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Croton *Codiaeum variegatum* (L.) Blume cultivars characterized by leaf phenotypic parameters

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ABSTRACT

Croton *Codiaeum variegatum* (L.) Blume is a popular ornamental foliage plant that displays an anomalous range of variations in its leaf size, shape and color pattern. By fixing such leaf variations, more than 300 cultivars have been produced in the world. Until now, however, only a limited number of studies have been made to describe the leaf phenotypic diversity. Here we investigated the diversity of leaf phenotype in croton cultivars with a numerical taxonomic approach. One-way analysis of variance confirmed significant phenotypic variations in the croton cultivars for 12 quantitative leaf parameters. Among the numerical parameter tested, the leaf index that is the ratio of leaf length to leaf width showed the highest variability. High coefficient of variation values were observed in petiole length, leaf area and leaf down quarter width. In contrast with leaf morphology, the composition of leaf pigments that contribute to leaf coloration did not show diversity. Based on the analyses of the leaf parameters, we clustered the croton cultivars into four major groups. The results of this study provide a numerical basis for classification of croton cultivars. A possible involvement of transposon is discussed in terms of somatic mutation that results in the leaf phenotypic diversity.

1. Introduction

Codiaeum variegatum (L.) Blume, commonly known as “croton”, is an ornamental shrub that originates in tropical forests (Govaerts et al., 2000). A wide range of variations in leaf shape and coloration has fascinated breeders, landscapers, horticulturists and gardeners, and a huge number of cultivars have been fixed for commercial production (Chen and Stamps, 2006). More than 300 cultivars are available in the ornamental horticulture industry (Brown, 1995). Crotons have now become one of the most popular ornamental tropical shrubs even in Europe and U.S. as much as *Ficus benjamina*.

Diversity in croton’s leaf-shape is quite enormous. The leaf shapes include ovate to linear, entire to deeply lobed, appendiculate at the middle connected by midrib. Coloration and color pattern on the leaves is also a prominent characteristic to distinguish each cultivar. The phenotypic diversity observed in croton leaves is of a great interest in plant science because virtually all types of leaf morphology can be seen in one species; the plasticity in leaf phenotype is extremely high (Shimoji et al., 2006). Until now, however, the mechanism for creating such high diversity in the leaves has remained obscure. Since seed propagation of crotons resulted in different phenotypes (Sharma and Bal, 1958), the present cultivars have been mostly fixed by vegetative propagation (Chennaveeraiah and Wagley, 1985). It appears that genetic instability is associated with the leaf phenotypic diversity. Brown (1995) suggested that somatic mutations and/or cross pollination by ants may be involved in the mechanism for creating such high diversity. Ogunwenmo et al. (2007) reported that chromosomal variability could account for the wide range morphological variation in the croton *C. variegatum*. A high variation in chromosome numbers and karyotypes may attribute to the morphological diversity among the cultivars of this species (Deng et al., 2010a). Recently, Deng et al. (2010b) reported the genetic relationship of *C. variegatum* cultivars using AFLP markers, and suggested that croton cultivars are genetically highly polymorphic. In addition to these aspects of basic biology, there have been horticultural studies to account for the plasticity: effect of fertilizers on growth responses (Chase and Poole, 1989), micropropagation of shoots (Marconi and Radice, 1997), effect of growth hormone on axillary shoot production (Orlikowska et al., 2000), and in vitro propagation of croton (Nasib et al., 2007). Since there is no criterion to identify each cultivar, to date, no one can compare the results reported the above on scientific basis. To clarify the mechanism for high phenotypic diversity, it is essential to establish systematic classification to distinguish the cultivars.

Abbreviations: CV, coefficients of variation; LI, leaf index; LMW, leaf middle width; LUW, leaf upper quarter width; LDW, leaf down quarter width; LBA, leaf base angle; LTA, leaf tip angle; PL, petiole length; LA, leaf area; LP, leaf perimeter.

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Table 1
List of the parameters studied for morphological characterization of *C. variegatum* cultivars.

Parameters	Measurement unit and description	Abbreviation
1. Leaf length	cm	L
2. Leaf width	cm	W
3. Leaf index (L/W)	–	LI
4. Leaf middle width (1/2)	cm	LMW
5. Leaf upper quarter width (3/4)	cm	LUW
6. Leaf down quarter width (1/4)	cm	LDW
7. Leaf base angle	°	LBA
8. Leaf tip angle	°	LTA
9. Petiole length	cm	PL
10. Leaf area	cm^2	LA
11. Leaf perimeter	cm	LP
12. Leaf area/perimeter	–	LA/LP
13. Leaf margin-undulate	normal = 0, undulate = 1	LMU
14. Leaf margin-spiral	normal = 0, spiral = 1	LMS
15. Leaf margin-appendiculate	normal = 0, appendiculate = 1	LMA
16. Leaf margin-lobed	normal = 0, lobed = 1	LML
17. Leaf shape	oblanceolate = 0, linear = 1, oval = 2, elliptic = 3, oblong = 4, spiral = 5, hastate = 6, acicular = 7, appendiculate = 8	LS
18. Leaf color-yellow on leaf	green = 0, yellow on leaf = 1	LCY
19. Leaf color-red on leaf	green = 0, red on leaf = 1	LCR
20. Leaf color pattern-line blotch	no leaf blotch = 0, line blotch along vein = 1	LCLB
21. Leaf color pattern-spot blotch	no leaf blotch = 0, spot blotch on vein = 1	LCSB
22. Petiole color	green = 0, yellow = 1, pink = 2, red = 3	PC
23. Vein color	green = 0, yellow = 1, pink = 2, red =3	VC

Leaf parameters have been traditionally used for identification and classification of croton cultivars. Brown (1995) categorized the croton cultivars into nine groups based on leaf types: (1) broad leaf; (2) oak leaf; (3) semi-oak leaf; (4) spiral leaf; (5) narrow leaf; (6) very narrow leaf; (7) small leaf; (8) interrupted leaf; and (9) recurved leaf. Classification based on leaf types is subjective and may depend on individual preference. Moreover, a single parameter does not adequately reflect the overall phenotypic expression of cultivars. For these reasons, phenotypic characterization based on numerical criteria has become a common tool to identify and classify cultivars (Revilla and Tracy, 1995; Rakonjac et al., 2010).

The aim of this study was to describe the high leaf phenotypic diversity in croton on scientific basis. Here we characterize croton cultivars by leaf morphology with several parameters that have been applied for numerical taxonomy. The results of this study confirm an enormous diversity in leaf phenotype of croton. A possible mechanism for high leaf phenotypic diversity observed in croton is discussed in terms of transposable elements, a mobile gene that may cause the instability of the genome.

2. Material and methods

2.1. Plant material

Okinawa (26′15″N, 127′45″W) is a subtropical island of Japan that is a world showcase for *Codiaeum* (Brown, 1995). Croton cultivars are favored in Okinawa as garden plants, landscape plants and indoor pot plants. We collected 29 croton cultivars from Okinawa Island (Fig. 1). In this study, we do not use English names for describing cultivars because they are not consistent over the information sources. Instead, we designated the croton cultivars by temporary code and number in order to avoid potential confusions. Each code (C1–C29) was assigned to representative cultivars (Fig. 1).

2.2. Leaf parameter analysis

Using 23 leaf parameters listed in Table 1, we analyzed 12 cultivars (C2, C3, C7, C9, C11, C12, C13, C16, C20, C25, C26, and C28 shown in Fig. 1) for morphological characterization. Following the guidelines provided by the International Plant Genetic Resources Institute (IPGRI, 1999), we randomly collected 10 fully expanded leaves from three different trees and used for analysis. The harvested fresh leaves were scanned with a flatbed scanner (CanoScan LiDE 80) to obtain the digital information in shape and color. NIH ImageJ software (http://rsb.info.nih.gov/ij) was applied to obtain quantitative parameters (Usher et al., 2010). Ratio of leaf length to leaf width (leaf index) and leaf area to leaf perimeter were calculated from the measured parameters. Qualitative parameters (Table 1) were scored according to the methods reported by Sneath and Sokal (1973) and by Fajardo et al. (2008).

Descriptic statistics (mean, standard deviation) for quantitative parameters were carried out with KaleidaGraph (version 4.0). A one-way analysis of variance (ANOVA) was performed to test the significant difference among the cultivars for all the quantitative parameters. Coefficients of variation (CV) were calculated to compare relative variation in each parameter. Data were also subjected to principal component analysis (PCA) and cluster analysis. Prior to PCA, data were transformed by standardized to zero mean and unit variance according to Iezzoni and Pritts (1991). Correlation matrix among the parameters was calculated from the standardized data matrix. PCA was carried out with XLSTAT Pro 2010 software version 3.02 (http://www.xlstat.com/). Cluster analysis was performed based on standardized original data of the 23 leaf parameters using MVSP version 3.1 (http://www.kovcomp.co.uk/mvsp/). Gower General Similarity Coefficient algorithm was employed to determine the phenetic similarities among cultivars through Unweighted Pair-Group Method with Arithmetic average (UPGMA) clustering procedure.

2.3. Microscopic observation

Croton cultivars (C12 and C20 in Fig. 1) were processed with a handy microtome to make transverse leaf sections as we previously described (Yamasaki et al., 1995). Microscopic observations of leaf sections under visible or BV (blue violet) light were conducted with Nikon MICROPHOT-FXA microscope.

2.4. Red pigment analysis

Each 0.2 g of croton leaves (C2, C3, C9, C10, C12, C20, C26 and C28 in Fig. 1) was crushed with liquid nitrogen to make a fine powder. 1 ml of 1% HCl methanol was added to the powdered leaves and