ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 23(2) 2024, 93–101
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2023.5280
ORIGINAL PAPER
Received: 29.09.2023
Accepted: 01.04.2023
Issue published: 30.04.2024

REGENERATIVE POTENTIAL AND ITS VARIABILITY IN DIFFERENT TOPOPHYSICAL ZONES OF *Kalanchoe daigremontiana* LEAVES IN *in vitro* CULTURE CONDITIONS

Krystyna Winiarczyk[1] ✉, Dominika Czerska[1], Bożena Denisow[2], Ewelina Chrzanowska[2], Jacek Pietrusiewicz[1]

[1] Maria Curie-Sklodowska University, Institute of Biological Sciences, Department of Cell Biology, 19 Akademicka Street, 20-033 Lublin, Poland

[2] University of Life Sciences in Lublin, Department of Botany and Plant Physiology, 15 Akademicka Street, 20-950 Lublin, Poland

ABSTRACT

Plants of the genus *Kalanchoe* are the most frequently purchased potted plants because of their decorative qualities and easy cultivation. Modern commercial plants have unique shapes that are usually not genetically determined; hence, *in vitro* cultures are used to propagate such specimens. The efficiency of regeneration is significantly affected by the type of explant used when that explant was previously located on one of several parts of a complex plant organ. The study aimed to investigate the influence of the topophysical location of collected explants on the regeneration efficiency of adventitious shoots. Various regeneration pathways were observed in the *in vitro* micropropagation of *Kalanchoe daigremontiana* leaf fragments, i.e., direct and indirect organogenesis from the callus stage. The results show a dependent relationship between the location of the collected explant and its regenerative potential. The most remarkable regenerative capacity of *K. daigremontiana* was found in leaf blade fragments taken from the middle and proximal zones. In addition, the explants collected from the marginal zone of the leaf blade were notably more valuable than those from the part located near the midrib. The research results indicate that the growth and development of new seedlings propagated vegetatively with the *in vitro* method depends on their location on the mother plant.

Key words: *Kalanchoe daigremontiana*, topophysis, *in vitro* micropropagation

INTRODUCTION

The genus *Kalanchoe* includes approximately 140 species and numerous interspecific hybrids. The sizeable morphological diversity determines the attractiveness of these plants to the horticultural market. Not only is the exceptional durability of their flowers appreciated, but plants with unique morphological features, such as surface cariculations of leaves, are also becoming increasingly popular. In some cases, mutations result in teretecylindrical, variously flattened, generally apically in-rolled, or peltate leaves in *Kalanchoe* plants. These unique, original shapes are usually not genetically determined, and such specimens can only be reproduced in *in vitro* cultures [Izumikawa et al. 2006]. Globally, plants of the genus *Kalanchoe* are the

✉ krystyna.winiarczyk@umcs.pl

most frequently purchased potted plants because of their decorative qualities and easy cultivation. The sales of plants of this genus reached €62 million in 2015 [Kuang et al. 2021], and the global annual *Kalanchoe* trade in 2018 was estimated at €67–69 million [Smith and Shtein 2022]. In response to the high demand for *Kalanchoe* plants in the marketplace, large enterprises have been established to produce these plants and carry out breeding work. The effects of this work are impressive, e.g., the small tubular structures formed on the adaxial surface of the leaves of *Kalanchoe neglecta* or highly original tomentose leaves formed by *Kalanchoe beharensis* "Baby's Bottom" [Smith 2020]. In the case of F1 hybrids (first-generation offspring from different parents), sterile amphidiploids of these hybrids are formed. In such cases, the only effective method of propagation is micropropagation. Since experimenters most often want to regenerate whole plants in *in vitro* cultures, they are more interested in shoot organogenesis (caulogenesis) than root organogenesis (rhizogenesis) [Dimitrova and Nacheva 2021, Kuang et al. 2021].

Kalanchoe daigremontiana demonstrates that a plant's ability to regenerate from a single cell or a group of cells manifests not only in tissue and cell *in vitro* culture but also in natural conditions in specialized somatic cells of the parent plant. In *K. daigremontiana*, vegetative embryos – propagules – form along the entire length of the leaf edge. It can, therefore, be assumed that there are sufficient levels of the phytohormones in the entire leaf blade responsible for forming vegetative embryos. Based on the above information, it could be argued that the effectiveness of regeneration in *K. daigremontiana* does not depend on the topophysical specifics of the collected explant. This study examines both this theory and another, assuming that, *in planta*, the level of phytohormones in this species, especially auxins, is sufficiently high, and it is not necessary to add synthetic auxin to the medium for regeneration in *in vitro* cultures [Wendling et al. 2015]. Verifying these theories may help develop optimal conditions for regenerating Kalanchoe plants, particularly those of sterile species incapable of sexual reproduction. The knowledge of the relationship between the location of the collected explant and its propagation potential may be a valuable tool for the vegetative propagation of plants. The most significant advantages of this propagation technique include high spatial efficiency with simultaneous reduction of costs (micropropagation can produce even a million progeny plants in one year) and improvement of the growth rate and quality of pathogen-free micro-plants [Previati et al. 2008]. The main scientific objective was to investigate the influence of the topophysical location of the collected explants on the efficiency of regeneration of adventitious shoots in *in vitro* cultures.

MATERIAL AND METHODS

Plant material *Kalanchoe daigremontiana* Raym.-Hamet & H.Perrie (Index Seminum 52) collected from the Botanical Garden of Maria Curie-Skłodowska University in Lublin. The plants were obtained in 2010 and then kept in the Faculty of Biology and Biotechnology greenhouse of Maria Curie-Skłodowska University in Lublin. Throughout the entire period, the plants were propagated vegetatively, i.e., genotypically homogeneous. *K. daigremontiana* plants were grown in a greenhouse illuminated by natural light, with temperatures above 15°C in winter but not thermally regulated in summer. The observations were carried out on a group of 50 mother plants. The experiments' leaves were properly developed without any pathological changes with specific dimensions: 15–25 cm length and approximate 3 mm thickness. The research was conducted on 20 randomly selected plants with three replications. From a single explant, from 1 to 7 embryos were assessed depending on the efficiency of embryogenesis. Macroscopic observations were imaged using a Nikon SMZ 74ST stereoscopic microscope, and photographic documentation was made using the Delta Pix Color digital camera with a dedicated program.

Two media were used for the experiment in vitro, without hormones: MS [Murashige and Skoog 1962] and the addition of hormones: K2C medium recommended by Frello et al. [2002]. Composition of K2C medium with growth regulators: IAA – 3-indolyl acetic acid IAA: 0.8 mg/l; BAP – 6-benzylaminopurine BAP: 0,5 mg/l. The same medium was used for all subsequent passages, and the footing of the seedlings was observed on this medium.

Histological analysis of embryos and vegetative structures formed on the callus was performed using

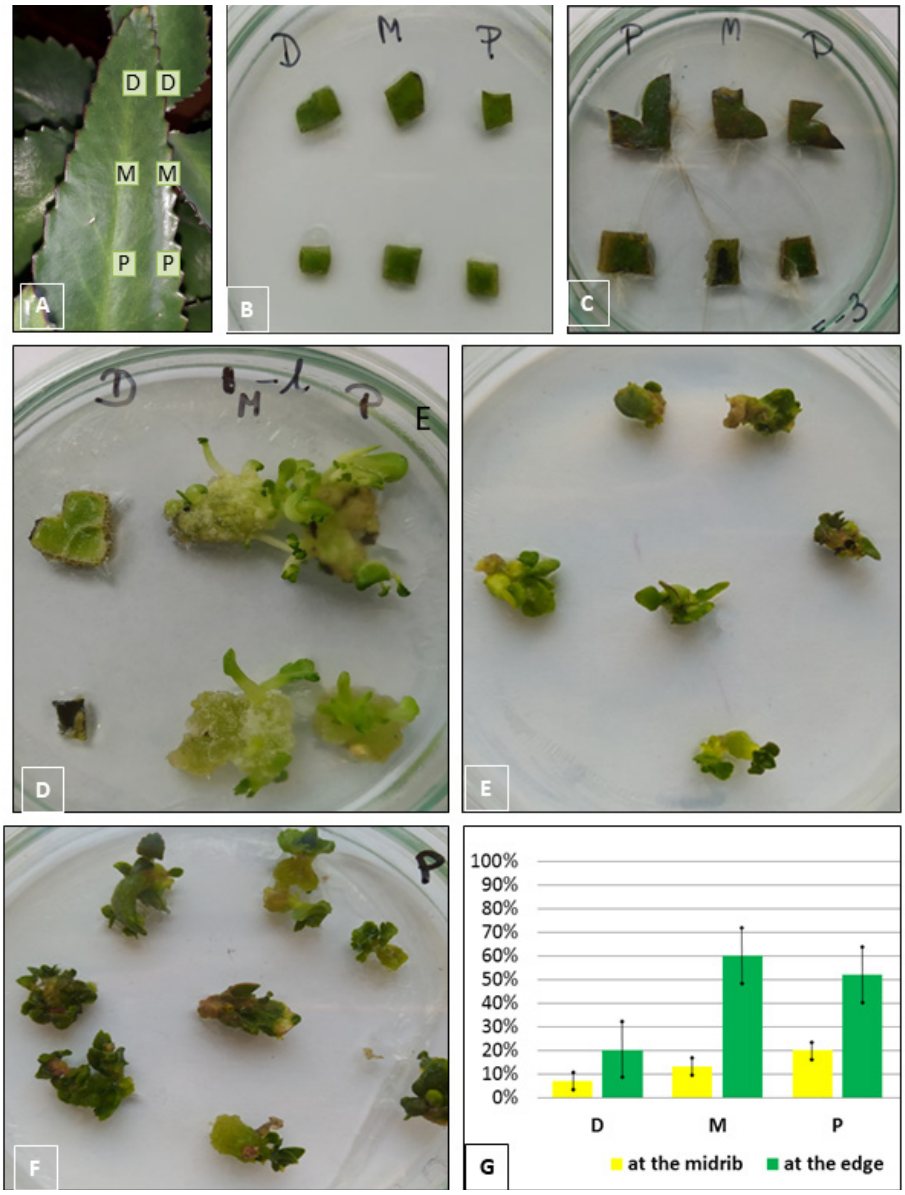

Fig. 1. Stages of the *K. daigremontiana* regeneration and final efficiency.
A. Primary explants were collected from three different topophysical zones: distal (D), central (C), and proximal (P). In each zone, two additional parts were separated: at the midrib and the edge of the leaf blade.
B. Dish with explants on the day of establishing the culture. The upper row contains fragments taken from the part at the edge of the leaf blade; in the lower row, there are leaf fragments taken from the zone near the midrib.
C. Culture at 30 days after plating on MS medium without growth regulators. Numerous young roots growing from explants taken from the leaf blade's marginal part and the zone near the midrib are visible. The average root length ranged from 3 to 23 mm.
D. Culture at 30 days after plating on MS medium supplemented with 0.8 mg/L IAA and 0.5 mg/L BAP. Two regeneration pathways were observed on single explants: indirect regeneration from the callus stage (arrow) and direct organogenesis with the formation of shoot structures (bold arrow).
E. Microshoot fragments ten days after separation from primary explants from zone M.
F. Microshoot fragments ten days after separation from primary explants from zone P.
G. Percentage of initiated regeneration depending on the zone from which the explants were collected

a light microscope (Nikon), and photographic documentation was made with a digital camera and NIS-Elements BP software using the Extended Depth of Focus (EDF) module.

Depending on the efficiency of embryogenesis, from 3 to 10 embryos were collected and analyzed for microscopic observations. After the last *in vitro* passage, the micro seedlings were planted in Paper Pots (Ceres, Poland) and placed in plastic, closed containers with a transparent cover to maintain high humidity. Acclimatization was carried out in a phytotron at a temperature of 23°C and a 16-hour photoperiod. After five days, the plants were gradually hardened to lower air humidity. After two weeks, the young seedlings were transferred to the greenhouse.

***In vitro* experiment.** The leaf blade of *K. daigremontiana* was divided into three topophysical zones: distal, central, and proximal. Fragments of 0.5 × 0.5 cm were isolated from each zone. Each zone was additionally divided into two parts: the part at the midrib and the part at the edge of the leaf blade. Leaf fragments were placed on one plate (Fig. 1 A). The leaves were surface sterilized for 10 min in 5% sodium hypochlorite and then rinsed several times in sterile water. Next, 50 × 50 mm sections of a leaf were cut out with a scalpel. The explants were transferred to K2C medium dishes with composition as used by Frello et al. [2002]. They were incubated in a growth chamber at 25°C illuminated at 80 µmol photon m^{-2} s^{-1} for 16 h and kept in the dark for 8 h. The material was passaged after 14 and 30 days was stored in the same conditions. After embryos and micro shoots emerged, the material was placed in a chamber at 23°C with a 16-hour photoperiod. The seedlings were transferred to plastic pots with a diameter of 8 cm. The acclimatization in the greenhouse conditions lasted four weeks. The plants were grown in pots with universal soil in a greenhouse with natural sunlight.

Microscopic preparation and staining. Young somatic embryos were separated from the explants and fixed in Carnoy's fixative. The material was embedded in paraffin blocks cut longitudinally and transversely into 7-micrometer-thick sections using a Leica model RM2125RTS rotary microtome. The sections were glued to the slides with Haupt's adhesive. The slides were stained using the safranin and permanent green method [Ruzin 1999]. The anatomical documentation was performed using a light microscope (Nikon Eclipse Ni), and photographic documentation was made with a digital camera Nikon DS-Ri2 and NIS-Elements BP software using the Extended Depth of Focus (EDF) module.

RESULTS

The first stage of our research consisted of determining the regeneration ability of explants placed on the MS medium without adding phytohormones (Fig. 1B). The culture was maintained for 30 days. It turned out that, regardless of the topophysical location of the collected material, no callus induction was noted on any of the explants, and only rhizogenesis was observed (Fig. 1C). Therefore, we decided to use MS medium with the addition of 0.8 mg/L indole acetic acid and 0.5 mg/L benzylaminopurine. This medium is recommended for *in vitro* culture experiments on *Kalanchoe* species. After 30 days of cultivation of the *K. daigremontiana* explants, various regeneration pathways were observed, i.e., direct and indirect organogenesis from the primary callus stage (Fig. 1D). Additionally, two paths of regeneration were observed on single explants: indirect regeneration from the callus stage and direct organogenesis with the formation of shoot microstructures. At the same time, it was noted that 80% of the explants taken from the zone near the midrib died, and only a few undertook regeneration, while the number of micro shoots was – from 1 to 5. Callus initiation on these explants was also poor. Much better results were observed on explants from the edges of the leaf blade. The number of shoot structures in individual explants was estimated and cross-referenced to the topophysical zone. In the next stage of the experiment, the first passage was performed on the same K2C medium. After 30 days, the most significant numbers of micro shoots and somatic embryos were observed on explants from the proximal and middle part of the *K. daigremontiana* leaf blade (Fig. 1E). A quantitative and qualitative analysis of the effects of *K. daigremontiana* micropropagation indicated that micro shoots and somatic embryos were formed most abundantly on explants collected from the middle zone (about 60% of such explants achieving this) and the proximal zone (about 52%). In turn, the lowest number of these struc-

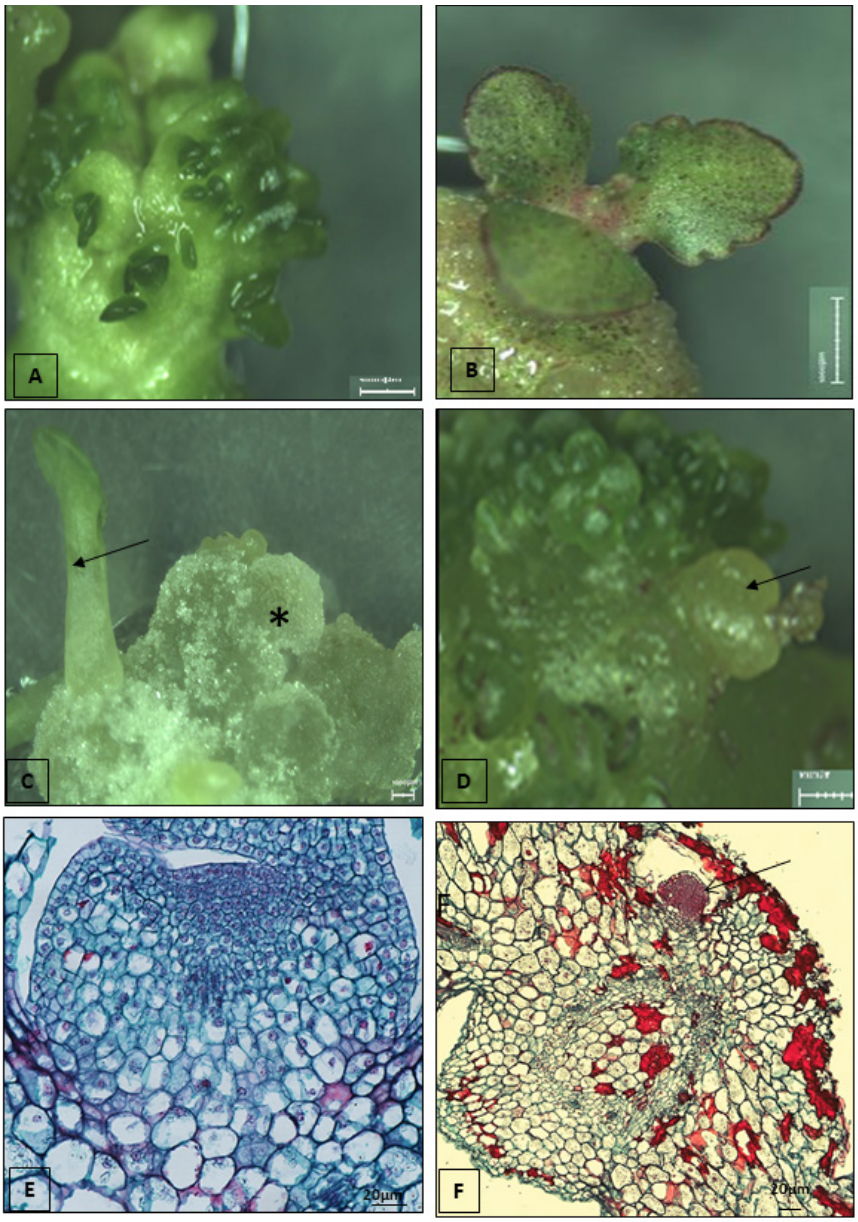

Fig. 2. Different regeneration pathways on the explants taken from the proximal and middle parts: direct (A, B) and indirect from the callus stage (C), direct somatic embryogenesis (D). Pictures taken with the stereomicroscope A-E and LM (F–H).
A. Young 10-day-old micro shoots formed from the proximal part due to direct organogenesis.
B. Well-developed 30-day-old micro shoot before rooting with visible characteristic serration of the edge of the leaf blade (from the proximal part).
C. Indirect caulogenesis. Elongated microshoot on callus tissue.
D. Direct somatic embryogenesis at the globular/heart-shaped stage (arrow).
E. Longitudinal section of the apical micro shoot meristem with leaf primordia on both sides. A layer of protoderm is visible in the central zone of the meristem, and a group of columnar core stem cells is visible below. Underneath, there are larger parenchymal cells with a developed vascular bundle.
F. Globular somatic embryo. The embryo comprises small cells with dense, highly stained cytoplasm and a visible protoderm layer. Groups of differentiated non-embryogenic somatic cells are visible around the embryo

tures was found on explants collected from the distal zone (about 20% of explants from this site displaying any formation) and from the edges of the leaf blade (from 7 to 20%) (Figs 1F and G). As shown by the results, during the vegetative propagation of the studied species in *in vitro* cultures, regeneration proceeded via direct organogenesis (Figs 2 A, B, and D) and indirectly from the callus stage (Fig. 2C). Embryoid structures, globular embryos, and micro shoots were formed (Fig. 2D). At a later stage, these structures developed into young plants with characteristic serrations of the leaf margins (Fig. 2B). After isolation, the young plants were acclimatized to the conditions in the greenhouse, where further cultivation was carried out. All plants obtained in *in vitro* cultures developed normally and did not differ phenotypically from mother plants. The young somatic embryos obtained in the experiment were analyzed in histochemical preparations (Figs 2 E and F). Their anatomical structure was correct and typical of dicot embryos. In the apical meristems of the shoot, zones of intense mitotic divisions were distinguished, which ensured the specific histological organization of the young plant (Fig. 2E). Leaf buds were developed on both sides of the apical meristem of the micro shoot. In the central zone of the meristem, a layer of protoderm covered the stem cells of the tunic and body, and a group of columnar core stem cells was visible below. Underneath were larger parenchymal cells of the core with a developed vascular bundle. The embryo obtained from the micropropagation was composed of small cells with dense, highly stained cytoplasm with a visible layer of protoderm. Groups of differentiated non-embryogenic somatic cells were visible around the embryo.

DISCUSSION

Seeds propagated the first varieties of plants of the genus Kalanchoe, and the resulting plants were phenotypically very diverse. Currently, most hybrid varieties are propagated vegetatively to ensure the uniformity of flower form, habit, growth, and color. Another valuable advantage of the *in vitro* method is the possibility of using virtually any non-meristematic plant organ (or its fragment) as a source of explants. Through micropropagation, numerous genetically identical plants can be obtained in *in vitro* cultures relatively quickly. The effectiveness of this micropropagation method depends on many factors, e.g., the composition of the medium, type of explant, and genotype. It has been proved in some species that the efficiency of regeneration may also depend on the location of the explant on the mother plant, which is the so-called phenomenon of topophysis [Zalewska and Miler 2010].

Regeneration of plants propagated vegetatively using the *in vitro* technique can proceed directly (embryos with cotyledons and first true leaves) and indirectly from the callus stage. For instance, indirect organogenesis occurs in *Kalanchoe rhombopilosa* Mannoni & Boiteau, and it has been shown that stem segments are the recommended explants for *in vitro* shoot and root multiplication [Kertrung and Junkasiraporn 2018, Pacholczak and Nowakowska 2020]. However, research conducted by Sanikhani et al. [2006] showed that explants of *Kalanchoe blossfeldiana* taken from petioles were more efficient than those taken from leaves. In addition, Smith and Nightingale [1979] showed that plants of this species obtained *in vitro* were highly branched and had short internodes, which is commercially a precious attribute.

The ability of *K. daigremontiana* to form somatic embryos outside the ovary provides an attractive model system for studying somatic embryogenesis in nature. Crucial is the ability of vegetative cells to acquire the competence to create embryos without fertilization. As demonstrated in this species, the ability to regenerate from somatic cells occurs not only in *in vitro* tissue and cell culture but also in specialized somatic cells of the parent plant in natural conditions [Garcês and Sinha 2009].

Based on this unique ability to form somatic embryos on leaves, we attempted to assess the ability of *K. daigremontiana* explants to regenerate in a medium without synthetic hormones [Kertrung and Junkasiraporn 2018]. After 30 days of cultivation, we noted that regardless of the zone from which the material was taken, only rhizogenesis, i.e., the formation of numerous adventitious roots, was observed.]. The initiation and course of developmental processes in *in vitro* cultures depend on the type and mutual proportions of growth regulators present in the medium. There is a certain general regularity in the influence of auxins and cytokinins on organogenesis. A high le-

vel of auxins stimulates root formation, while a high concentration of cytokinins promotes shoot formation. The balance in the levels of both hormone classes influences the callus formation, i.e., undifferentiated or poorly differentiated tissue composed of parenchymatous cells [Su et al. 2011].

Sometimes, both processes, i.e., organogenesis and somatic embryogenesis, have been observed in *K. daigremontiana* leaf explants. Ludvová and Ostrolucká [1998] described the coexistence of indirect organogenesis and indirect embryogenesis in specific conditions of *Actinidia chinensis* Planch – leaf culture. Callogenesis, caulogenesis, rhizogenesis, and somatic embryogenesis have been observed on *Echeveria elegans* Bgr isolated leaves. (Crassulaceae) [Solis et al. 2013]. Somatic embryogenesis has been found to occur more frequently in younger tea plant leaves [Kato 1996]. Spontaneous variability is often observed among *in vitro*-propagated plant individuals [Schwaiger and Horn 1987]. Somaclonal changes can be caused either by spontaneous changes occurring during the stages of unorganized callus growth or by the genetic heterogeneity of the explants. Before starting mass propagation of plants, it is essential to determine the extent of such variations.

The differentiation of the explants depending on the zone (distal, central, and proximal) allowed verification of the thesis that the growth and development of new *K. daigremontiana* seedlings propagated vegetatively depend on their location on the mother plant. There are few studies on the correlation between the site of explant excision and regenerative capacity. Previous experiments were conducted on flowering plants such as rose [Le Bris et al. 1998] and chrysanthemum [De Ruiter 1996]. However, in woody plants, a strong relationship was found between the location of the shoot used as an explant and its ability to root [Peer and Greenwood 2001]. Based on the results of studies on the chrysanthemum, it was found that the regenerative activity of explants depended on their topophysical location and differed depending on whether a fragment of a shoot or a leaf was used. There was no regeneration on the explants taken from the proximal part of the leaf, whereas the most significant number of adventitious shoots was formed on explants taken from the distal part of the leaf [Zalewska and Miler 2010, Miler and Zalewska 2014].

The morphological and anatomical analysis of *K. daigremontiana* explants showed the greatest regenerative potential in the explants originating from the proximal and middle parts of the leaf. In addition, the expansive leaf sections taken from the marginal zone of the leaf blade turned out to be much more valuable than those from the part near the midrib. These results support the conclusion that, in *K. daigremontiana*, there is a relationship between the location of the collected explant and its regenerative potential. The greatest regenerative potential in this species was obtained from explants collected from the edge of the leaf blade in the middle (M)‑and proximal (P) zones. Such observations coincide with the *K. daigremontiana* leaf development pattern; as the leaves mature from tip to base, the divisions cease first in the upper part and then on the side. The mitotic divisions persisted for the longest time in the marginal part of the leaf basal zone. Similar observations were reported in a study on tobacco [Cantrill et al. 2001]. *In vitro* cultures are the primary tool for biotechnologists' work in the callus multiplication process to create new varieties and interspecies hybrids and obtain valuable metabolites and biopharmaceuticals [Radomir et al. 2022]. Bidabadi and Jain [2020] point out that many events during the plant regeneration process can be controlled by manipulating the signaling pathways associated with phytohormone interaction, explant injury, and programmed cell death. Nevertheless, questions remain about how explants transmit endogenous and environmental signals and induce or maintain cell differentiation. The research results presented in this study are part of the trend expanding the knowledge of *in vitro* plant regeneration for more comprehensive applications in crop improvement, commercial applications, and acquisition of secondary metabolites [Pacholczak and Nowakowska 2020, Buyun et al. 2021].

CONCLUSIONS

Micropropagation in *in vitro* cultures is highly recommended for rapid propagation of new *Kalanchoe cultivars*, as every leaf, shoot tip, and stem section can be used as an explant for cultivation. In plants of the genus *Kalanchoe*, the effects of in vitro micropropagation may vary depending on the species and even the variety.

The results of our research demonstrate different regeneration pathways, i.e., direct and indirect organogenesis from the callus stage, in *K. daigremontiana*. The final effect of *K. daigremontiana* micropropagation is also significantly influenced by topophysis. Therefore, it is crucial to know not only which organ but also from which of its parts the explant is collected, as in the case of the leaf.

SOURCE OF FUNDING

This research was funded by the Ministry of Education and Science of Poland, LKR/S/49/2023

AUTHOR CONTRIBUTIONS

KW, BD — supervised the experiment and preparation of the manuscript; DC, JP, EC performed the experiment. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTERESTS

All authors declare that they have no conflict of interest.

REFERENCES

Bidabadi, S.S., Jain, S.M. (2020). Cellular, molecular, and physiological aspects of *in vitro* plant regeneration. Plants (Basel), 9(6), 702. https://doi.org/10.3390/plants9060702. PMID: 32492786; PMCID: PMC7356144.

Buyun, L., Ivannikov, R., Batsmanova, L., Taran, N., Kovalska, L., Gurnenko, I., Maryniuk, M. (2021). Structural adaptation and antioxidant response of *Guarianthe bowringiana* (O'Brien) Dressler & WE Higgins (Orchidaceae Juss.) seedlings during ex vitro acclimatization. Acta Agrobot., 74(1), 7422. https://doi.org/10.5586/aa.7422

Cantrill, L.C., Overall, R.L., Goodwin, P.B. (2001). Changes in symplastic permeability during adventitious shoot regeneration in tobacco thin cell layers. Planta, 214, 206–214. https://doi.org/10.1007/s004250100620

De Ruiter, H.A. (1996). Development of chrysanthemum cuttings: the influence of age and position of the axillary buds. Ann. Bot., 77(1), 99–104.

Dimitrova, N., Nacheva, L. (2021). An optimized micropropagation protocol by ex vitro rooting of pear rootstock OHF 333 (*Pyrus communis* L.). Acta Agrobot., 74(1), 744. https://doi.org/10.5586/aa.744

Frello, S., Venerus E., Serek M. (2002). Regeneration of various species of Crassulaceae, with special reference to Kalanchoe. J. Hortic. Sci. Biotechnol., 77(2), 204–208. https://doi.org/10.1080/14620316.2002.11511480

Garcês, H., Sinha, N. (2009). The 'mother of thousands'(*Kalanchoë daigremontiana*): a plant model for asexual reproduction and CAM studies. Cold Spring Harb. Protoc., 10. https://doi.org/10.1101/pdb.emo133

Izumikawa, Y., Nakamura, I., Mii, M. (2006). Interspecific hybridization between *Kalanchoe blossfeldiana* and several wild Kalanchoe species with ornamental value. Acta Hort., 743, pp. 59–65. https://doi.org/10.17660/ActaHortic.2007.743.7

Kato, M. (1996). Somatic embryogenesis from immature leaves of *in vitro* grown tea shoots. Plant Cell Rep., 15, 920–923. https://doi.org/10.1007/BF00231588

Kertrung, T., Junkasiraporn, S. (2018). *In vitro* propagation of *Kalanchoe rhombopilosa* (Crassulaceae). NU Int. J. Sci., 15(1), 37–48.

Kuang, Y., Lu, C.H., Hsu, F.C. (2021). Restoring fertility for novel interspecific hybrids between *Kalanchoe garambiensis* and *K. nyikae* using colchicine treatment. Plants, 10, 209, 16 pp. https://doi.org/10.3390/plants10020209

Le Bris, M., Michaux-Ferrière, N., Jacob, Y., Poupet, A., Barthe, P., Guigonis, J.M., Le Page-Degivry, M.T. (1999). Regulation of bud dormancy by manipulation of ABA in isolated buds of *Rosa hybrida* cultured *in vitro*. Funct. Plant Biol., 26(3), 273–281. https://doi.org/10.1071/pp98133

Ludvová, A., Ostrolucká, M.G. (1998). Morphogenic processes in callus tissue cultures and de novo regeneration of plants in *Actinidia chinensis* Planch. Acta Soc. Bot. Pol., 67(3–4), 217–222. https://doi.org/10.5586/asbp.1998.025

Miler, N., Zalewska, M. (2014). Somaclonal variation of chrysanthemum propagated *in vitro* from different explants types. Acta Sci. Pol. Hortorum Cultus, 13(2), 69–82.

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Phys. Plant., 15(3), 473–497.

Pacholczak, A., Nowakowska, K. (2020). The effect of biostimulators and indole-3-butyric acid on rooting of stem cuttings of two ground cover roses. Acta Agrobot., 73(1), 7314. https://doi.org/10.5586/aa.7314

Peer, K.R., Greenwood, M.S. (2001). Maturation, topophysis and other factors in relation to rooting in Larix. Tree Physiol., 21(4), 267–272. https://doi.org/10.1093/treephys/21.4.267

Previati, A., Benelli, C., Da Re, F., Giannini, M. (2008). *In vitro* production of virus-free chrysanthemum stock plants for cut flowers. Propag. Ornam. Plant, 8, 167–169.

Radomir, A.M., Stan, R., Pandelea, M.L., Vizitiu, D.E. (2022). *In vitro* multiplication of *Mentha piperita* L. and comparative evaluation of some biochemical compounds in plants regenerated by micropropagation and conventional method. Acta Sci. Pol. Hortorum Cultus, 21(4), 45–52. https://doi.org/10.24326/asphc.2022.4.5

Ruzin, S.E. (1999). Plant microtechnique and microscopy. Vol. 198. Oxford University Press, New York, p. 322.

Sanikhani, M., Frello, S., Serek, M. (2006). TDZ induces shoot regeneration in various *Kalanchoe blossfeldiana* Poelln. cultivars in the absence of auxin. Plant Cell Tiss. Organ Cult., 85, 75–82. https://doi.org/10.1007/s11240-005-9050-6

Solís, J. J., Reyna, M., da Feria, M., Cardona, M. A., Rojas, D. (2013). *In vitro* propagation of *Echeveria elegans*, a species of the flora endangered Mexican. J. Environ. Sci. Engineer. B, 2(9B), 555–558.

Smith, G.F. (2020). Plant smart. The big easy. Gardening with Kalanchoe. Veld Flora, 106(3), 40–43.

Smith, G.F., Shtein, R. (2022). A review of horticulturally desirable characters in Kalanchoe (*Crassulaceae* subfam. Kalanchooideae): variable and deviating vegetative and reproductive morphologies useful in breeding programmes. Haseltonia, 28(1), 106–119. https://doi.org/10.2985/026.028.0110

Smith, R.H., Nightingale, A.E. (1979). *In vitro* propagation of Kalanchoe. HortScience, 14, 20.

Su, Y.H., Liu, Y. B., Zhang, X.S. (2011). Auxin–cytokinin interaction regulates meristem development. Mol. Plant, 4(4), 616–625. https://doi.org/10.1093/mp/ssr007

Schwaiger, G., Horn, W. (1987). Somaclonal variations in micropropagated Kalanchoe hybrids. Acta Hort., 226, 695–698. https://doi.org/10.17660/ActaHortic.1988.226.98

Wendling, I., Brooks, P.R., Trueman, S.J. (2015). Topophysis in *Corymbia torelliana*× *C. citriodora* seedlings: adventitious rooting capacity, stem anatomy, and auxin and abscisic acid concentrations. New For., 46, 107–120. https://doi.org/10.1007/s11056-014-9451-7

Zalewska, M., Miler, N. (2010). Topophysis in adventitious shoots regeneration *in vitro* in chrysanthemum. Biotechnologia, 2(89), 89–95.