African Journal of Biotechnology Vol. 10(19), pp. 3762-3765, 9 May, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2060
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Adaptation of regenerants of *Vaccinium corymbosum* L. and *Vaccinium vitis-idaea* L. to *ex vitro* conditions

Elena Kutas

Central Botanical Garden of the NAS of Belarus, 220072 Minsk, Surganova, 2v, Republic of Belarus. E-mail: vinogradova-kira@tut.by. Tel: (+375 17) 284-15-89. Fax: (+375 17) 284-14-84.

Accepted 22 February, 2011

The benchmark analysis of the structured-functional particularities of regeneration has introduced varieties of *Vaccinium corymbosum* L. and *Vaccinium vitis-idaea* L. under *in vitro* and *ex vitro* conditions. The anatomical structures of the leaves of introduced varieties of *V. corymbosum* and *V. vitis-idaea*, cultivated in aseptical culture, greenhouse and open ground were studied. First, it is shown that the condition of cultivation superimposes the imprint on the structure and function of regeneration; secondly, the structured-functional organization regeneration (a mobile system) can be reformed in accordance with the changed condition surrounding ambiences. The differences in construction and functions of the sheet plants growing in an aseptic culture, in hothouses or open ground conditions, are indicative of the plastic sheet, which is an organ capable of reconstructing its structure and function adequately to the condition of cultivation that is theoretically, a guarantor to successful adaption of the plants when carrying them from *in vitro* (the cultural container) to *ex vitro* (the greenhouse and open ground) conditions.

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

INTRODUCTION

In the foundation of clonal micropropagation of plants, there are two completely different stages (*in vitro* and *ex vitro*). During the first stage (*in vitro*), vital functions of the material that is 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 *ex vitro* system, quite different from *in vitro* conditions. In *ex vitro* conditions, the plants have to pass from a heterotrophic to autotrophic nutrition, 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 *ex vitro* conditions is critical in most cases and entails death of plants. From this study's point of view, the comparative analysis of the *ex vitro* and *in vitro* conditions of the structural and functional peculiarities of regenerants will help to understand and prevent the cause of death of plants during the adaptation period.

Researches conducted by Brainerd et al. (1981) on leaf anatomy and water stress with plump plants, *in vitro* and *ex vitro*, showed that the loss of water occurred three times faster with plants obtained via *in vitro* culture than 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 researches by Grout (1975) and Sutter and Langhans (1979), the leaves are deprived of wax bloom with plants cultivated *in vitro*, and stoma function is imperfect as a result of the open-closed mechanism's failure. Similar conclusions about stoma functioning were obtained by Lee et al. (1988), Brainerd et al. (1982) and Wardle and Short (1983).

According to the data given by Bunning and Sagromsky (1948), O'Leary and Knecht (1981) and 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 of the stomata of plants *ex vitro* conditions. In the study's opinion, such behaviour of stomata *ex vitro* conditions is quite justified because in cultural retorts, a very high constant relative humidity rate is kept (over 90%), and the temperature and illumination degrees are not expected to

fall over the limit because they are being controlled. However, if any condition should occur in the cultural container, the stomata reaction will follow in response to the changes of the given conditions.

From this point of view, the failure clearly overtakes some researchers seeking to interfere with the efficient performance of stomata responding to conditions in which they are found. For instance, the use of antitranspirants during transfer of plants from *in vitro* to *ex vitro* conditions promoted a decrease of photosynthesis caused by a deterioration of plant growth (Danies and Kozlowski, 1974).

According to researches by Fabbri and Sutter (1986), the leaf structure of wild strawberry, which formed *in vitro* culture, was characterized by a relatively thin leaf plate, under developed palisade cells, big air cavities, and weakly developed cuticular integument. At the same time, the leaf of wild strawberry, which formed *ex vitro* conditions, was differentiated into palisade and spongy tissues with a well-developed cuticular integument. Similar results were obtained by Donnelly and Vidaver (1984) when studying raspberry leaves regenerated *in vitro*. Waldenmeier and Schmidt (1990) observed histological differences in rhododendron leaves *in vitro* and *ex vitro* when tempering on them. The differences included absence of breathing pores and weakly-structured mesophyll with leaves *in vitro*. With the leaves found *ex vitro*, the anatomical structure of leaves changed. As a result, their thickness grew, the number of layers of epidermis and palisade tissue increased, and the cuticle appeared. The acclimatization by low humidity rate led to a clear differentiation of the tissue into palisade and spongy mesophyll.

The objective of this research is to study the adaptation of the regenerants of the introduced varieties of *Vaccinium corymbosum* L. (Dixi, Bluecrop) and *Vaccinium vitis-idaea* L. (Koralle).

MATERIALS AND METHODS

The leaves of *V. corymbosum* L. (Dixi, Bluecrop) and *V.vitis-idaea* L. (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 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 et al. (1979) and Lee et al. (1988).

RESULTS AND DISCUSSION

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

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

The leaves of plants transplanted into open ground did not differ from greenhouse leaves in a general structure. They had a leaf structure that is 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 reduced volume of ductus intercellularis when compared with the greenhouse leaves *in vitro* (Table 1).

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

A low intensity of photosynthesis was discovered by them through a very low illumination degree conjugated with the absence of clear differentiation of the leaf into palisade and spongy tissues in an *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 considerably change their anatomical and physiological features when compared to their double cultivated *ex vitro* conditions. The changes are accounted for by the influence of a specific environment in aseptic culture and a disappearance of the transfer of plants into *ex vitro* conditions due to a quick recovery of metabolism resulting from normal development of plants.

According to researches done by Donnely et al. (1984) and 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 for plants that survived during acclimatization using the stock of metabolites. The normal recovery of the structure and function occurs with the regenerants within a month after placing them in *ex vitro* conditions. To increase the survival rate of plants during adaptation,

Table 1. Quantitative indices of anatomical leaves structure of *V. corymbosum* and *V. 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 coefficient	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 coefficient	Length : width of cells of palisade tissue ratio	The number of stomata per 1 mm^2	Stoma size length x width (µm)
V. 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
V. 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

* No indices are shown for palisade coefficient and 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.

it is necessary to gradually decrease the relative air humidity and increase irradiation. This promotes increase of space occupied by palisade cells which, in turn, causes increase in intensity of photosynthesis.

Interesting researches were conducted by Solarova (1989) on the study of round-o'clock variability of CO_2 concentration in cultivating retorts, where the cultivated regenerants plants were obtained from leaf pieces. It turned out that CO_2 concentration in retorts increased in a 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 to 4 h, despite the low illumination degree (100 $\mu mol.m^{-2}.s^{-1}$). A conclusion was made by the author that the low CO_2 concentration in closed retorts for cultivation of regenerant plants is different in growth. Therefore, the decreased CO_2 concentration is one of the low photosynthetical intensity observed with regenerants plants in *in vitro* culture. The CO_2 concentration increases by transfer of plants into *ex vitro* conditions causing an increase in intensity of photosynthesis followed by the growth acceleration.

On the foundation of the comparative analysis of the structural and functional features of the regenerants in *in vitro* and *ex vitro* conditions, based on the written sources and results of the author's own researches, this study came to a conclusion that: (1) the *in vitro* and *ex vitro* cultivating conditions leave imprint on the structure and functions of regenerants, and (2) structural and functional organization on regenerants is a mobile system that has the ability to be transformed in accordance with the changed environmental conditions. This means that the differences in the structure and function of plant leaves' growth in the aseptic culture, in greenhouse or open ground, testify to the flexibility of the leaf, that is, the organ has the ability 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 material at the critical point due to the use of techniques based on conclusions confirmed by the results of experimental researches. This was proven by the study's observations over the adaptation process of the introduced species of *V. corymbosum (*Dixi, Bluecrop, Herbert, Rancocas and Covill, Early blue*)* and *V. vitis-idaea (*Koralle, Masovia, Erntedank, Erntecrone and Erntezegen*)* when transferring them from *in vitro* to *ex vitro* conditions.

To prevent death of the material from over

transpiration (which refers not only to *V. corymbosum* and *V. vitis-idaea*) caused by reasons known to the study: (1) the humidity drops ex vitro conditions, and (2) the imperfect structural and functional organization of the leaf, in terms of ex vitro conditions, is needed first to increase the turgor of regenerants to its maximum value. It is achieved by plunging the material into a retort containing distillated water for 5 to 6 h.

The second essential condition is to keep high humidity rate in the greenhouse (not under 90%) and remove strong air flows, that is, elimination of any wind since they entail drying up the leaves because of quick evaporation. Absence of the 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 to 3 weeks of regenerant cultivation (before root formation), which means that the humidity rate will be strictly controlled, while the temperature will be kept similar to that seen when cultivating plants in *in vitro* conditions and in relatively low illumination degree (500 lx).

Thus, the high air humidity will not cause intensive transpiration preventing the plant from fading. High temperature (25°C) and low illumination degree (500 lx) favour low intensity of photosynthesis and stoppage of regenerant growth. Therefore, the stock of metabolites with the regenerant will be utilized for root formation. 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 complete: the cuticular layer will appear, the cells of epidermis will change their shape and the mesophyll of the leaf will change its texture. The leaf will acquire features of xeromorphic structure and the plant will not be hindered by the low air humidity and even by strong wind characteristic for open ground conditions.

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

To sum up, it can be concluded that the successful adaption of regenerant plants when transferring from *in vitro* to *ex vitro* conditions depends on the theoretical knowledge of the study and results of experimental researches on the one hand, and on the strict observance of simple techniques on the other hand.

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

REFERENCES

Brainerd KE, Fuchigami LK, Kwiatkowski S, Clark CS (1981). Leaf anatomy and water stress of aseptically cultured "Pixy" plume prown under diferent enviroments. Hort Sci. 16: 173-175.

Bunning E, Sagromsky H (1948). Die bildung des spaltöffununsgmusters in der blattepidermis. Z. Naturf., 36: 203-216.

Danies WJ, 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 DJ, Vidaver WE (1984). Leaf anatomy of red raspberry transferred from culture to soil. J. Americ. Soc. Hort. Sci. 109: 172-176.

Donnelly DJ, Vidaver WE, Colbow K (1984). Fixation of 14CO2 in tissue-cultured red raspberry prior to and after transfer to soil. Plant Cell. Tissue Organ. Cult. 3: 313-317.

Brainerd KE, Fuchigami LH (1982). Stomatal functioning of *in vitro* and greenhouse apple. Leaves in darkness, mannitol, ABA and CO_2. J. Exp. Bot. 33: 338-392.

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 BW, Millam S (1985). Photosynthetic development of micropropagated strawberry plantlets following transplanting. Ann. Bot. 55: 129-131.

Grout BW (1975). Wax development on leaf surfaces of *Brassica oleracea* var. Curravong regenerated from meristem culture. Plant Sci. Lett. 5: 401-405.

Lee N, Wetzstein HV, Sommer HE (1988). Quantum Flux 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.

O'Leary JW, Knechtt GN (1981). Elevated CO_2 concentration increases stomata numbers in *Pharsalus vulgaris* leaves. Bot. Gaz. 124: 438-441.

Penfound WT (1931). Plant anatomy as conditioned by light intensity and soil moisture. Amer. J. Bot. 18: 558-572.

Smith MA, Palta JP, MCcown BH (1986). Comparative anatomy and physiology af microcultured, seedling, and greenhouse groun Asian White Birch. J. Americ. Soc. Hort. Sci. 111: 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 RW (1979). Epicuticular wax formation on coronation 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* blattern bei der abhärtung von *Rhododendron*. Gartenbauwissenschaft, pp: 55: 49-54.

Wardle K, Short KC (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.