THE ANATOMICAL STRUCTURE OF REGENERANTS OF *VACCINIUM CORYMOSUM* L. AND *VACCINIUM VITIS-IDAEA* L. TO *IN VITRO* AND *EX VITRO* CONDITIONS

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Abstract

It is given benchmark analysis structured-functional particularities of regenerants untrodused varietes of *Vaccinium corymbosum* L. and *V. vitis-idaea* L. under *in vitro* and *ex vitro* condition.

The anatomical structures of the leaves of introduced varieties of *Vaccinium corymbosum* and *V. vitis-idaea* cultivated in the aseptical culture, greenhouse and open ground were studied.

It is shown that condition cultivation superimposes the imprint on structure and function regeneration - first; secondly, structured-functional organization regeneration - a mobile system and can reform in accordance with changed condition surrounding ambiences. The differences in construction and functions sheet plants, growing in aseptic culture, in condition of the hothouses or in open ground, are indicative of plastic sheet - an organ, capable to reconstruct its structure and function adequately condition of cultivation that theoretically is a guarantor to successful adapting the plants when carrying them from conditions *in vitro* (the cultural container) in condition *ex vitro* (the greenhouse and open ground).

Key words: aseptic culture, greenhouse, open ground, anatomical structure, blueberry, cowberry

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INTRODUCTION

In the foundation of clonal micropropagation of plants there are two completely different stages, *in vitro* and *ex vitro*. During the first of them (*in vitro*) 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 transverred from *in vitro* conditions the second stage begins *ex vitro* system, which is quite different from *in vitro* conditions.

Ex vitro conditions the plants have to pass from heterotrophic nutrition to autotrophic conjugated with structural and functional transformation of the organism in new conditions. They must adjust themselves to changeable environmental factors inherent to them.

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

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

According to researches by Grout (1975), Sutter and Langhans (1979) the leaves are deprived of wax bloom in plants cultivated *in vitro* and stoma function is imperfect because of failure of 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) the stoma development is influenced by such factors as CO_2 concentration in the retort, 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 behaviour of stomata under *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 responsible to over falls because of being controlled. Should any condition in cultural container occur, the stomata reaction will follow in respond 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 breathing of banana in vitro system. The authors come to a conclusion that leaves function stomata well if banana shoots 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 is.

From this point of view the failure is clear overtaking some researchers seeking to intertere wich efficient performance of stomata responding to conditions under which they are. For instance, the use of antitranspirants during transfer of plants from *in vitro* to *ex vitro* conditions promoted decreasing of photosynthesis caused by worsening of plant growth (Danies and Kozlowski, 1974).

According to researchs by Fabbri and Sutter (1986) the leaf structure of wild strawberry formed *in vitro* culture, was chracterised by a relatively thin leaf plate, under developed palisade cells, big air cavities, weakly developed cutucular integument. At the same time the leaf of wild strawberry formed under *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 gistological differences of rhododendron leaves *in vitro* and *ex vitro* when tempering them. The differences included absence of breathing pores, weakly-structured mesophyll with leaves *in vitro*. In the leaves *ex vitro* the anatomical structure of leaves changed: their thickness grew, the number of layers of epidermis and palisade tissue increased, the cuticle is appeared. The acclimatization by low humidity rate led to a clear differentiation of the tissue into palisade and spongy mesophyll.

The object of the study served of regenerants the introduced varieties of *Vaccinium corymbosum* L. (Dixi, Bluecrop) and *V. vitis-idaea* L. (Koralle) cultivated in the aseptical culture, greenhouse and open ground.

MATERIALS AND METHODS

The leaves of regenerants the introduced varieties of *Vaccinium corymbosum (*Dixi, Bluecrop*)* and *V. vitis-idaea* (Koralle) were preserved in alcohol-acetic acid (3:1). The cross sections were made in the middle part of the leaf, at microtome by histological techniqaue and by razor. The sections were cleared with chloral hydrate then stained with Genevez and Sudan III reagents (Braune et al. 1979, Toma and Rugină 1998, Verzar-Petri 1979). The thickness of leaf was measured by micrometer.

The analyzes of anatomical structure was realized according to previously methods described by Brainerd et al. (1981), Grout (1975), Sutter et al. (1979), Lee et al. (1988).

RESULTS, DISCUSSION AND CONCLUSION

The researches conducted by us on dependence of internal leaf structure under cultivating conditions showed that regenerants of introduced species of *Vaccinium corymbosum* (Dixi, Bluecrop) and *V. vitis-idaea* (Koralle) cultivated under *in vitro* conditions, had no clear differentiation of mesophyll into palisade and spongy tissues, had a thin leaf plate, weakly

developed cuticular integument and under developed stoma apparatus entailing continuous opening of 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 quantitative indices of the leaf structure. Thus leaves of plants from open ground had a thicker leaf plate, more layers of palisade tissue, longer cells, reduced volume of ductus intercellularis in compared with the greenhouse leaves and *in vitro* (Table 1).

It should be pointed out that the differences in leaf structure are conjugated with their functional differences. An example is a thorough research on comparative anatomy and physiology of Asian birch (*Betula platyphylla*) cultivated in the greenhouse on aseptic culture, conducted by Smith et al. (1986). The authors come to conclusion about weak development of vascular system under condition *in vitro* followed by as increased sensitivity of such plants to water stress inherent in *ex vitro* conditions.

A low intensity of photosynthesis was discovered by them by a very low illumination degree conjugated with the absence of clear differentiation of the leaf into palisade and spongy tissues *in vitro* culture.

After transfer of plants into *ex vitro* conditions (greenhouse) the researchers observed the increase in photosynthesis intensity and changes in leaf anatomy. In their opinion, the plants grown in aseptic conditions change considerably their anatomical and physiological features compared to their doubles cultivated *ex vitro* conditions. The changes are accounts for by the influence of a specific environment in aseptic culture and disappear transfer of plants into *ex vitro* conditions due to a quick recovery of metabolism resulting from normal development of plants.

According to researches 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 transfer of leaves from *in vitro* culture was observed 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 increasing of space occupied by palisade cells wich in turn causes increase in intensity of photosynthesis.

Interesting researches were conducted by Solarova (1989) on study of round-o'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 period and was connected to the regenerant size and sucrose content in the medium. The concentration in retorts decreased in light period and the illumination reached the compensation point in 3-4 hours despite the low illumination degree (100 $\mu mol.m^{-2}. s^{-1}$). The

author made a conclusion that the low CO_2 concentration in closed retorts for cultivation of regenerants plants induces different 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 under *ex vitro* conditions causing an increase of intensity of photosynthesis followed by the growth acceleration.

On the foundation of comparative analysis of structural and functional features of the regenerants under *in vitro* and *ex vitro* conditions based on written sources and results of our own researches we came to a conclusion: 1) that *in vitro* and *ex vitro* cultivating conditions leave imprint on 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 a successful adaptation of plants when transferring them from *in vitro* to *ex vitro* conditions.

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

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

The second essential condition is to keep high humidity rate in the greenhouse (not under 90%) and removal of strong air flows i.e. elimination of any wind since the wins entails drying up of leaves because of quick evaporation. Absence of wind and high humidity rate will cause steam pressure gradient between 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). It means to strictly control humidity rate, keep temperature 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. High temperature (25^0C) and low illumination degree (500 lx) favour low intensity of photosynthesis and stop 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*

Cultivar	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 to complete the structural transformation of the leaf: the cutucular layer will appear, the cells of epidermis will change their shape, the mesophyll of the leaf will change its texture. The leave will acquire features of xeromorphic structure and the plant will not be frightened by the low air humidity and even by strong wind characteristic under open ground condotions.

The procedures mentioned strictly implemented by us when transferring the introduced species of *V. corymbosum* and *V. 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.

To sum it up it can be concluded that the successful adaption of regenerant plants when transferring from *in vitro* to *ex vitro* conditions depends on the one hand on our theoretical knoweledge, results of experimental researches and on the other hand , on the strict observance of simple techniques.
The confirmation is a case of 100% adaptation of regenerant plants of introduced species of *V. corymbosum* and *V. vitis-idaea* not only under greenhouse conditions but also under open ground conditions.

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