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 9, Issue 4 -2022

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.04.004

Adaptation of regenerants of introduced plantlets Vaccinium ssp. to *ex vitro* conditions.

Elena Kutas
Central Botanical Garden of the NAS of Belarus, 220072 Minsk, Surganova, 2v,
Repulic of Belarus.
E-mail: *vinogradova-kira@tut.by*.

Abstract

This research is a comparative analysis of structural and functional peculiarities of regenerants of the introduced varieties of *Vaccinium corymbosum*L. and *V. vitis-idaea* L. in the conditinon *in vitro* and *ex vitro*. It is shown that first, culture conditions leave its mark on structure and function of the regenerants; second, the structural and functional regenerant organization is a mobile system and can be reconstructed in accordance with the changed conditions of the environment. The differences in leaf texture and function in plants grown in aseptic culture in the condition of the greenhouse or in the open ground are indicative of leaf plasticity. A leaf is an organ that can change its structure and function according to the culture conditions and in theory this fact is a guarantor of successful adaptation of plants when carrying them from the condition *in vitro* (the cultural container) to the condition *ex vitro* (the greenhouse and open ground).

Keywords: aseptic culture, greenhouse, open ground, anatomical structure, *Vaccinium corymbosum, V.vitis-idaea.*

Introduction

In the foundation of the clonal micropropagation of plants there are two completely different stages, *in vitro* and *ex vitro*.

During the first of them *(in vitro)* the vital functions of the material being propagated occur in a closed sterile space on the nutrient medium under strictly controlled conditions. After the regenerants are transferred from *in vitro* conditions the second stage begins – the *ex vitro* system, quite different from *in vitro* conditions.

Under *ex vitro* conditions the plants have to pass from heterotrophic nutrition to autotrophic conjugated with a structural and functional transformation of the organism in new conditions. They must adjust them selves to changeable environmental factors not inherent to them.

The transition of plants from *in vitro* to in *ex vitro* conditions is critical in most cases and entails the death of plants. From our point of view the comparative analysis of structural and functional peculiarities of regenerants in *ex vitro* and *in vitro* conditions will help to understand and to prevent the cause of death of plants during the adaptation period.

Research conducted by Brainerd et al.(1981) on leaf anatomy and water stress with plump plants in *in vitro* and *ex vitro* conditions showed that the loss of water occurs three times faster with plants obtained in vitro culture compared with plants obtained from the greenhouse. The thickness of palisade cells was much lower with regenerants raised in aseptical conditions than that of regenerants from the greenhouse and open ground.

According to research by Grout (1975), Sutter and Langhans (1979) the leaves are deprived of wax bloom with plants cultivated in vitro and stoma function is imperfect because of failure of the open-closed mechanism. The similar conclusions about stoma functioning were obtained by Lee et al. (1988), Brainerd and Fuchigami (1982), Wardle and Short (1983).

According to data by Bunning and Sagromsky (1948), O'Leary and Knecht (1981), Penfound (1931) stoma development is influenced by such factors as CO_2 concentration in the retort, and water regime, and hormone level.

The stomata of plants *in vitro* conditions are usually open which is not true in respect with stomata *ex vitro* conditions. In our opinion, such behavior of stomata *ex vitro* conditions is quite justified because in cultural retorts a very high constant relative humidity rate is kept (over 90%), temperature and illumination degree are not liable to overfalls because of being controlled. Should any condition in retorts occur, the stomata reaction will follow in response to the changes of the given conditions.

The true confirmation of this are the results of experiments obtained by Schoch et al. (1989) during the study of photosynthesis and the breathing of the banana *in vitro* system. The authors come to the conclusion that leaves function stomata well if the banana shoots are cultivated *in vitro* conditions, i.e. they respond to light and close under water stress. That means stomata react adequately to the conditions in which a plant finds itself.

The use of antitranspirants during the transfer of plants from *in vitro* to *ex vitro* conditions promoted the decreasing of photosynthesis caused by a worsening of plant growth Danies and Kozlowski (1974).

.

According to research by Fabbri and Sutter (1986) the leaf structure of wild strawberry formed *in vitro* culture, was characterized by a relatively thin leaf plate, underdeveloped palisade cells, big air cavities, and weakly-developed cuticular integument. At the same time the leaf of wild strawberry formed in *ex vitro* conditions was differentiated into palisade and spongy tissues with a well-developed cuticular integument.

The similar results were obtained by Donnelly and Vidaver (1984) when studying raspberry leaves regenerated *in vitro*.

Waldenmeier and Schmidt (1990) observed histological differences of rhododendron leaves *in vitro* and *ex vitro* when tempering them. The differences included absence of breathing pores, and a weakly-structured mesophyll with leaves *in vitro*. With the leaves *ex vitro* the anatomical structure of the leaves changed: their thickness grew, the number of layers of epidermis and palisade tissue increased, the cuticula appeared. The acclimatisation by low humidity rate led to a clear differentiation of the tissue into palisade and spongy mesophyll.

Materials and Methods

The object of the study was the introduced varieties of *Vaccinium corymbosum* L. ('Dixi', 'Bluecrop') and *V.vitis-idaea* L. ('Koralle'). The leaves of *V. corymbosum* L. ('Dixi', 'Bluecrop') and *V. vitis-idaea* L. ('Koralle') were preserved in alcohol-acetic acid (3:1). The crosssections were made in hemiddle part of the leaf, at microtome, by histological technique and razor. The sections were cleared with chloral hydrate and then stained with Genevez and Sudan III reagents. The thickness of the leaf plate and other indices of anatomical leaves structure were measured by micrometer. The analysis of the anatomical structure was realized according to the previous method described by Brainerd et al. (1981), Grout (1975), Sutter and Langhans(1979), Lee et al. (1988).

Results and Discussion

The research conducted by us on the dependence of internal leaf structure on cultivating conditions showed that regenerants of the introduced species of *Vaccinium corymbosum* ('Dixi', 'Bluecrop') and *Vaccinium vitis-idaea* ('Koralle') cultivated *in vitro* conditions, had no clear differentiation of mesophyll into palisade and spongy tissues, had a thin leaf plate, weakly developed cuticular integument and underdeveloped stoma apparatus entailing continuous opening of the stomata and over-transpiration.

The leaves developed in the greenhouse had a clear mesophyll differentiation into palisade and spongy mesophyll, had cuticular integument, well-developed stoma apparatus enabling normal transpiration.

The leaves of plants transplanted into open ground did not differ from greenhouse leaves in general structure. They had a leaf structure clearly differentiated into palisade and spongy mesophyll, a well-developed cuticular integument and a stoma apparatus. However, it should be pointed out that the difference was observed in the change of the quantitative indices of the leaf structure. Thus leaves from open ground had a thicker leaf plate, more layers of palisade tissue, longer cells, and a reduced volume of ductus intercellulavis compared with the greenhouse leaves and *in vitro* (Table 1).

It should be pointed out that the differences in leaf structure are conjoined with their functional differences. An example is a thorough research on comparative anatomy and physiology of Asian White Birch cultivated in the greenhouse on an aseptic culture, conducted by Smith et al. (1986). The authors come to the conclusion that a weak development of the vascular system in *in vitro* conditions followed by an increased sensitivity of such plants to water stress inherent to *ex vitro* conditions. A low intensity of photosynthesis and a very low illumination degree conjoined with the absence of a clear differentiation of the leaf into palisade and spongy tissues *in vitro* culture. After the transfer of plants into *ex vitro* conditions (greenhouse) the researchers observed an increase in photosynthesis intensity and changes in the leaf anatomy. In their opinion, the plants grown in aseptic conditions change considerably their anatomical and physiological features compared to their doubles cultivated in *ex vitro* conditions. The changes are accounted for by the influence of a specific environment in the aseptic culture and disappear after the transfer of plants into *ex vitro* conditions due to a quick recovery of metabolism resulting from the normal development of plants.

According to research by Donnely et al. (1984), Grout and Millam (1985) the photosynthetical activity is lower with *in vitro* shoots compared to that of *ex vitro* shoots. The minimum photosynthetical activity until 14 days after the transfer of leaves from *in vitro* culture it was observed that plants survive during acclimatization using the stock of metabolites. The normal recovery of structure and function occurs with the regenerants within a month after placing them into *ex vitro* conditions. To increase the survival rate of plants during adaptation it is necessary to gradually decrease the relative air humidity and increase irradiation. This promotes the increasing of space occupied by palisade cells,

which in turn causes an increase in the intensity of photosynthesis.

Interesting research was conducted by Solarova (1989) on the round the clock variability of CO_2 concentration in cultivating retorts where in regenerants plants were cultivated obtained from leaf pieces. It turned out that CO_2 concentration in retorts increased in dark periods and was connected to the regenerant size and sucrose content in the medium. The concentration in retorts decreased in light periods and the illumination reached the compensation point in 3-4 hours despite the low illumination degree (100 mc mol/m^{-2}, s^{-1}). The conclusion was made by the author that the low CO_2 concentration in closed retorts for cultivation of regenerant plants causes different rates of growth.

Therefore, the decreased CO_2 concentration is one of the low photosynthetical intensity observed with regenerants plants *in vitro* culture. The CO_2 concentration increases by transfer of plants into *ex vitro* conditions causing an increase in the intensity of photosynthesis followed by growth acceleration.

On the foundation of the comparative analysis of structural and functional features of the regenerants *in vitro* and *ex vitro* conditions based on written sources and the results of our own research we came to the conclusions: 1) *in vitro* and *ex vitro* cultivating conditions leave an imprint on the structure and functions of regenerants, 2) structural and functional organization on regenerants is a mobile system able top transform in accordance with the changed environmental conditions. That means that the differences in structure and function of plant leaves growth in the aseptic culture, in the greenhouse or in open ground testify to the flexibility of the leaf – the organ able to transform its structure and function according to the cultivating conditions. This is theoretically the guarantor of the successful adaptation of plants when transferring them from *in vitro* to *ex vitro* conditions.

In practice we managed to avoid losses of material at the critical point thanks to using techniques based on conclusions confirmed by the results of experimental research. This was proved by our observations on the adaptation process of the introduced species of *Vaccinium orymbosum* ('Dixi', 'Bluecrop', 'Herbert', 'Rancocas', 'Covill', 'Early blue') and *Vaccinium vitis-idaea* ('Koralle', 'Masovia', 'Erntedank', 'Erntecrone', 'Erntezegen') when transferring them from *in vitro* into *ex vitro* conditions (Sidorovich, Kutas, 1996).

To prevent death of the material from over-transpiration (refers not only to *Vaccinium corymbosum* and *Vaccinium vitis-idaea)* were caused by the following reasons: 1) the humidity drop in *ex vitro* conditions, 2) imperfect structural and functional organization of the leaf in terms of *ex vitro* conditions. It is needed to increase the turgor of regenerats to its maximum value. This is achieved by plunging of the material into a retort containing distillated water for 5-6 hours.

The second essential condition is to keep a high humidity rate in the greenhouse (not under 90%) and the removal of strong air flows i.e. elimination of any wind since the wind entails drying up of leaves because of quick evaporation. Absence of wind and a high humidity rate will cause a steam pressure gradient between the leaves and air.

It is essential to create *in vitro* identical conditions in the greenhouse in the first 2-3 weeks of regenerant cultivation (before root formation) This means that the humidity rate must be to strictly controlled, the temperature kept similar to that when cultivating plants *in vitro* conditions and relatively low illumination degree (500 lx).

Thus, the high air humidity will not cause intensive transpiration preventing the plant from fading. A high temperature (25°C) and low illumination degree (500 Lx) favor the low intensity of photosynthesis and the stoppage of regenerant growth. The stock of metabolites with the regenerant will be utilized for root formation.

Table 1 − Quantitative indices of anatomical leaves structure of *Vaccinium corymbosum* and *Vaccinium vitis-idaea* cultivated in the aseptical culture, greenhouse and open ground*

Grade	Aseptic culture (*in vitro*) 4000 Lx			Greenhouse >15000 Lx					Open Ground > 50000 Lx				
	Leaf thickness, µm	The number of stomata per 1 mm^2	Stoma size length x width, µm	Leaf thickness, µm	Palisade cofficient	Length:width of cells of palisade tissue ratio	The number of stomata per 1 mm^2	Stoma size length x width, µm	Leaf thickness, µm	Palisade cofficient	Length:width of cells of palisade tissue ratio	The number of stomata per 1 mm^2	Stoma size length x width, µm
Vaccinium corymbosum													
'Bluecrop'	76±2	16±1	15x11	154±16	0,75	1,8:1	251±11	25x17	210±11	0,87	2,5:1	260±12	23x16
'Dixi'	85±3	16±1	15x12	173±13	0,71	1,9:1	250±9	26x16	221±12	0,9	2,7:1	265±10	24x15
Vaccinium vitis-idaea													
'Koralle'	91±4	19±1	16x10	286±9	0,63	2,61:1	410±20	24x15	450±19	0,86	3,31:1	430±23	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.

After root formation it is necessary to gradually decrease the air humidity around the regenerant and increase the illumination degree. This will enable the structural transformation of the leaf to be completed: the cuticular layer will appear, the cells of epidermis will change their shape, the mesophyll of the leaf will change its texture. The leaf will acquire features of xeromorphic structure and the plant will not be influenced by the low air humidity and even by strong wind characteristic for open ground conditions.

The procedures mentioned strictly implemented by us when transferring the introduced species of *Vaccinium corymsosum* and *Vaccinium vitis-idaea* from *in vitro* to *ex vitro* conditions allowed us to preserve the viability of plants and to secure their 100% survival and adaptation.

Conclusion

The successful adaptation of regenerant plants when transferring from *in vitro* to *ex vitro* conditions depends on the one hand on adequate theoretical knowledge (results of experimental research) and, on the other hand, on the strict observance of simple techniques.

The confirmation is the case of 100% adaptation of regenerant plants of the introduced species of *Vaccinium corymbosum* and *Vaccinium vitis-idaea* not only in greenhouse conditions but also in open ground conditions.

References

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

Brainerd, K.E., Fuchigami, L.H. 1982. Stomatal functioning of in vitro and greenhouse apple leaves in darkness, mannitol, ABA and CO_2. J. Exp. Bot. 33: 388-392.

Bunning, E., Sagromsky, H. 1948. Die Bildung des Spaltöffunungsmusters in dea Blattepidermis. Z. Naturf.36:203-216.

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. 99:297-304.

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

Grout, B.W. 1975. Wax Development on Leaf Surfases of Brassica Olezaceavar. 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.

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

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

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

Schoch, P., Lefevre, B., Teisson, C. and Genry, I. 1989. Photosynthese et respiration de bananier *in vitro.* Photosynthetica. 23:113-118.

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

Smith, M.A., Palta, J.P. and MCcown, B.H. 1986. Comparative Anatomy and Physiology of Microcultured, Seedling, and Greenhouse

groun Asian White Birch. J. Americ. Soc. Hort. Sci. 111: 437-442.

Solarova, Jrmila. 1989. Photosynthesis of plant regenerants diurnal variation in CO2 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 Unterschiede zwischen in vitro und *ex vitro* Blatternbei der Abhärtung von Rhododendron. Gartenbauwissenschaft. 55:49-54.

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.

Access this Article in Online	
Quick Response Code	Website: www.ijarbs.com Subject: Plant Tissue Culture
DOI:10.22192/ijarbs.2022.09.04.004	

How to cite this article:
Elena Kutas. (2022). Adaptation of regenerants of introduced plantlets Vaccinium ssp. to *ex vitro* conditions. Int. J. Adv. Res. Biol. Sci. 9(4): 31-37.
DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.04.004