

Kalanchoë daigremontiana as a Model Plant for the Study of Auxin Effects in Plant Morphology

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The ability to regenerate a plant from a single cell is known as totipotency, and such phenomenon occurs not only in *in vitro* tissue and cell culture but in specialized somatic cells that are part of whole plants in *ex vitro* natural conditions, constituting this asexual or vegetative processes their only means of reproduction [1].

A large number of studies have been conducted in order to understand the mechanisms by which shoots, plantlets and vegetative propagules are produced. According to the first studies reported by Yarbrough [2, 3], in the fern species *Camptosorus rhizophyllus* (which has two different kinds of leaves), shoots are produced at the tip of long acuminate leaves, while in the species *Tolmiea menziesii* shoots are originated in a notch near the junction of the petiole and the leaf blade; in both cases, the shoots are produced from meristematic tissue and once they are mature are naturally detached from the leaf and fall to the ground originating a new plant. Also, in the orchid *Malaxis paludosa*, cells at the tip of mature leaves have an intense meristematic activity which originates embryo-like structures that once the mature leaf is wilted the small shoot detaches originating a new plantlet [4]. This phenomenon has been widely studied in plants of the Crassulaceae family, mainly in the genus *Kalanchoë*. According to the capacity to asexually produce such plants, species of this genus have been classified in four categories: a) plants which do not produce plantlets (*K. marmorata*, *K. rhombopilosa*, *K. tomentosa* and *K. thyrsoiflora*), b) plants that spontaneously produce plantlets (*K. daigremontiana*), c) plants that produce plantlets by the action of an environmental stress (*K. pinnata*, *K. fedstchenkoii*, *K. strepethantha*, *K. prolifera* and *K. crenata*) and d) plants that produce spontaneously plantlets by the action of stress and/or (*K. gastonis-bonniieri*) [5]. According to studies conducted by Batygina et al. [6] in the species *Kalanchoë daigremontiana* (formerly called *Bryophyllum daigremontianum*) and *Kalanchoë pinnata* (formerly called *Bryophyllum calycinum*) the development of the propagules is through somatic embryogenesis where the globular, heart and torpedo stages can be recognized as they occur in zygotic embryos, however, González-Hernández [7] found that the plantlets produced in the leaf margins of *K. daigremontiana* are produced by two mechanisms: organogenic, in which the vascular tissue of the shoot is linked to the vascular strands of the leaf, and somatic embryogenesis where no vascular connections are present between the embryo and the mother leaf (Figure 1). Moreover, molecular studies suggest that both organogenesis and embryogenesis programs are involved in plantlet formation in the leaf notches of this species [8].

Out of all species that belong to the genus *Kalanchoë* the one that has been widely studied and used as a model plant is precisely *K. daigremontiana* mainly due to the high number of plantlets produced in the notches along the leaf margins as opposed to other species that produce few plantlets primarily at the leaf tip [5,8].

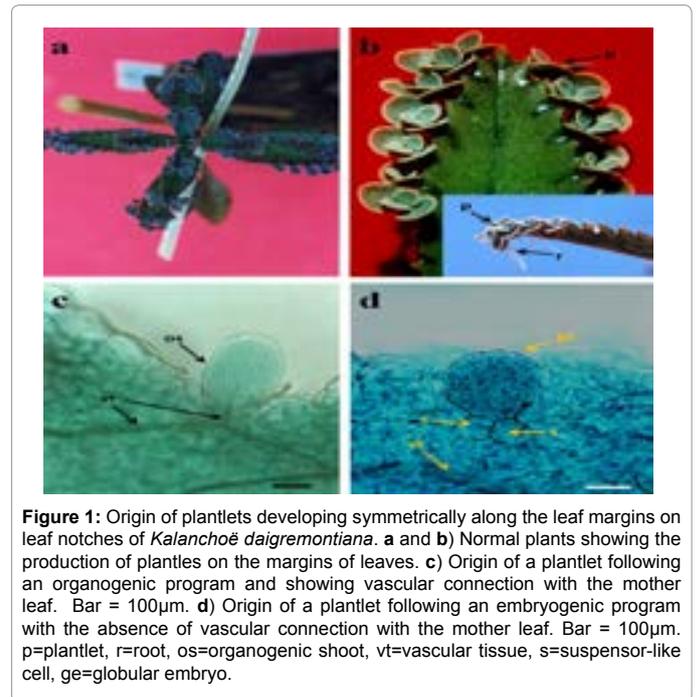


Figure 1: Origin of plantlets developing symmetrically along the leaf margins on leaf notches of *Kalanchoë daigremontiana*. a and b) Normal plants showing the production of plantlets on the margins of leaves. c) Origin of a plantlet following an organogenic program and showing vascular connection with the mother leaf. Bar = 100µm. d) Origin of a plantlet following an embryogenic program with the absence of vascular connection with the mother leaf. Bar = 100µm. p=plantlet, r=root, os=organogenic shoot, vt=vascular tissue, s=suspensor-like cell, ge=globular embryo.

On the other hand, the most abundant auxin occurring in plants is indole-3-acetic acid (IAA) that is involved in a good number of physiological processes in growth and development such as embryo and fruit development, organogenesis, vascular tissue differentiation, root patterning, cell division and elongation and apical dominance [9]. There exist multiple pathways for plant and microbial IAA biosynthesis which have been discovered and elucidated by the use of modern biochemical, genetic and molecular techniques. These have demonstrated tryptophan-dependent and tryptophan independent paths for IAA biosynthesis. The tryptophan-dependent routes have tryptophan as a common initial trough different intermediates such as Indole-3-acetamide, tryptamine, Indole-3-acetaldoxime and Indole-3-

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pyruvic acid finalizing with IAA synthesis. On the other hand, some putative tryptophan-independent routes for IAA biosynthesis have chorismate as a precursor with the possible intermediates Indole-3-acetonitrile and Indole-3-pyruvate [10]. It is important to note that also chorismate is the precursor of the plant hormone salicylic acid which mediates plant defences against pathogens [11] and other kinds of abiotic stress [12].

As mentioned above, this hormone plays an important role in the life of a plant. Recently, its movement from the plant tip downward has been studied by many researchers mainly in the model plant *Arabidopsis thaliana*. The polar auxin transport is an important mechanism that influences cell polarity, patterning of the embryo sac, apical development and general plant morphology, cell elongation and embryo development among many others [13-15]. There are several compounds that inhibit the polar auxin transport, among them, some of the most used in experiments for the study of the action of auxins are 9-hydroxyfluorene-9-carboxylic acid (HFCA) and 2,3,5-triiodobenzoic acid (TIBA). Liu, et al. [16] showed that auxin polar transport is essential for the establishment of bilateral symmetry in *in vitro* cultured Indian mustard (*Brassica juncea*) embryos with the use of TIBA and HFCA. Similarly, the effect of the same compounds on *K. daigremontiana* has been studied and showed similar results to those reported for *B. juncea*. Furthermore, *K. daigremontiana* is a good plant model in teaching the action of auxins and their inhibition on plant morphology (Figure 2).

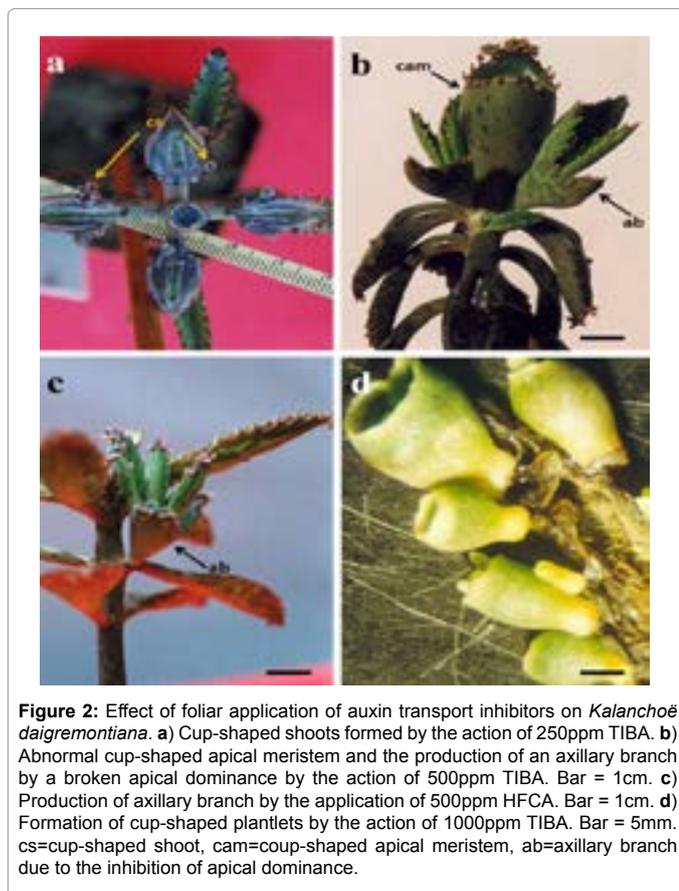


Figure 2: Effect of foliar application of auxin transport inhibitors on *Kalanchoë daigremontiana*. a) Cup-shaped shoots formed by the action of 250ppm TIBA. b) Abnormal cup-shaped apical meristem and the production of an axillary branch by a broken apical dominance by the action of 500ppm TIBA. Bar = 1cm. c) Production of axillary branch by the application of 500ppm HFCA. Bar = 1cm. d) Formation of cup-shaped plantlets by the action of 1000ppm TIBA. Bar = 5mm. cs=cup-shaped shoot, cam=coup-shaped apical meristem, ab=axillary branch due to the inhibition of apical dominance.

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