International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 11, Issue 4-2024

Review Article

DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.04.008

# Adaptation of regenerants of sterile cultures to *ex vitro* conditions - A review

**Elena Kutas**
Central Botanical Garden of the NAS of Belarus, 220072 Minsk, Surganova, 2v,
Republic of Belarus. E-mail: *vinogradova-kira@tut.by*.
Tel: (+378 14) 358-15-89.Fax: (+378 14) 378-14-84.

## Abstract

A comparative analysis of the structural and functional features of regenerants *in vitro* and *ex vitro* conditions is presented, based on literary data and materials of their own research. The anatomical structure of the leaves of plants grown in aseptic culture, in greenhouse conditions and in the open ground is considered. It has been shown that *in vitro* and *ex vitro* growing conditions leave an imprint on the structure and functions of regenerants− firstly; secondly, the structurally functional organization of regenerants is a mobile system, and it can be rebuilt in accordance with changed environmental conditions. Differences in the structure and function of the leaves of plants grown in aseptic culture, in greenhouse conditions and in the open ground, indicate the plasticity of the leaf, an organ capable of reconstructing its structure and function adequately to the conditions of cultivation, which theoretically is a guarantee of successful adaptation of plants when transferring them from *in vitro* to *ex vitro* conditions.

**Keywords:** adaptation, regenerants, *in vitro*/*ex vitro* conditions

## Introduction

Clonal micro-reproduction of plants is based on two fundamentally different stages: *in vitro* and *ex vitro*. In the first of them, the vital activity of the propagated material takes place in a sterile enclosed space, on a nutrient medium under strictly controlled conditions. After the transfer of regenerants from *in vitro* conditions, the second stage of the regenerants' vital activity begins in the *ex vitro* system, that is, in greenhouse and open ground conditions completely different from *in vitro* conditions.

In *ex vitro* conditions, plants are forced to switch from a heterotrophic type of nutrition to an autotrophic one, which is associated with a structural and functional restructuring of the body in new conditions. The transition of plants from *in vitro* to *ex vitro* conditions is critical in most cases and is associated with plant death (Jones, 1987; Conner and Thomes, 1981; Kramarenko et al., 1988; Grout and Aston, 1977; Burdasov et al., 1988; Conner and Conner, 1984). From our point of view, a comparative analysis of the structural

and functional characteristics of regenerants *in vitro* and *ex vitro* conditions will help to understand the cause of plant death during adaptation and prevent it.

## Structural and functional features of regenerants in *in vitro* and *ex vitro* conditions

According to research by Noé and Bonini (1996) in vitro-developed leaf cells of micropropagated high bush blueberry (*Vaccinium corymbosum* L.) cv. 'Bluetta' were circular and small, the spongy parenchyma was discontinuous and disorganized and formed by 1 - 2 layers of cells with large intercellular spaces and the palisade to spongy mesophyll thickness ratio was1:1.5. After rooting ex vitro, the first leaves formed under natural conditions showed substantial changes in the anatomical characteristics. After 6 months, the plants produced leaves similar to those in field-grown plants. The palisade cells were rectangular, the spongy parenchyma was formed by 3 - 4 layers of cells and the intercellulars were around the stomata. Leaves from field-grown plants lost24 % of water during 15 min after excision while leaves from in vitro shoots lost about 50 % of water in the same time. Leaves from in vitro shoots showed a higher number of smaller stomata (361 permm$^2$), with the guard cells forming a circular ring; the stomata frequency in field-grown leaves was 241per mm$^2$and the guard-cells were elliptical.

Review by Pospíšilová et al.,(2007) focused on the changes essential for stabilization of water relations of plantlets (development of cuticle, epicuticular waxes, and functional stomatal apparatus leading to effective regulation of transpiration); the improvement of photosynthetic apparatus (changes in chlorophyll 'a' and 'b' contents, photosynthetic efficiency and net photosynthetic rate) ensuring fully autotrophic growth with the rate corresponding to naturally grown plants; occurrence of photoinhibition, and possibilities of improvement of *ex vitro* transfer by *in vitro* hardening or by application of abscisic acid (ABA) and/or $CO_2$ enrichment.

Studies conducted by Brainerd et al., (1981) on the study of leaf anatomy and water stress in plum plants under *in vitro* and *ex vitro* conditions showed that water loss occurs three times faster in plants obtained *in vitro* culture compared with plants from a greenhouse. In regenerates grown under aseptic conditions, the thickness of the front garden cells was significantly less compared to regenerants from the greenhouse and open ground.

According to the results of research by Grout (1975), Sutter and Langhans (1979), in plants cultivated *in vitro* conditions, the leaves are devoid of wax coating, and the stomata work is imperfect due to a violation of the mechanism in their opening and closing. Lee et al. (1988), Brainerd and Fuchigami (1982), Wardle and Short (1983) came to a similar conclusion about the work of scientists.

According to Bunning and Sagromsky (1948); O'Leary und Knecht (1981); Penfound (1931), the development of stomata is influenced by factors such as the concentration of $CO_2$ in the vessel, the water regime and hormonal levels. Stomata of plants under *in vitro* conditions are usually in an open state, which cannot be said about stomata under *ex vitro* conditions (Brainerd and Fuchigami, 1981; Wetzstein and Sommer, 1982a, 1983b). From our point of view, such behavior of stomata *in vitro* is quite justified, since a very high level of relative humidity (more than 90%) is constantly maintained in culture vessels, temperature and illumination are not subject to fluctuations, since they are under controlled conditions. However, it is necessary to change the conditions in the culture vessels, as the stomata will react to these changes.

A real confirmation of this is the results of experimental studies obtained by Schoch et al., (1989) in the process of studying photosynthesis and respiration of bananas in the *in vitro* system. The authors conclude that when banana shoots are grown*in vitro*, the stomata on the leaves function normally, that is, they react to light and close when creating water stress. Therefore, stomata react adequately to the conditions in which the

plant is located. From this point of view, the failure that befell some researchers who sought to artificially interfere with the precise work of stomata corresponding to the conditions in which they are located becomes understandable. For example, the use of antitranspirants during the transfer of plants from *in vitro* to *ex vitro* conditions contributed to a decrease in photosynthesis, which was a consequence of a deterioration in plant growth (Danies and Kozlowski, 1974).

According to Fabbri and Shutter (1986), the structure of the strawberry leaf formed *in vitro* culture was characterized by a relatively thin leaf blade, underdeveloped palisade cells, large air-bearing cavities, and underdeveloped cuticle. At the same time, the strawberry leaf formed in *ex vitro* conditions was differentiated into columnar and spongy tissue, and had a well-developed cuticle cover. Similar results were obtained by Donnelly and Vidaver (1984) in the study of raspberry leaves formed *in vitro*.

Waldenmaier and Schmidt (1990) observed histological differences between rhododendron leaves *in vitro* and *ex vitro* during their hardening. These differences consisted in the absence of respiratory pores, weakly structured mesophyll in the leaves *in vitro*, and in the leaves *ex vitro*, there was a change in the anatomical structure of the leaves: their thickness increased, the number of layers of epidermis and palisade tissue, cuticle formed. Acclimatization at low humidity led to a clear differentiation of the tissue into columnar and spongy parenchyma.

Studies conducted by Sidorovich and Kutas (1996) to study the internal structure of the leaf depending on cultivation conditions showed that regenerants of introduced varieties of high blueberry ('Dixi', 'Bluecrop') and cranberry ('Koralle') grown *in vitro* did not have a clear differentiation of mesophyll into columnar and spongy tissue, had a thin leaf lamina, underdeveloped cuticle, underdeveloped stomatal apparatus, which contributed to the constant opening of stomata and excessive transpiration.

The leaves of plants developing in greenhouse conditions had a clear differentiation of the mesophyll into columnar and spongy parenchyma, cuticle cover, and a developed stomatal apparatus, which contributed to the normal provision of transpiration. The leaves of plants planted in the open ground, according to the general plan of the structure, did not differ from the leaves of greenhouse plants. They had a clearly differentiated leaf structure into columnar and spongy parenchyma, well-developed cuticle cover, stomatal apparatus.

However, it should be noted that there was a difference in the change in quantitative indicators of the leaf structure. Thus, leaves from the open ground had a thicker leaf blade (400 microns), more layers of columnar tissue, longer cell length, and a smaller volume of intercellular cells compared with leaves from a greenhouse (286 microns) and an aseptic culture (91 microns) (see Table).

The studied plant varieties reacted to the cultivation conditions by changing both quantitative parameters and the internal structure of the leaf. Open ground conditions with increased solar insolation and relatively low air humidity contributed to an increase in the thickness of the leaf blade, the palisade coefficient, the length of columnar tissue cells, the number of stomata per 1 $mm^2$ of the leaf surface, and greenhouse conditions with reduced solar insolation and relatively high air humidity led to a decrease in the magnitude of these indicators.

Table *. Quantitative indices of anatomical leaves structure of *Vaccinium corymbosum* and *Vaccinium vitis-idaea* cultivated in the a septical culture, greenhouse and open ground (Sidorovich and Kutas, 1996).

| Indicators of anatomical structure | Type, variety | | |
|---|---|---|---|
| | *Vaccinium corymbosum* | | *V. vitisidaea* |
| | 'Bluecrop' | 'Dixi' | 'Koralle' |
| Aseptic culture (*in vitro*) 4000 Lx | | | |
| Leaf thickness, µm | 76±2 | 85±3 | 91±4 |
| The number of stomata per 1 $mm^2$ | 16±1 | 16±1 | 19±1 |
| Stoma size (length x width), µm | 15x11 | 15x12 | 16x10 |
| Greenhouse 15000 Lx | | | |
| Leaf thickness, µm | 154±16 | 173±13 | 286±9 |
| Palisade cofficient | 0,75 | 0,71 | 0,68 |
| Length: width of cells of palisade tissue ratio | 1,8:1 | 1,9:1 | 2,6:1 |
| The number of stomata per 1 $mm^2$ | 251±11 | 250±9 | 410±20 |
| Stoma size (length x width), µm | 25x17 | 26x16 | 24x15 |
| Open Ground 50000 Lx | | | |
| Leaf thickness, µm | 210±11 | 221±12 | 450±19 |
| Palisade cofficient | 0,87 | 0,9 | 0,86 |
| Length: width of cells of palisade tissue ratio | 2,5:1 | 2,7:1 | 3,31:1 |
| The number of stomata per 1 $mm^2$ | 260±12 | 265±10 | 430±23 |
| Stoma size (length x width), µm | 23x16 | 24x15 | 21x14 |

*In the Table no indices are shown of palisade coefficient and of palisade tissue cells with the leaves of plants from aseptic culture, since the mesophill of the leaf was not differentiated into palisade and spongy mesophills

*In vitro* cultivation conditions, characterized by relatively high air humidity in culture vessels, low illumination and a heterotrophic type of nutrition, contributed to a decrease in the thickness of the leaf blade, a reduction in the number of stomata per 1 $mm^2$ of the leaf surface, and the absence of differentiation into columnar and spongy parenchyma. The *in vitro* leaf structure has all the signs of a leaf of a plant growing in the shade (homogeneous mesophyll consisting of cells of only spongy parenchyma having an isodiametric shape; a thin leaf blade; a small number of stomata per 1$mm^2$ leaf surface; absence of cuticle).

It should be said that the differences in the structure of the sheet are associated with their functional differences. An example of this is a thorough study on the comparative anatomy and physiology of the Asian birch grown in aseptic culture and in a greenhouse, conducted by Smith et al. (1986).The authors come to the conclusion

about the weak development of the vascular system in *in vitro* conditions and, as a result, about the increased sensitivity of such plants to water stress, characteristic of *ex vitro* conditions. They found a low intensity of photosynthesis at a very low level of illumination, which is associated with the lack of clear differentiation of the leaf into columnar and spongy tissue in *in vitro* culture. After transferring the plants to *ex vitro* conditions (in a greenhouse), the researchers observed an increase in the intensity of photosynthesis and changes in the anatomy of the leaf. In their opinion, plants grown under aseptic conditions significantly change their anatomical and physiological properties compared to their counterparts cultivated in *ex vitro* conditions. These differences are mainly the result of exposure to a specific environment in aseptic culture and disappear after the transfer of plants to *ex vitro* conditions due to the rapid restoration of metabolism as a consequence of normal plant development.

According to research by Donnelly et al. (1984), Grout and Millam (1985) photosynthetic activity in red raspberry and strawberry shoots in *in vitro* culture is lower compared to photosynthetic activity in *ex vitro.* The leaves formed *in vitro* had a maximum photosynthetic activity up to 14 days after their transfer from the culture *in vitro.* The authors conclude that plants survive during acclimatization using a supply of metabolites. The normal restoration of structure and function occurs in regenerants within a month from the moment they are placed in *ex vitro* conditions. In order to increase the survival rate of plants during adaptation, it is necessary to gradually reduce the relative humidity of the air and increase irradiation. This contributes to an increase in the area occupied by the palisade cells, which in turn has an effect on increasing the intensity of photosynthesis.

Interesting studies were conducted by Solarova (1989) to study the daily variability of $CO_2$ concentration in culture vessels as a result of photosynthetic activity of plants. Using the potentiometric method, changes in the concentration of $CO_2$ in culture vessels in which regenerating plants obtained from pieces of leaves were grown were determined. It turned out that during the dark period, the concentration of $CO_2$ in the vessels increased and was associated with the size of the regenerants and the sucrose content in the medium. During the light period, the $CO_2$ concentration in the tubes decreased and, despite the low illumination (100 mmol/ $m^{-2} \cdot s^{-1}$), after 3-4 hours of illumination it reached the compensation point. The author concludes that the low concentration of $CO_2$ in closed vessels for the cultivation of regenerating plants is the main limiting factor constraining their growth.

Therefore, one of the reasons for the low intensity of photosynthesis observed in regenerating plants in *in vitro* culture is the reduced concentration of $CO_2$ under these conditions.

When transferring plants in *ex vitro* conditions, the concentration of $CO_2$ increases, which increases the intensity of photosynthesis and, as a result, accelerates their growth.

## Conclusion

Based on a comparative analysis of the structural and functional characteristics of regenerants in *in vitro* and *ex vitro* conditions, based on literature data and materials from our own research, we came to the conclusion that the conditions of cultivation *in vitro* and *ex vitro* leave an imprint on the structure and function of regenerants− this is firstly; secondly, the structurally functional organization of regenerants is a mobile system, and it can be rebuilt in accordance with the changed environmental conditions. This means that differences in the structure and function of the leaves of plants grown in aseptic culture, in greenhouse conditions and in the open ground, indicate the plasticity of the leaf − an organ capable of rebuilding its structure and function adequately to the conditions of cultivation, which theoretically is a guarantee of successful adaptation of plants when transferring them from *in vitro* conditions to *ex vitro* conditions.

In practice, as our observations of the adaptation process of introduced varieties of high blueberry ('Dixi', 'Bluecrop', 'Herbert', 'Rancocas', 'Covill', 'Earlyblue') and cowberry ('Koralle', 'Masovia', 'Erntedank') have shown when transferring them from *in vitro* to *ex vitro* conditions, we managed to avoid losses of varieties at a critical moment for them due to compliance with technical techniques based on conclusions confirmed by the results of experimental studies.

In order to prevent the death of regenerants from excessive transpiration (this applies not only to blueberries and lingonberries), which occurs for reasons known to us: 1) due to a sharp decrease in humidity in *ex vitro* conditions and 2) due to the imperfect structural and functional organization of the leaf from the point of view of *ex vitro* conditions, first of all it is necessary to raise the turgor of regenerants to the maximum value. This is ensured by immersing regenerants in a vessel with distilled water for 5-6 hours.

The second prerequisite is the creation of high humidity in the greenhouse (at least 90%) and the elimination of strong air flows, that is, the exclusion of any wind, since the wind contributes to the drying of leaves due to the rapid return of moisture. The absence of wind and high humidity will contribute to the creation of a vapor pressure gradient between the leaves and the air.

In the first 2-3 weeks of cultivation of regenerants (before the formation of roots) in the greenhouse, it is necessary to create conditions identical to *in vitro* conditions. This means strictly controlling humidity, maintaining a temperature similar to that at which regenerants were cultivated *in vitro*, and a relatively low illumination intensity (500 Lx).

Thus, high humidity in the air will not contribute to intensive transpiration, which will save the plant from wilting. High temperature ($25^0$ C) and low intensity of illumination (500 Lx) are favorable conditions for low intensity of photosynthesis and suspension of regenerate growth. The stock of metabolites available in the regenerate will be used for root formation.

After the formation of roots, it is necessary to gradually reduce the humidity of the air around the regenerants and increase the intensity of lighting. This will complete the structural restructuring of the leaf: the cuticle layer will appear, the cells of the epidermis will change their shape, and changes in the structure of the mesophyll of the leaf will occur. The leaf will acquire the features of a xeromorphic structure, and the plant is no longer afraid of low humidity and even strong wind, characteristic of open ground conditions.

These procedures, strictly performed by us when transferring plants of introduced varieties of high blueberry and lingonberry from *in vitro* to *ex vitro* conditions, allowed us to preserve the viability of plants and ensure their 100% survival and adaptation.

## References

Brainerd, K.E., Fuchigami, L.H. 1982. Stomatal functionoig of *in vitro* and greenhouse apple. Leaves in darkness, mannitol, ABA and $CO_2$.J. Exp. Bot. 33: 338-392.

Brainerd, K.E., Fuchigami, L.H. 1981. Acclimatization of aseptically cultured apple plants to low relative humidity. J. Amer. Soc. Hort. Sci. 106: 515-518.

Brainerd, K.E., Fuchigami, L.K., Kwiatkowski, S. and Clark, C.S. 1981. Leaf anatomy and Water Stress of Aseptically Cultured "Pixy" Plume Grown under Diferent Enviroments. Hort Science.16:173-175.

Bunning, E., Sagromsky, H. 1948. Die Bildung des Spaltoffnungs musters in der Blattepidermis. Z. Naturf. 36: 203-216.

Burdasov, V.M., Ilyina, E.I., Kononova, N.V. 1988. Features of microclonal reproduction of apple trees. Biology of cultured cells and biotechnology. Novosibirsk. 2: 360.

Conner, L.N.,Conner, A.J. 1984.Comparative water loss from leaves of *Solanum lociniatum* plants cultured *in vitro* and *in vivo*. Plant Sci. Lett. 36(3):241-246.

Conner, A.J., Thomes, M.B. 1981.Re-establishing plantlets from tissue culture: a review. Comb. Proc. Int. Plant Prop. Soc. 3: 342-357.

Danies, W.J., Kozlowski, T. 1974. Short- and long-term effects antitranspirants on water relation and photosynthesis of woody plants. J. Americ. Soc. Hort. Sci. 99(4): 297-304.

Donnelly, D.J., Vidaver, W.E. and Colbow, K. 1984. Fixation of $14CO_2$ in tissue-cultured red raspberry prior to and after transfer to soil. Plant Cell. Tissue Organ.Cult.3: 313-317.

Donnelly, D.J., Vidaver, W.E.1984. Leaf anatomy of red raspberry transferred from culture to soil. J. Americ. Soc. Hort. Sci. 109: 172-176.

Fabbri, A., Shutter, E. 1986. Anatomical changes in persistent leaves of tissue cultured strawberry plants after removal from culture. Scientia Hort. 28: 331-337.

Grout,B.W.W., Aston, M.J. 1977. Transpanting cauliflower plants regenerated from meristem culture. I. Water loss and water transfer related to changes in leaf wax and to xylem regeneration. Hort. Res. 17(1): 1-7.

Grout, B.W.W. 1975. Wax Development on Leaf Surfases of *Brassica olezacea* var. *Curravong* regenerated from meristem culture. Plant Sci. Lett.5: 401-405.

Grout, B.W., Millam, S.1985. Photosynthetic development of micropropagated strawberry plantlets following transplanting. Ann. Bot. 55: 129-131.

Jones, O.P. 1987. Reproduction of economically important woody plants *in vitro*. Biotechnology of agricultural plants. 1:142-152.

Kramarenko, L.A., Kataeva, N.V., Skvortsov, A.K. 1988. Clonal micropropagation of *Armeniaca vulgaris.* Biology of cell culture and biotechnology. Novosibirsk, 2: 321.

Lee N., Wetzstein, H.V., Sommer, H.E.1988. Quantum Tlux Density Effect on the Anatomy and Surface Morphology of *in vitro*- and *in vivo*- developed Sweetgum Leaves. J. Americ. Soc. Hort. Sci.113:167-171.

Noé, N.,Bonini, L. 1996. Leaf anatomy of high bush blueberry grown *in vitro* and during acclimatization to *ex vitro* conditions. Biol. Plant. 38:19-25.

O'Leary, J.W., Knecht, G.N. 1981. Elevated $CO_2$ concentration increases stomata numbers in *Phaseolus vulgaris* leaves. Bot. Gaz. 124 (4): 438-441.

Penfound, W.T. 1931. Plant anatomy as conditioned by light intensity and soil moisture. Am. J. Bot. 18:558-572.

Pospíšilová, J., Synková, H., Haisel, D., Semorádová, Š. 2007. Acclimation of Plantlets to *Ex Vitro* Conditions: Effects of Air Humidity, Irradiance, $CO_2$ Concentration and Abscisic Acid (a Review). Acta Horticulturae.748: 29-38.

Schoch, P., Lefevre, B., Tession, C., Gengy, J. 1989. Photosintheseet respiration de bananier *in vitro*. Photosynthetica.23(1): 113-118.

Sidorovich, E.A., Kutas, E.N. 1996. Clonal micropropagation of new fruit and berry plants. Minsk, Science and Technology:1-246.

Smith, M.A., Palta, J.P., McCown, B.H. 1986. Comparative Anatomy and Physiology of Microcultured, Seedling and Greenhouse grown Asian White Birch. J. Amer. Soc. Hort. Sci. 111(3): 437–442.

Solarova, J. 1989. Photosynthesis of plant regenerants diurnal variation in $CO_2$ concentration in cultivation vessels resulting from plantlets photosynthetic activity. Photosynthetica. 23:100-107.

Sutter, E., Langhans, R.W. 1979. Epicuticular wax formation on cornation plantlets regenerated from shoot-tip culture. J. Americ. Soc. Hort. Sci. 104: 493-496.

Waldenmaier, S., Schmidt, G. 1990. Histologische Unterchiede zwischen *in vitro* und *ex vitro* Blatternbei der Abhartung von Rhododendron. Gartenbauwissenschaft.55(2): 49-54.

Wardle, K., Short, K.C. 1983.Stomatal response of in vitro cultured plantlets, Responses in epidermal strips of Chrysanthemum to

environmental factors and growth regulators. Biochem. Physiol. Pflanzen. 178: 619-624.

Wetzstein, H.Y., Sommer, H.E. 1982a. Leaf anatomy of tissue cultured *Liquidambar styraciflue* (Hamamelidaceae) during acclimatization. Amer. J. Bot. 69(10): 1579-1586.

Wetzstein, H.Y., Sommer, H.E.1983b. Scanning electron microscopy of *in vitro Liquidambar styraciflue* plantlets during acclimatization. J. Amer. Soc. Hort. Sci. 108:475-480.

**Access this Article in Online**

Website: www.ijarbs.com

Subject: Horticultural Sciences

Quick Response Code

DOI:10.22192/ijarbs.2024.11.04.008

How to cite this article:
Elena Kutas. (2024). Adaptation of regenerants of sterile cultures to *ex vitro* conditions - A review. Int. J. Adv. Res. Biol. Sci. 11(4): 68-75.
DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.04.008