

THE INFLUENCE OF STRESS CONDITIONS ON ACCLIMATION OF MAGNOLIA *Magnolia × soulangiana* SOUL.- BOD.¹

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Introduction

Stress conditions negatively influence physiological and biochemical processes and therefore stress research plays an important role in micropropagation. Tissue cultures promptly respond to changed environmental conditions, especially to water and heat stress. Due to action of stress factors, a rapid decrease of leaf water potential begins and therefore tissue cultures after transfer to changed cultivation conditions wilt rapidly. Plant adaptation to changed environmental conditions occurs slowly at fast action of stress (heat stress), while at slow action of stress (water stress), the degree of plant dehydration changes simultaneously with changes of leaves water deficit and tissue conductance. Mutual interactions between stress effects and plants reaction are determined by the degree of tissue culture tolerance to stress.

Tissue cultures react to stress factors by different adaptation changes such as morphological changes, toxic substances production, accelerated senescence and start of enforced dormancy, leaves movement (by rolling) and also changes in water relations affecting duration of adaptation (stomata density, thickness of palisade parenchyma).

Tissue cultures and regenerants react to adaptation stress also by changes of assimilate pigments and by rate of leaf senescence [MARIN et al. 1988]. Stress factors induce local stress reaction. Disorders in assimilation apparatus decrease growth, vitality, biomass formation, water and nutrition uptake and cause disorders in photosynthesis. Due to all these changes growth of tissue culture is decreased or even stopped [SHIBLI et al. 1999]. Plant adaptation to stress factors leads to adaptation syndrome (closed stomata, increased leaf diffusion resistance, cell and tissue osmotic adjustment) and consequently total plant production decreases. Water stress and low photosynthesis activity affect regenerants adapta-

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tion during transfer into soil conditions. Long-term effect of water stress causes changes in organs structure, and this fact negatively influences duration of regenerants adaptation to changed conditions. Water stress manifests itself the most expressively, because regenerants do not have developed cuticle and control mechanisms of water relations.

The aim of this work was to study stomata apparatus changes occurring on leaves of *Magnolia x soulangiana* SOUL.-BOD. tissue cultures and regenerants compared to donor tree.

Material and methods

The donor tree of *Magnolia x soulangiana* was about 100 years old. Primary explants were taken in spring. Juvenile shoots with removed leaves were sterilised in 0.1–0.3% HgCl_2 with three drops of TWEEN 20 (0.03–0.05%). The disinfectants were removed by 3 successive rinses into sterile water. The last rinse consisted of a 30 min. soaking. Shoots were aseptically divided into nodal segments (3–5 mm). The segments were placed on S-medium [STANDARDI, CATALANO 1985] or WPM, medium [LLOYD, McCOWN 1981]. Culture vessels of 100 ml contained 25 ml of culture medium supplemented with $0.3 \text{ mg}\cdot\text{dm}^{-3}$ benzylaminopurine (BA), $0.1 \text{ mg}\cdot\text{dm}^{-3}$ naphthaleneacetic acid (NAA), $20 \text{ g}\cdot\text{dm}^{-3}$ saccharose and $7.0 \text{ g}\cdot\text{dm}^{-3}$ agar-agar. The pH of all media was adjusted to 5.8 with $1 \text{ mol HCl}\cdot\text{dm}^{-3}$ before autoclaving. Explants were kept in an air-conditioned room with a 16 h light/8 h dark photoperiod, with a light intensity of $35\text{--}40 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Day temperatures were maintained at 22°C .

Axillary shoots 3.0 to 4.0 cm long were separated from the multiplication culture and rooted on S-medium supplemented with $1.0 \text{ mg}\cdot\text{dm}^{-3}$ indolebutyric acid (IBA). Regenerants were cultivated in a substrate of peat and sand (1 : 3) and placed in plastic pots in hotbeds.

Histological studies were carried out on leaves of 1-year old regenerants. The samples were taken from the third leaf at the end of June. Samples for the scanning electron microscope (SEM) were frozen in liquid nitrogen at -196°C without metal-coating. The results were processed with the program Lucia M (Nikon, Laboratory Imaging Ltd., Prague).

Results and discussion

Differences between leaves surface of tissue culture, regenerants and donor documented by stomata state and leaves anatomical structure are very clear (Fig. 1, 2 and 3). The leaf of tissue culture has markedly thinner layer of mesophyll cells. Chloroplasts occur mainly on the adaxial leaf side and are markedly smaller. Exoderma surface layers on the abaxial as well as on adaxial leaf side are very dimly developed. Thickening of tissue culture epidermal cell wall is limited. Epidermis contains big vacuolised cells with nucleus with nucleolus. Leaf mesophyll of tissue culture plant is homogenous compared to differentiated leaf of donor plant with palisade parenchyma mainly on the adaxial side. Epidermis has expressive polarity.



Photo 1A. Donor plant of magnolia
Fot. 1A. Roślina mateczna

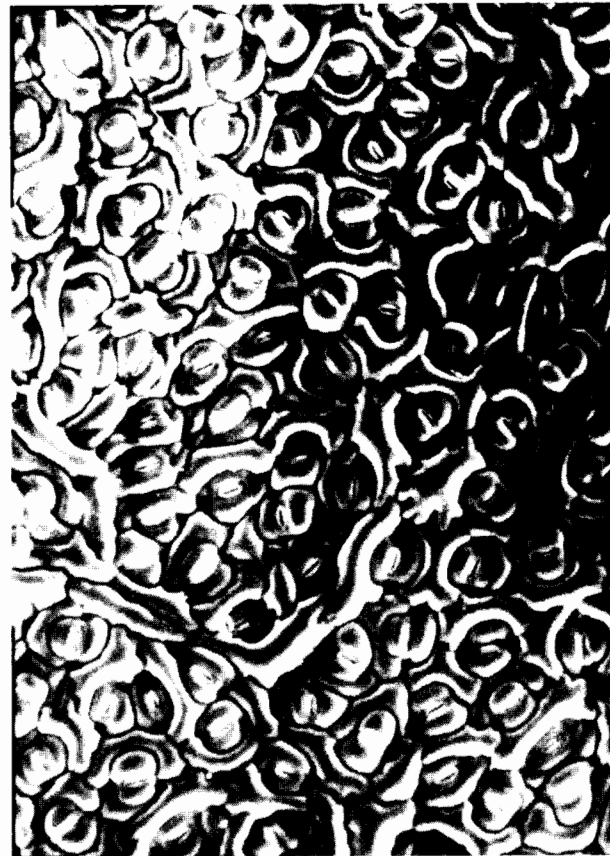


Photo 1B. Leaf surface of donor plant, (magnification 250 x)
Fot. 1B. Powierzchnia liści rośliny matecznej (powiększenie 250 x)



Photo 2A. Tissue cultures of magnolia
Fot. 2A. Kultury tkankowe magnolii



Photo 2B. Leaf surface of tissue cultures (magnification 250 x)
Fot. 2B. Powierzchnia liści z kultur tkankowych (powiększenie 250 x)



Photo 3A. Regenerants of magnolia
Fot. 3A. Zregenerowane rośliny magnolii



Photo 3B. Leaf surface of regenerant (magnification 250 x)
Fot. 3B. Powierzchnia liści z rośliny zregenerowanej (powiększenie 250 x)

Epidermal cells of donor plant leaves are smooth, isodiametrically shaped with many trichomes (Fig. 3). Stomata are ellipsoid and more numerous than those of the regenerant or tissue culture leaves. The guard cells of the donor leaves are identical with those of the regenerant leaves but narrower in comparison with the tissue culture leaves. The epidermal leaf surface of tissue culture and regenerant is smooth, with no trichomes. The length of tissue culture guard cells is smaller and the width is greater compared both to donor and regenerant (Tab. 1). The density of regenerant stomata (70 per mm²) and of the tissue culture (50 per mm²) is lower than that of the donor (152 per mm²). Stomata are also more rounded than those of the donor. The length of guard cells of regenerant leaf is lower than that of the tissue culture, but similar to the donor (Fig. 3). Similarly the width of guard cells is in line with the donor, but smaller than that of the tissue culture. Epidermal cells of tissue culture leaf are irregularly shaped with undulated cell walls.

Table 1; Tabela 1

Area of epidermal cell, number of stomata cells per leaf surface and size of guard cells from leaf of donor plant, tissue culture and regenerant plant of magnolia

Powierzchnia komórki epidermy, liczba komórek szparkowych oraz rozmiary komórek przyszparkowych liści magnolii pochodzących z rośliny matecznej, kultur tkankowych i rośliny zregenerowanej

Leaf; Liść	Area of epidermal cell (μm ²) Powierzchnia komórki epidermy (μm ²)	No. of stomata cells per mm ² Liczba komórek szparkowych na mm ²	Size of guard cells Rozmiary komórek przyszparkowych	
			length długość (μm)	width szerokość (μm)
Donor plant; Roślina mateczna	27	152	17.6	1.5
Tissue culture; Kultury tkankowe	80	50	13.0	5.9
Regenerant plant; Roślina zregenerowana	22	70	17.9	1.4

These results coincide with WETSTEIN and SOMMER [1983] and JURÁŠ [2001]. On the leaves of *in vitro* plants stomata were observed with smaller or larger surrounding guard cells [BLANKE, BELCHED 1989; CAPELLADES et al. 1990]. More abnormalities were observed in saucer magnolia tissue culture compared to regenerant and donor. The results confirm that although stomata of saucer magnolia grown *in vitro* are open, they do not function because of a high relative humidity. Due to defective regulation mechanisms stomata stay open, resulting in rapid leaf dehydration. The transpiration occurs preferentially through observed dysfunctional, deformed stomata which do not offer resistance to gas diffusion [BLANKE, BELCHED 1989]. Deformed stomata may cause dehydration and consequent loss of leaves after transfer of plants from *in vitro* to *in vivo* conditions. Although epidermis as well as stomata cells are already present on the leaves in *in vitro* conditions, they become physiologically functioning only after transfer into conditions of natural environment.

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Key words: donor plant, tissue cultures, regenerants, leaf cells

Summary

Under a scanning microscope, leaves from tissue cultures (after 8 weeks *in vitro* culturing), from 1 year old regenerants and from the 100 years old donor tree were studied. More abnormalities were observed in the tissue culture compared to regenerant and donor. During tissue culture transfer rapid, leaf dehydration occurs due to defective regulation mechanisms making stomata of tissue culture stay open. Epidermis as well as stomata cells were observed already on the leaves in *in vitro* conditions, but they become physiologically functioning only after transfer into conditions of natural environment.

WPLYW WARUNKÓW STRESU NA AKLIMATYZACJE
MAGNOLII *Magnolia × soulangiana* SOUL. – BOD.

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Słowa kluczowe: roślina mateczna, kultury tkankowe, regeneranty, komórki liści

Streszczenie

Przy zastosowaniu mikroskopu scanningowego porównywano cechy budowy komórek liści pochodzących z 100 letniej rośliny matecznej, 8 tygodniowych kultur tkankowych oraz 1 rocznych regeneratów. W regeneratach z kultur tkankowych obserwowano więcej nienormalności w budowie komórek epidermy, komórek szparkowych i komórek przyszparkowych w porównaniu z komórkami pochodzącymi z rośliny matecznej. Podczas przenoszenia tkanek w kulturze *in vitro* obserwowano odwodnienie tkanek liści a mimo to komórki szparkowe pozostawały otwarte. Komórki epidermy oraz komórki szparkowe były obecne na liściach hodowli *in vitro* jednak stawały się one fizjologicznie funkcjonalne tylko wtedy gdy zostały przeniesione do warunków naturalnych.

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