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## Morphological and Molecular Analysis of Three Celery Accessions

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### ABSTRACT

**Celery (*Apium graveolens* L.), a culinary herb and vegetable, is considered a good source of the essential oil and phenolic acids for use as a food, medicine, and flavoring agent. Substantial plant to plant variation within celery, however, indicates a high degree of genetic inconsistency that affects plant quality and yield. This study evaluated the fresh and dry weights and leaf characteristics of three celery genotypes grown in a greenhouse. The genotypes were also screened for polymorphic RAPD (Random Amplified Polymorphic DNA) markers. Celery Accessions were found to differ in fresh and dry weight. Our study demonstrated that RAPD technique could be a suitable tool for genotypes identification and classification in celery.**

### INTRODUCTION

Celery (*Apium graveolens* L.), a biennial plant in the Apiaceae family, is frequently used as a vegetable, spice, and natural medicine in Egypt. Leaves and stalks (petioles) of celery are frequent components of salads and the seeds are used for the treatment of various diseases (Halim et al., 1990; Shalaby and El-Zorba, 2010). The essential oil extracted from the celery seeds is a major flavoring agent in the food industry used to improve the taste and aroma of prepared foods, soups, meats, sauces, pickles, and vegetable juices (Sowbhagya, 2014). Celery is rich in nutrients that help maintain a healthy body (Kreck et al., 2006).

Health promoting constituents of the celery plant include dietary fiber; the vitamins A, B<sub>1</sub>, B<sub>2</sub>, B<sub>9</sub>, C, E, and K; the minerals calcium, iron, magnesium, manganese, phosphorus, potassium, and zinc; and the amino acid tryptophan. Celery seed oil contains two main groups of compounds: limonene-type mono-terpenes and butylphthalides (Tang, et al., 1990). A study by Lam and Zheng (1991) demonstrated that 3-n-butylphthalide and the related phthalide, sedanolide, increased the activity of glutathione-s-transferase (GST) in the liver, small intestinal mucosa, and forestomach of inbred A/J mice. GST is a particularly important phase II xenobiotic metabolizing enzyme and is often involved in the removal of reactive intermediates.

Celery, originating from the Mediterranean area of southern Europe and from the swamps of Egypt and Sweden, is comprised of three cultivated forms: celery (var. *dulce*), celeriac (var. *rapaceum*) and smallage (var. *sealinum*) (Yang and Quiros, 1993; Domblides, et al., 2008). Identification of these celery accessions has been primarily based on morphological and biochemical traits that extensive observations of mature plants and, in some situations, may lack definitive objectivity (Wrigley et al., 1987; Yang and Quiros, 1993; Khadari et al., 1995). For these reasons, molecular marker technology (RAPD), was used to access genetic diversity and relationships within the celery collections studied in this report (Hadrys et al., 1992; Hu and Quiros, 1991, Bai et al., 1998; Sun et al., 2001). The RAPD technique has been previously applied in the

evaluation of uniformity in androgenic celery (Yang and Quiros 1993), cauliflower (Stipic and Campion 1997), cabbage (Kaminski et al., 2003) and carrot (Mirostawa and Habdas, 2006). Briard et al., (2001) used RAPD markers for identification and genetic diversity of carrot cultivars and breeding lines.

The purpose of the current study was to demonstrate that cultivated forms of celery could be differentiated using the RAPD technique, and to assess genetic diversity among landraces of celery using RAPD markers and morphological traits.

## MATERIALS AND METHODS

**Plant material.** Celery (*Apium graveolens* L., Family Apiaceae) plants grown from seed of three accessions (Wild type, Balady, and Green Leaves) were used in this study. Seeds of the Wild type celery were collected in Egypt from plants growing in a wet, saline soil near the village of Albosaily (located in Behira Governorate), seeds of Balady were sourced from an Egyptian seed company (Harraz for Herbs, Oils, & Natural Extracts, Cairo), and seeds of Green Leaves, a commercial variety, were purchased in the U.S.A. (Reimer Seeds, Inc., Mount Holly, NC).

Replicate planting of the celery selections were sown on October 1, 2007 (planting 1), and January 15, 2008 (planting 2) (Table 1), into separate 50 cm x 50 cm square plastic flats filled 5 cm deep with a commercial growing media (LC1 Mix, Sun Gro Horticulture, Vancouver, Canada). After seeding, the flats with seeds were placed in a controlled environmental chamber (Model EF7, Conviron, Winnipeg, Canada) set at 20°C with a 14 h day/10 h night cycle using a mixture of fluorescent and incandescent bulbs (PAR = 135  $\mu\text{mol m}^{-2}\text{s}^{-1}$  and R-FR ratio = 1.92) at the University of Massachusetts, Amherst, MA for germination.

Table 1. Replicate planting schedule for celery selections.

Schedule	Seeding date	Transplanting date	Harvest dates
Planting 1	Oct. 1, 2007	Nov.1, 2007	Dec. 15, 2007 Jan. 15, 2008 Feb. 15, 2008
Planting 2	Jan. 15, 2008	Feb 15, 2008	Apr. 1, 2008 May 1, 2008 Jun. 1, 2008

At seven days after seedling emergence, the foliage of 10 randomly selected seedlings was harvested from the Planting 1 of each celery accession for use in molecular analysis. At 31 days after seeding, seedlings from each accession (three replicates of five plants each) were randomly selected and transplanted (one plant per 3.8 L plastic pot) into a peat moss, vermiculite, and perlite commercial growing mix (Pro-Mix BX, Pro-Mix Inc., Quakertown, PA). After transplanting, the pots containing the plants were transferred into a greenhouse (natural daylight, minimum temperature of 20±2°C). Vigorous plant growth was maintained by daily watering and biweekly treatment with a water soluble (28-8-16) fertilizer.

**Morphological analysis.** The growth of each accession was characterized using randomly selected samples from the third harvest of Planting 1 and Planting 2 (Table 1). Plants were harvested by cutting the stem at 2 cm above the growth media surface using a knife, and the fresh tissue was immediately weighed on an electronic balance (Mettler-Toledo, Inc., Westfield, MA). The third inner stalk from the outside of each celery bunch (the collection of leaves) was then removed as a test leaf for comparative measurements among the celery accessions.

The leaf length (petiole with attached blade) and petiole width (at leaf base) of the test plant were measured to the nearest millimeter with a ruler. The leaf area of the test leaf (petiole with attached blade) was determined using a digital leaf area meter (LI-300 portable area meter (Li-Cor, Lincoln, NE). Dry weight of the celery leaves was determined after drying the fresh leaves at 55°C for 48 h and then weighing according to procedures outlined in the AOAC (1980).

**DNA analysis.** To characterize and compare the DNA of the three celery accessions, total genomic DNA, extracted from the randomly selected celery seedlings collected at seven days after seed germination using the modified method of Doyle and Doyle (1990) and Mady et al. (2013). Extraction chemicals were purchased from Sigma-Aldrich (St. Louis, MO), random primers (Table 2) and the marker (0.1-10.0 kb 2-Log DNA ladder) were purchased from New England Biolabs, Inc. (Ipswich, MA) and

DNA polymerase was purchased as a Taq Polymerase kit from Takara Bio Co., Inc. (Shiga, Japan).

Table 2. Primers used in determining banding patterns of celery accessions.

Primer	Sequence	Primer	Sequence
1	GTCATGGAC	4	CTACTGCCGT
2	CCCAAGGTCC	5	AGTCGTCCCC
3	CCCGATTGCG	6	GTGCCTAACCC

The polymerase chain reactions (PCR) were completed according to instructions included with the primers until reproducible bands were obtained. The cycling program for the PCR reaction was 95°C for 3 min, 35 x (95°C, 50 sec, 37°C, 45 sec, 72°C, 1.2 min); 72°C, 10 min. Separation of the PCR products was by electrophoresis at 50 volts on a 1.5 % agarose gel using 40 mL of 1X TBE buffer containing 6 µL ethidium bromide. The products were visualized under UV light to observe the banding patterns for each amplicon and determine the level of polymorphism.

**Statistical analysis.** Within each planting, all experimental measurements were replicated three times with five plants for each accession. Tests for significant differences among the accessions were analyzed using ANOVA (Snedcor and Cochran, 1980). Following a significant F test, means were separated using a least significant differences (LSD) test.

## RESULTS

**Morphology.** Statistically significant differences in leaves were observed among the celery accessions (Table 3). The Wild type had the largest leaf area in in both Planting 1 and Planting 2. The Wild type also exhibited significantly longer leaves as compared with the other accessions in Planting 2. Although the differences were not significant, the wild type exhibited the most leaves, leaf length, and petiole width of the three accessions. The Balady accession exhibited the smallest values for all measured leaf characteristics in both Plantings.

No significant differences were detected among the three celery genotypes for either fresh or dry weight (Table 4). Except for the first harvest in Planting 2, moisture content of the celery leaves ranged from 90.5% to 93.8%. Moisture content of the leaves in the first harvest of Planting 2 ranged from 86.4% to 89.6%.

Table 3. Leaf characteristics of celery accessions.

Morphological characteristics <sup>1</sup>	Accessions			Significance
	Planting 1			
	Wild type	Balady	GreenLeaves	LSD <sub>0.05</sub>
Leaf length (cm)	34.17	28.87	30.63	ns <sup>2</sup>
Petiole width (cm)	4.43	3.43	3.53	ns
Leaves per plant (No.)	31.00	25.00	29.00	ns
Leaf area (cm <sup>2</sup> /plant)	20.72	12.29	13.47	5.23
	Planting 2			
Leaf length (cm)	38.23	28.77	31.67	7.29
Petiole width (cm)	4.60	3.07	3.50	ns
Leaves per plant (No.)	32.00	28.33	30.00	ns
Leaf area (cm <sup>2</sup> /plant)	22.40	12.12	13.05	5.12

<sup>1</sup>Means of three harvests from each planting for each accession.

<sup>2</sup>ns = no significant differences.

Table 4. Fresh and dry weights of celery accessions.

Accession	Planting 1 harvests <sup>1</sup>					
	Fresh weight (g)			Dry weight (g)		
	1st	2nd	3rd	1st	2nd	3rd
Wild type	34.4	40.4	42.3	3.01	3.63	3.53
Balady	27.9	32.6	29.7	2.47	2.37	2.43
Green Leaves	32.8	36.0	29.4	3.12	3.13	2.33
LSD at 5%	ns	ns	ns	ns	ns	ns
Accession	Planting 2 harvests <sup>1</sup>					
	Fresh weight (g)			Dry weight (g)		
	1st	2nd	3rd	1st	2nd	3rd
Wild type	32.2	42.5	31.7	3.62	3.80	2.57
Balady	29.8	40.4	28.3	4.06	3.60	2.40
Green Leaves	29.3	38.7	31.2	3.05	2.40	2.43
LSD at 5%	ns	ns	ns	ns	ns	ns

<sup>1</sup>Means of three harvests from each planting for each accession.

**Genotype characterization.** DNA analysis demonstrated genetic variability among the three celery accessions used in this study. Using six decamer primers, a total of 108 bands were obtained from the electrophoretic separation of the genomic DNA (Figure 1). The bands varied with the selection of primers, yielding 12 monomorphic and 24 polymorphic bands (Table 5).

The number of polymorphic fragments per primer ranged from 67% (Primers 1 and 2) to 87% (Primer 5). The fragment size varied between 200 and 4900 base pairs. The observed bands are distinguished as 64 bands present and 44 bands absent. All primer combinations detected polymorphism among the three studied celery accessions.

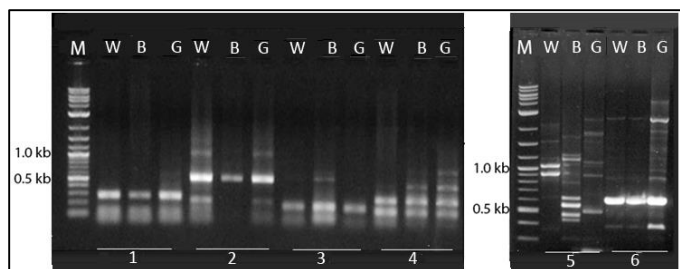


Figure 1. DNA banding patterns of three celery accessions using six primers.

M = Marker 0.1-10.0 kb 2-Log DNA ladder.

W = Wild type, B = Balady, & G = Green leaves accessions.

Numbers 1 through 6 refer to the primers used in the contiguous underlined gel lanes (W, B, & G).

Table 5. Presence and absence of DNA bands among the celery accessions.

Primers	Wild type <sup>1</sup>		Balady <sup>1</sup>		Green leaves <sup>1</sup>		Band loci	Polymorphism		
	+	-	+	-	+	-		Mono bands <sup>2</sup>	Poly bands <sup>2</sup>	% <sup>3</sup>
1	1	2	1	2	3	0	3	1	2	67
2	3	0	1	2	3	0	3	1	2	67
3	2	2	4	0	2	2	4	2	2	50
4	2	2	3	1	4	0	4	2	2	50
5	4	11	8	7	8	7	15	2	13	87
6	4	3	4	3	7	0	7	4	3	43
Total	16	20	21	15	27	9	36	12	24	67

<sup>1</sup>Number of bands observed (+) and absent (-) for each primer within each accession.

<sup>2</sup>Monomorphic bands and polymorphic bands.

<sup>3</sup>Percentage polymorphism.

The six primers used in the study were able to detect 19 positive unique bands (Table 6). Most of these bands, a total of ten, were observed in the accession Green leaves. In contrast, only one unique band was observed in the Wild type accession and Balady had eight unique bands. Only four negative unique bands were discerned.

The similarity interaction percentage between primers and accessions revealed that celery Wild type exhibited 67.57% similarity with both Balady and Green leaves, whereas the similarity of Balady with Green leaves was 43.25% (Table 7).

Table 6. Unique bands and size detected by use of six primers in three celery accessions.

Primer	Positive unique bands			Negative unique bands		
	Wild type	Balady	Green leaves	Wild type	Balady	Green leaves
1	-	-	0.5 kb 0.4 kb	-	-	-
2	-	-	-	-	1 kb 0.3 kb	-
3	-	0.6 kb, 0.5 kb	-	-	-	-
4			0.5 kb	0.4 kb		
5	4.3 kb	3.5 kb, 2.0 kb 0.8 kb, 0.6 kb 0.55 kb 0.4 kb	4.9 kb, 3.6 kb 0.5 kb 0.22 kb 0.2 kb	-	3.1 kb	-
6	-	-	6 kb 1 kb	-	-	-
Total Bands	1	8	10	1	3	-

Table 7. The similarity of DNA matrix among the celery accessions.

Accession	Similarity/Dissimilarity (%)		
	Wild type	Balady	Green leaves
Wild type	--	67.57	67.43
Balady	32.43	--	43.25
Green leaves	32.43	56.75	--

## DISCUSSION

The morphological characteristics of the three tested celery accessions were consistently different only in leaf area over the two plantings. In contrast, the molecular markers revealed distinct genetic differences within the plants. The similarity in plant morphology while significant variations existed within the DNA genotypes cannot be readily explained, but may be related to the evaluation of mostly non-coding regions of the genome. The regulation of morphological characteristics is most likely controlled by expressed sequences and their interactions (Karimi, et al., 2012). The RAPD primers used in our study may not have included the genes that code for morphological characteristics (Garcia et al., 2002).

The positive and negative unique bands in the DNA analysis enabled the studied celery genotypes

to be distinguished from each other. The origin of these DNA bands may be attributed to mutations at the priming site. Lack of studies with DNA markers on celery, however, prevents a detailed genetic analysis of most traits having economic importance.

Earlier research by Yang and Quiros (1993) observed 6.1% polymorphic marker bands within 21 *Apium graveolens* var. *dulce* cultivars using 28 RAPD primers. Adding one celeriac (*Apium graveolens* var. *rapaceum*) and one annual small age (*Apium graveolens* var. *secalinum*) to their study increased the ratio of polymorphisms to 9.3%. Domblides et al. (2008) were able to detect 42% polymorphic bands in 12 cultivars with six RAPD primers.

Our study suggests the importance of positioning the Egyptian wild type as a new celery accession. The growth of the wild type in saline soil with a high water table could be helpful in future breeding programs for the production of celery varieties tolerant to environmental stresses.

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