НАЦИОНАЛЬНАЯ АКАДЕМИЯ НАУК БЕЛАРУСИ

ЦЕНТРАЛЬНЫЙ БОТАНИЧЕСКИЙ САД

КУЛЬТУРА БРУСНИЧНЫХ ЯГОДНИКОВ: ИТОГИ И ПЕРСПЕКТИВЫ

Материалы Международной научной конференции
Минск, 15-19 августа 2005 года

Минск 2005

УДК 581.522.4:634.739.3:631.5
ББК

Культура Брусничных ягодников: итоги и перспективы: Материалы Международной научной конференции. Минск, 15-19 августа 2005 г. – Минск: 2005. - … с.

Представлены результаты исследований учёных Беларуси, России, Украины, Эстонии, Польши, Словакии, Чехии. В них отражена экологическая проблематика и перспективы развития нетрадиционного ягодоводства, интродукции и селекции, биотехнологии и переработки ягодных растений сем. *Брусничные* в Беларуси и странах ближнего и дальнего зарубежья.

Материалы конференции изданы при финансовой поддержке Белорусского республиканского фонда фундаментальных исследований.

ADAPTATION OF REGENERANTS OF ADAPTATION OF *VACCINIUM CORYMBOSUM* L. AND *VACCINIUM VITIS IDAEA* L. TO IN VIVO (EX VITRO) CONDITIONS

E.KUTAS, I.MALACHOVA.
Central Botanical Garden of National Academy of Science of Belarus, 220072, Minsk, st. Surganova 2v. Belarus.

Abstract

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

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 soil, 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 virto (the hothouse and open soil).

Key words: Adaptation, introduction varieties, *Vaccinium corymbosum* L., *V.vitis-idaea* L., ex vitro.

Introduction

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

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 trasferred from in vitro conditions the second stage begins in vivo system quite different from in vitro conditions.

In vivo 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 uninherent to them.

The transition of plants from in vitro to in vivo conditions is critical in most cases entails death of plants. From our point of view the comparative analysis of structural and functional peculiarities of regenerants in vivo 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 in vitro and in vivo 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 sells 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), 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 open-closed mechanism. The similar conclusions about stoma functioning were obtained by Lee et al., (1988). According to data by Bunnig and Sagromsky (1948), 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 in vivo conditions. In our opinion, such behaviour of stomata in vitro conditions is quitre 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 conditions changes in retorts 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 leave 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 interfere with efficient performance of stomata responding to conditions in which they are. For instance, the use of antitranspirants during transfer of plants from in vitro to in vivo conditions promoted decreasing of photosynthesis caused by worsening of plant growth (Danies and Kozlowski, 1974).

According to researches by Fabbri and Sutter (1986) the leaf structure of wild strawberry formed in vitro culture, was characterised 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 in vivo 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. Waldenmaier and Schmidt (1990) observed gistological differences of rhododendron leaves in vitro and in vivo when tempering them. The differences included absence of breathing pores, weakly-structured mesophill with leaves in vitro. With the leaves in vivo the anatomical structure of 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 mesophill.

Material and methods

The Object of the study served the introduced varieties of *Vaccinium corymbosum* L. (Dixi, Bluecrop) and *V.vitis idaea* L. (Koralle). The Studies of the anatomical structure of the sheet held at generally accepted methods (Brainerd et al, 1981; Grout, 1975; Sutter and Langhans, 1979; Lee et al., 1988).

Results end discussions

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

The leaves developed in the greenhouse, had a clear mesophill differentiation into palisade and spongy tissue, 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 mesophill, a vell-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 from openground had a thicker leaf plate, more layers of palisade tissue, longer cells, 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 conjugated with their functional differences. An example is a thorough research on comparative anatomy and physiology of Asiatic Birch cultivated in the greenhouse on aseptic culture, conducted by Smith et al., (1986). The authors come to a conclusion about a weak development of vascular system in vitro condition followed by as increased sensitivity of such plants to water stress inherent to in vivo 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 in vivo 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 in vivo conditions. The changes are accounts for by the influence of a specific environment in aseptic culture and culture and disappear transfer of plants into in vivo conditions due to a quick recovery of metabolism resulting from normal development of plants.

According to researches by Donnelly et al., (1984) Grout and Millam (1985) the Photosynthetical activity is lower with in vitro shoots compared to that of in vivo shoots. The minimum photosynthetical activity until 14 days after trancfer of leaves from in vivo culture was observed with leaves formed in vitro. The authors make a conclusion that the plants survive during acclimatisation using the stock of metabolites. The normal recovery of structure and function occurs with the regenerants within a month after placing them into in vivo 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 which in turn causes increase in intensity of photosynthesis.

Table 1. **Quantative Indices of Anatomical Structure of Leaves of *Vaccinium corymbosum*, *Vaccinium vitis-idaea* in the Aseptical Culture, Greenhouse, 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 L.							
Bluecrop	76±2	16±1	15×11	154±16	0,75	1,8:1	251±11	25×17	210±11	0,87	2,5:1	260±12	23×16
Dixi	85±3	16±1	15×12	173±13	0,71	1,9:1	250±9	26×16	221±12	0,9	2,7:1	265±10	24×15
						Vaccinium vitis-idaea L.							
Koralle	91±4	19±1	16×10	286±9	0,63	2,61:1	410±20	24×15	450±19	0,86	3,31:1	430±23	21×14

Notes: *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 differenttiated into palisade and spongy mesophills.

Interesting researches were conducted by Solarova (1989) on stady of round-o'clock variability of CO_2 concentration in cultivating retorts where in regenerant 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 CO_2 concentration in retorts decreased in light period and the illumination reached the compensation point in 3-4 hours despite the low illumination derree (100 mc 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 the basic growth different.

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

On the foundation of comparative analysis of structural and functional features of the regenerants in vitro and in vivo conditions based on written sources and results of our own researches we came to a conclusion that in vitro and in vivo cultivating conditions (1) leave imprint on structure and functions of regenerants (2) structural andfunctional organisation on regenerants is a mobile system able to transform in accordance with the changed environmental conditions. That means that the differences in structure and function of plant leaves grown inthe 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 successful adaptation of plants when transferring them from in vitro to in vivo 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 researches. This was proved by our observations over adaptation process of introduced species of *Vaccinium corymbosum* (Dixi, Bluecrop, Herbert, Rancocas, Covill, Earlyblue) and *Vaccinium vitis-idaea* (Koralle, Masovia, Erntedank) when transverring them from in vitro into in vivo conditions. To prevent death of the material from overtranspiration (refers not only to *Vaccinium corymbosum* and *Vaccinium vitis-idaea*) caused by the reasons known to us: 1) the humidity drop in vivo conditions, 2) imperfect structural and functional organisation of the leaf in terms on in vivo 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 conditions 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 wind 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°C) and low illumination degree (500

Lx) favour low intensity of photosynthesis and stoppage of regenerant growth. The stock of metabolites with the regenerant will be utilised 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 to complete the structural transformation of the leaf: the cuticular layer will appear, the cells of epidermis will change their shape, the mesophill of the leaf will change its texture. The leaf will acquire features of xeromorphic structure and the plant will not be frightened 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 corymbosum* and *Vaccinium vitis-idaea* from in vitro to in vivo conditions allowed us to preserve the viability of plants and to secure their 100% survival and adaptation.

Conclusions

To sun it up it can be concluded that the successful adaption of regenerant plants when transferring from in vitro to in vivo 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 *Vaccinium corymbosum* and *Vaccinium vitis-idaea* not only in greenhouse conditions but also in open ground conditions.

References

Brainerd K.E. et al. Leaf anatomy and Water Stress of Aseptically Cultured "Pixy" Plum Grown under Different Environments //Hort Sci. 1981. V.16, N 2. P.173-175.

Grout B.W. Wax Development on leaf surfaces of Brassica regenerated from meristem culture // Plant Sci. Lett. 1975. V.5, N 6. P.401-405.

Sutter E., Langhans R.W. Epicuticular wax formation on cornation plantlets regenerated from shoot-tip culture //J.Amer.Soc.Hort.Sci. 1979. V.104. N 4. P.493-496.

Lee N. Et al. Quantum Flux Density Effect on the Anatomy and Surface Morphology of in vitro- and in vivo- developed Sweetgum Leaves //J.Amer.Soc.Hort.Sci. 1988. V.113. N 1. P. 167-171.

Bunning E., Sagromsky H. Die Bildung des Spaltoffnungsmusters in der Blattepidermis //Z.Naturf. 1948. V.36. S.203-216.

Schoch P. et al. Photosynthese et respiration de bananier in vitro //Photosynthetica 1989. V.23. N 1. P.113-118.

Danies W.J., Kozlowski T. Short- and long-term effects antitranspirants on water relations and photosynthesis of woody plants //J. Amer. Soc. Hort. Sci. 1974. V. 99, N 4. P.297-304.

Fabbri A., Sutter E. Anatomical Changes in persistent leaves of tissue cultured strawberry plants after removal from culture //Scientia Hort. 1986. V.28, P.331-337.

Donnelly D.J. and Vidaver W.E. Leaf anatomy of red raspberry transferred from culture to soil //J. Amer. Soc. Hort. Sci. 1984. V.109. P.172-176.

Waldenmaier S., Schmidt G. Histologische Unterschiede zwischen in vitro und ex-vitro-Blattern bei der Abhärtung von Rhododendron //Gartenbauwissenschaft 1990 Bd. 55, N 2. S.49-54.

Smith M.A., et al. Comparative Anatomy and Physiology at Microcultured, Seedliny and Greenhousegrown Asian White Birch. //J.Americ.Soc.Hort.Sci. 1986. V. 111, N 3. P.437-442.

Donnedy D.J.et al. Fixation of $^{14}CO_2$ in tissue-cultured red raspberry prior to and after transfer to soil //Plant Cell.Tissue Organ.Cult. 1984. V.3, P.313-317.

Grout B.W., Millam S. Phothosynthetic development of micropropagated Strawberry plantlets following transplanting //Ann.Bot. 1985. Vol.55, P.129-131.

Solărovă Jrmila. Photosynthesis of plant regenerants diurnal variations in CO_2 concentration in cultivation vessels resulting from plantlets photosynthetic activity //Photosynthetica 1989. V. 23, N 1. P.100-107.

АДАПТАЦИЯ РЕГЕНЕРАНТОВ ИНТРОДУЦИРОВАННЫХ СОРТОВ ГОЛУБИКИ ВЫСОКОЙ И БРУСНИКИ ОБЫКНОВЕННОЙ К УСЛОВИЯМ EX VITRO.

Е.КУТАС, И.МАЛАХОВА

Резюме

Дан сравнительный анализ структурно-функциональных особенностей регенерантов интродуцированных сортов голубики высокой и брусники обыкновенной в условиях in vitro и ex vitro.

Показано, что условия культивирования накладывают отпечаток на структуру и функцию регенерантов – это во-первых; во-вторых, структурно-функциональная организация регенерантов – мобильная система и может перестраиваться в соответствии с изменившимися условиями окружающей среды. Различия в строении и функции листьев растений, выращенных в асептической культуре, в условиях оранжереи или в открытом грунте, свидетельствуют о пластичности листа - органа, способного перестраивать свою структуру и функцию адекватно условиям культивирования, что теоретически является гарантом успешной адаптации растений при переносе их из условий in vitro (культуральных сосудов) в условия ex virto (оранжерея и открытый грунт).