{16:0}$; 6.71 to 12.64%], stearic acid [$C_{18:0}$; 3.35% - 7.65%], oleic acid [$C_{18:1}$; 18.42 to - 46.09%] and linoleic acid [$C_{18:2}$; 35.38 to 64.05%] (Fig. 1C-F) were the primary fatty acids in the oil, with the latter being predominant. The fatty acid composition observed in the current study is comparable to that previously reported in *C. pepo* for palmitic (9.5 to 14.5%), stearic (3.1 to 7.4%), oleic (21.05 to 46.9%) and linoleic (35.6 to 60.8%) acid (Murkovic et al., 1996). In comparison with major oil crops, the degree of unsaturation (oleic acid and linoleic acid) in the current study (78.6 to 86.1%) was similar to that of soybean (84.4%) and sunflower (88.6%) (Baboli and Kordi, 2010). The high level of unsaturated fatty acids may contribute towards a reduced risk of arteriosclerosis and heart-related ailments (Wassom et al., 2008). However, the high linoleic acid content in pumpkin seed lowers the heat stability of the derived oil thus making it unsuitable for cooking at high temperatures.

Genetic diversity analysis. Genetic diversity using a set of 39 SSR markers revealed a total of 102 alleles averaging 3.92 alleles per loci. The mean polymorphic information content (PIC) was 0.44, with eleven SSR markers having a PIC of ≥ 0.5. CMTp133 had the highest PIC and tied with CMTp177 and CMTp205 for most alleles (7). The markers revealed a mean gene diversity and major allele frequency of 0.48 and 0.65, respectively. The weighted neighbor joining dendrogram grouped the genotypes into three major clusters (Fig. 2). Cluster I consisted primarily of hulled seed accessions of low oil but high protein that are grown for edible flesh. On the other hand, clusters II and III consisted primarily of accessions with reduced hulls that had high oil, but low protein. Due to their genetic similarities to edible-flesh cultivars, PI's 267660, 267661, and 267664 form ideal sources for reduced-hull trait in conventional hulled-seed pumpkins.

Horticultural performance. The two cultivars exhibited two distinct growth habits. 'Beppo' had long vines, while 'Naked

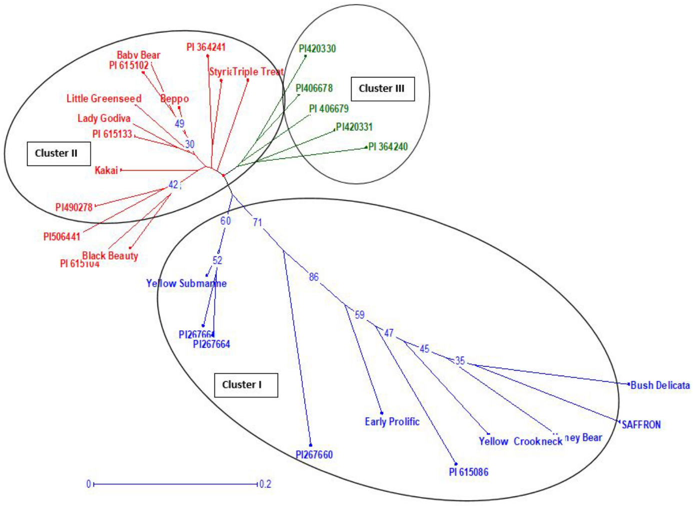

Fig. 2. Dendrogram showing clustering of 35 pumpkin accessions into three main groups.

Bear' had short vines (Fig. 3). Although a 4 ft in-row spacing was adequate for both cultivars in the current study, growers may be able to increase the planting density for 'Naked Bear' due to its bush type habit. Male flowers for 'Beppo' cultivar opened significantly earlier than those of 'Naked Bear' (Table 1). Similarly, 'Beppo' was significantly earlier in female flowering than 'Naked Bear'. The fruit load on 'Naked Bear' plants ranged from 1 fruit/plant to 4 fruit/plant, with an average fruit yield of 2.3 fruits/plant (Table 1). These yields were significantly higher than those for 'Beppo', which had an average yield of 1.3 fruit/plant and a range of 1 to 2 fruit/plant. Seed yield/fruit ranged from 246 to 361 for 'Beppo', and 177 to 398 for 'Naked Bear'. The average seed yield/fruit in 'Beppo' (313.5) did not significantly differ from that of 'Naked Bear' (274.1) (Table 1). However, based on the difference in fruit yield/plant between 'Beppo' (1.3 fruit/plant) and 'Naked Bear' (2.3 fruit/plant), it is expected that overall seed yield/plant would be much higher in 'Naked Bear'. 'Beppo' had round fruits with orange-colored rind that were heavily ridged with contrasting green stripes (Fig. 4). Fruits of 'Naked Bear' were small and round, with a solid orange skin color. 'Beppo' fruit had significantly greater weight, length, width, and flesh diameter than those of 'Naked Bear' (Table 1). The flesh of both cultivars was cream-white (Fig. 4)

Fig. 3. Growth habit for 'Beppo' and 'Naked Bear' seed-oil pumpkins.

Fig. 4. Rind and flesh color of 'Beppo' and 'Naked Bear' seed-oil pumpkins.

Table 1. Horticultural characteristics of two seed-oil pumpkin cultivars in south Florida.

	Cultivar	
Trait	Beppo	'Naked Bear'
Days to first male flower	41.9 a[z]	44.6 b
Days to first female flower	47.7 a	50.8 b
Fruit weight (kg)	2.8 a	0.6 b
Fruit length (cm)	16.0 a	9.0 b
Fruit width	20.2 a	12.3 b
Flesh thickness	1.9 a	0.7 b
Fruit yield/ plant	1.3 a	2.3 b
Seed number/ fruit	313.5 a	274.1 a

[z]Means followed by same letter are not significantly different within rows.

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