Somaclonal Variation and its Effect on Foliar Epidermal Characters of *Caladium humboldtii* Schott

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ABSTRACT

The ornamental value of *Caladium* species cannot be overemphasized and tissue culture is increasingly being employed in their propagation. Somaclonal variation is also exploited for the generation of new cultivars for the ornamental market. These variations essentially affect leaf morphology. Therefore, to see if there are corresponding anatomical differences, foliar epidermal studies were carried out on parent plant, *Caladium humboldtii* Schott, and a somaclonal variant (*C. humboldtii* 'Sakpere') derived from tuber explants cultured on full strength Murashige & Skoog's (1962) medium supplemented with 3% (w/v) sucrose and 0.4 mg/L 2,4-D combined with 1.0 mg/L kinetin. Morphological differences observed were in the shape and colour of leaves of the *in vitro* derived plantlets. Foliar epidermal studies revealed significant differences in size of epidermal cells, stomatal index and stomatal size of the parent plant and the somaclonal variant. Circular-shaped stomata were encountered in *C. humboldtii*, these were sparse to absent in *C. humboldtii* 'Sakpere'.

KEY WORDS

Somaclonal variation, leaf morphology, anatomy, cultivar, 2, 4-D.

INTRODUCTION

The family Araceae (aroids) has more than 110 genera and 2500–3000 species. About one-third of the plants grown commercially worldwide for ornamental foliage belong to this family and *Caladium humboldtii* Schott is one such plant. *Caladium humboldtii* is known by its petioles (10–24 cm long) and its ovate, small blades (5–9 cm long, 2–4.5 cm broad), which are dull green with white blotches and spots above, and paler below. It has a freely suckering habit and it lacks inflorescences (Croat & Lambert, 1986). The ornamental value of caladiums (*Caladium* × *hortulanum* Birdsey) depends, to a great extent, on leaf characteristics including shape, color, color pattern, and venation pattern. Broadly, caladium leaves are classified into three shapes: fancy, strap, and lance (Z. Deng & B. K. Harbaugh, 2005). *Caladium humboldtii* is an example of lance-leaved caladiums.

Caladium leaf shapes are closely associated with plant growth habit, stress tolerance, tuber yield, etc. Generally, strap- or lance-leaved plants are much shorter in plant height, sprout more leaves from similarly-sized tubers, are more tolerant of sun and low temperatures, and produce smaller tubers than fancy-leaved plants. After orchids, foliage plants are the second most important group that benefits from

tissue culture in general (Debergh, 1994). With the development of many new techniques for breeding, many breeders now take advantage of the ability to regenerate whole plants from single cells. This is because *in vitro* selection makes it possible to isolate cells in culture with desirable traits and may exploit somaclonal variation (Larkin & Scowcroft, 1981). The phenomenon of high variability in individuals from plant cell cultures or adventitious shoots has been named somaclonal variation. Somaclonal variation is not restricted to, but is particularly common in callus-derived regenerants.

Somaclones were regenerated from 3 wheat cultivars, 'Glennson', 'Pavon' and 'PAK-1617', when callus was initiated from seeds cultured on Linsmaier & Skoog (LS) basal medium plus 2 mg/l 2,4-D, 2% sucrose and 1% agar (Mohmand, 1991). A wide range of somaclonal variation was found among potato plants regenerated from protoplast, stem and tuber tissue callus cultures. Many of the primary regenerants differed from the donor plant, and each other as well. Differences in general appearance include leaf size, shape and number. Sixteen randomly chosen regenerants also showed mutual differences in soluble protein patterns (Berljak, 1991).

Stace (1984) emphasized that characters of the leaf are second only to those of flowers and fruits in taxonomic studies and that they are also strictly comparable over a wider taxonomic range than are floral organs. Moreover, they are generally present on the plant for a much greater part of its life span. For these reasons, they are valuable not only in making primary taxonomic decisions, but also in the determination of incomplete plants e.g. sterile specimens, archaeological remains and fragmentary fossils. Metcalfe & Chalk (1979) expounded the use of microscopic characters for reflecting true relationships among species.

The taxonomic value of epidermal morphology is well documented in botanical literature (Dilcher, 1974; Palmer & Gerbeth-Jones, 1986; Adedeji & Faluyi, 2001). Quantitative epidermal characters such as stomatal index, stomatal size and epidermal cell size have also been used extensively (Obiremi & Oladele, 2001; Adedeji & Illoh, 2004; Adedeji 2004)

MATERIALS AND METHODS

Tuber, leaf and petiole explants were collected from greenhouse grown caladium plants and were washed under running tap water to remove dirt and reduce microbial population. The tubers were dehusked and selected for a healthy appearance (i.e. without malformations or presence of necrotic spots). The explants were then surface sterilized with 0.7% (w/v) sodium hypochlorite solution for 10 minutes. Two drops of Tween 20 were added to the sterilizing solution as a surfactant. After 10 minutes, the explants were rinsed three times in sterile distilled water. Tuber explants were cut into small cubes of about 1 cm^2, using sterilized scalpel. The leaves were then trimmed into pieces of about 2 cm^2 and the petioles were cut into pieces about 2 cm long. The explants were cultured on full strength Murashige & Skoog's (1962) medium (MS medium) supplemented with 3% (w/v) sucrose and 0.4 mg/L 2,4-D combined with 1.0 mg/L kinetin. The media was solidified with 0.8% (w/v) agar (Oxoid Agar No 1, Code L11) pH adjusted to 5.7 ± 0.1 (with 0.1 N HCl and/or 0.1 N NaOH, using pH meter) prior to autoclaving. Cultures were maintained at 25°C ± 2°C in the dark.

For foliar epidermal studies, portions of the leaves were taken from the median part (midway between the tip and the base) of the two cultivars. The portions were decolourized by boiling in 90% ethyl alcohol for 15 minutes and then washed in 5–6 changes of water to remove traces of alcohol. The scrape technique of Metcalfe (1960) was used whereby the required epidermis was obtained by scraping it away from the mesophyll. Epidermal peels of both adaxial and abaxial surfaces were made by placing the desired epidermal surfaces face down on a glass slide and scraping off with a sharp blade or a dissecting knife, all tissues above the desired

epidermis until the epidermis was reached. The leaf material being scraped was intermittently irrigated with water and the adhering loose tissues were removed. The epidermal surface was placed on a clean glass slide with the surface desired placed upward.

The epidermal peels were stained in 1% aqueous safranin O for 5–10 minutes and rinsed carefully in water to remove excess stain. The peels were washed in 3–4 changes of water and mounted in dilute glycerine solution. Stomatal index was calculated. It is the percentage proportion of the number of stomata (the guard cell) to the other epidermal cells present on a leaf portion. The Stomatal Index is expressed by the formula,

$$S.I. = \frac{S}{E + S} \times 100\%$$

Where,

S. I. = Stomatal Index

S = number of stomata per unit area

E = number of ordinary epidermal cells plus the subsidiary cells in the same unit area

All measurements were made with the aid of an ocular micrometer. These measurements were later multiplied with the ocular constant to convert to µm. Statistical t-test for the significance of the difference between the quantitative characters of the two cultivars was employed.

RESULTS

Leaf explants of *C. humboldtii* cultured on MS medium supplemented with 0.4 mg/L 2,4-D and 1 mg/L kinetin did not show any response to the growth regulators used. Creamy wet-looking callus was derived from petiole explants after 4 weeks in culture. Plantlets were however generated from tuber explants after 12 weeks in culture. They were transferred into sterilized sawdust (Plate 1B and C) and later to the soil. After some weeks of growth, in the plant shape and colour of leaves (Plate 1A). The plants were separated into 3 groups based on variation in leaf shape and colour; normal plantlets (that looked exactly like the parent plant), plantlets with only colour deviation and plantlets with shape and colour deviation (Plate 1D). The plantlet with shape and colour deviation was picked for the foliar epidermal study, it has been deposited at the Obafemi Awolowo University herbarium (IFE) and is hereafter referred to as *Caladium humboldtii* 'Sakpere'. The parent plant, *Caladium humboldtii* Schott has sagittate-lanceolate leaves with the leaf apex being acuminate and the leaf base sagittate. The somaclonal variant (*C. humboldtii* 'Sakpere') however has orbicular leaves with mucronate apex and sagittate base (Plate 1A).

CALADIUM HUMBOLDTII 'SAKPERE'

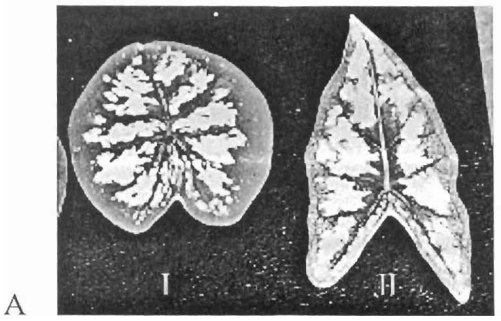
Epidermal cells largely polygonal in shape with straight anticlinal walls on both surfaces, 100.0–142.5 µm long and 47.5–87.5 µm wide on adaxial surface; 65.0–95.0 µm long and 35.0–62.5 µm wide on abaxial surface (Fig 1A and B).

Stomata: amphistomatic leaf surface, largely paracytic, occasionally anisocytic to anomocytic, often elliptic in shape. Stomatal index: adaxial 0%–5%, abaxial 6%–10.1%; Stomatal size–adaxial 437.5–787.5 µm^2, abaxial 343.8–612.5 µm^2. Raphides present.

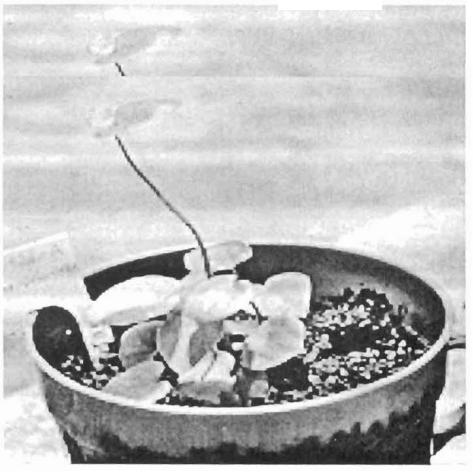
CALADIUM HUMBOLDTII

Epidermal cells are largely polygonal in shape with straight anticlinal walls on both surfaces, 50.0–70.0 µm long and 40.0–62.5 µm wide on adaxial surface; 32.5–62.5 µm long and 30.0–47.5 µm wide on abaxial surface (Fig 1C and D).

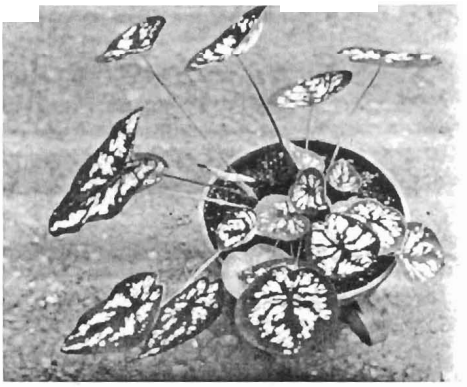
Stomata: amphistomatic leaf surface, largely paracytic, very occasionally anisocytic to anomocytic, circular in shape, but often elliptic too. Stomatal index: adaxial 0–1.4%, abaxial 3.5–8.5%; Stomatal size – adaxial 350.0–618.8 µm^2, abaxial 270.0–



A



B



C

D

Plate 1. (A) Variation in leaf shape: I - Plantlet with shape and colour deviation. II - Parent plant. (B) Plantlets in sterilized sawdust. (C) Plantlets showing variation. (D) Plantlets in soil: I - Normal plantlet. II - Plantlet with shape and colour deviation.

DISCUSSION

The potential instability of adventitious regeneration has been mentioned for some monocotyledonous genera where the incidence of axillary meristems is relatively low (Rice *et al.*, 1992). Lecoufle (1981) found a new near-albino cultivar, which is completely different from the mother plant out of the several thousands of tissue cultured *C. humboldtii* plants identical to the original. This he named *Caladium*

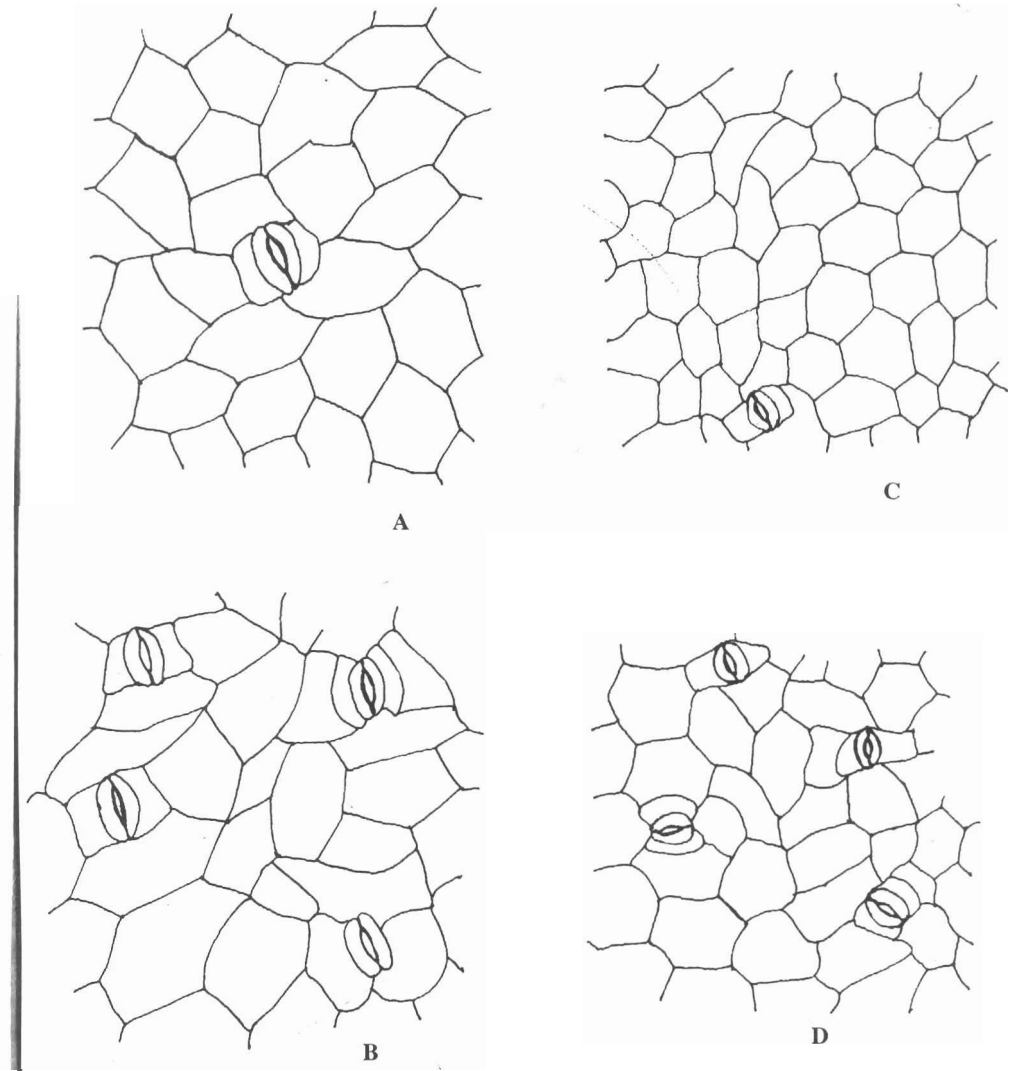


Fig. 1. A—Epidermal cells on adaxial surface of *Caladium humboldtii*. 'Sakpere'. B—Epidermal cells on abaxial surface of *Caladium humboldtii*. 'Sakpere'. C—Epidermal cells on adaxial surface of *Caladium humboldtii*. D—Epidermal cells on abaxial surface of *Caladium humboldtii*.

'Marcel'. According to Deng & Harbaugh (2005), leaf colour is an important characteristic in *Caladium* apart from shape and venation pattern. It is determined by the colour of veins (main, secondary and peripheral), interveinal areas, spots, and/or blotches. They observed that the leaf main vein colour seems to be stable under different environments, on different plants, or at different developmental stages. This stability of color expression in leaf main veins has been useful for cultivar description and identification, besides its ornamental significance.

Ahmed *et al.* (2002), recorded variations in shape and colour pattern of regenerated plants of *C. bicolor*. Mujib *et al.*, (2000) also reported chlorophyll deficiency in a sizeable number of regenerated plantlets of *C. bicolor*. The variations observed in this

experiment and used for foliar epidermal studies include, leaf shape and color intensity in interveinal areas (more green areas with greater intensity). The Protein band in the parent plant and somaclonal variant (*C. humboldtii* 'Sakpere') has been reported to be different (Sakpere & Adebona, 2007). There is however little information on how these variations affects foliar epidermal characters in these species and if it can be used to separate cultivars.

Many authors have used foliar epidermal characters, most especially stomatal index as a taxonomic tool. According to Olatunji (1983), stomatal index is highly constant for a certain species and can be used for species delimitation. Adedeji & Illoh (2004) used stomatal index to separate ten species of *Hibiscus* found in Nigeria into two groups. *Emilia praetermissa*, an allotetraploid hybrid of the other two species in the genus was observed to have intermediate values of the stomatal indices of the two putative parents, suggesting hybridization of the stomatal indices in the allotetraploid hybrid (Adedeji, 2004). A statistical t-test conducted on the values of the stomatal indices of the two cultivars, *C. humboldtii* and *C. humboldtii* 'Sakpere', revealed that at 95% probability level, there is significant difference in the stomatal index of the two cultivars with *C. humboldtii* 'Sakpere' having higher values, affirming that the two cultivars are different.

The epidermal cells in the two cultivars of *C. humboldtii* studied are generally polygonal on both surfaces with straight anticlinal walls. A statistical t-test conducted on the cell size revealed that there is significant difference in the epidermal cell sizes of the two cultivars. Stomata size also follow the same trend with *C. humboldtii* 'Sakpere' having higher values. Stomata shape has been known to delimit species (Adedeji, 2004). In this study, circular-shaped stomata were encountered in *C. humboldtii*, these were sparse to absent in *C. humboldtii* 'Sakpere'.

In conclusion, it has been shown that epidermal cell size, stomata shape, stomatal index and stomatal size can be affected by somaclonal variation. These can also be used as a tool for cultivar delimitation.

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