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INFLUENCE OF LIGHT CONDITIONS ON OXIDATIVE STRESS IN MAIZE CALLUS

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Introduction

Some explants have not been successfully cultured *in vitro* because of their low frequency of callus induction. In early stages of callus induction (6 weeks), tissues of explants cultured *in vitro* sometime degenerate and change their colour as the result of phenol oxidation, yielding yellow-brown products (quinones). It is suggested that production of reactive oxygen (for example H_2O_2) species may be an important factor in this process. The reactive oxygen species (ROS) are inevitably produced in higher plant cells during normal metabolism. Their accumulation is enhanced by exposure to environmental stresses including light [KARPIŃSKI et al. 1997]. Overproduction of reduced and chemically reactive ROS results in oxidative stress and cellular damage. On the other hand, ROS also play a positive role in plant growth and development. Some of the responses promoted by H_2O_2 are its participation in the polymerisation of lignin, suberin and possibly other cell wall components and the induction of defence-related genes. Hydrogen peroxide is a membrane-permeable molecule that has been demonstrated to function as a diffusible intercellular signal [MORITA et al. 1999]. It appears that H_2O_2 accumulation or balance between H_2O_2 formation and degradation might play a key role in early stages of *in vitro* culture and in next stages of culture (after 6 weeks) – in processes of embryogenesis and plant regeneration.

The aim of the first experiment was to study the effect of medium composition (MS and N6) on intercellular H_2O_2 content in maize genotype (two single cross maize hybrids: K103 × K85 and KOC 9431).

In the second experiment we analysed influence of light spectral on intracellular H_2O_2 level and total content of phenols. Increase in proportion between the red (600–700 nm) and blue (400–500 nm) component of light spectrum may induce changes in development regeneration plant from callus culture [KADKADE, JOPSON 1978; CHEE 1986].

Materials and methods

The callus was obtained from immature embryos of two maize lines K103 × K85 and KOC 9431. That two maize forms are differentially cold sensitive: KOC 9431 – chilling-resistant and K103 × K85 – chilling-sensitive. Immature

embryos were aseptically dissected from seeds and placed on a solid agar medium containing the inorganic components according to MS – MURASHIGE and SKOOG [1962] or N6 – CHU et al. [1975] and supplemented by 2,4-D (2 mg·dm^{-3}). The media were different in the level of total inorganic nitrogen: MS contained 60,01 meq·dm^{-3} of NH_4^+ + NO_3^- and N6 contained 34,99 meq·dm^{-3}. The cultures were grown in the dark at 25°C.

First experiment

After 2, 4 and 6 weeks of culture callus induction was observed. After 6 weeks the cellular H_2O_2 content was measured.

Second experiment

K103 × K85 callus was subjected to light of various spectral composition: red, blue (obtained by passage of light through cut-off filters with different absorbance properties (Fig. 1A, B) and white (control) or darkness. After 6 weeks intracellular H_2O_2 levels and total sum of phenols were measured.

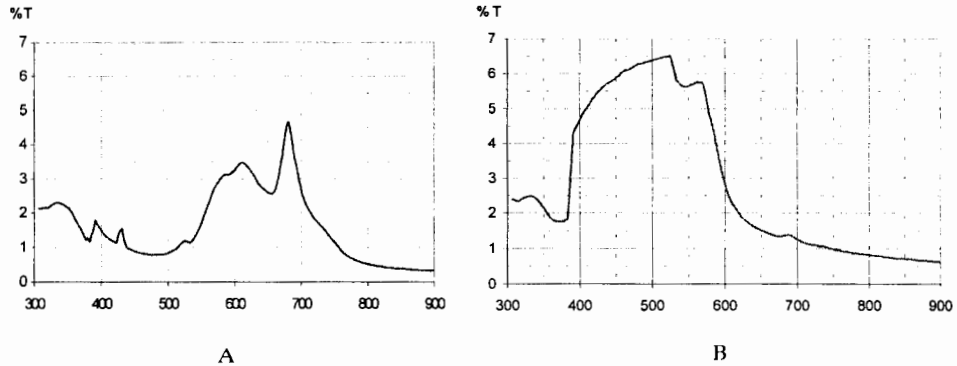

Fig. 1. % transmission (T) of red (A) and blue (B) spectral light composition: obtained by passage of light through cut-off filters with different absorbance properties

Rys. 1. % transmisji (T) światła czerwonego (A) i niebieskiego (B) otrzymanego po przepuszczeniu światła białego przez filtry o różnych właściwościach absorpcji

Phenolic estimation

250 mg of callus were boiled in 1 ml of 80% ethanol, then crushed in 3 ml of 80% ethanol and centrifuged at 2500·g for 20 min. The supernatant was mixed with 20% Na_2CO_3 and Folin-Ciocalteau reagent [SINGLETON, ROSSI 1965]. The absorbancy of samples was measured at λ=760 nm by means of spectrophotometer LKB Ultrospec II. The total phenolic content was calculated as milligrams of chlorogenic acid per 1 g of callus fresh matter.

H_2O_2 content

The cellular content was measured by a fluorometrical assay with homovanillic acid according to ISHIKAWA et al. [1993]. Samples (0.16 g) were homogenised in 0.6 ml of ice-cold 5% trichloroacetic acid (TCA) and centrifuged at 400·g for 5 min. The eluate was made to 1.2 ml with 5% TCA. For measurement 0.1 cm^3 of extract was added to an equal amount of 0.5 $mol \cdot dm^{-3}$ potassium phosphate buffer (pH 7.5). The reaction mixture (1 cm^3) contained 1.25 $mmol \cdot dm^{-3}$ homovanilic acid, 1 unit of horsedish peroxidase, 25 $mmol \cdot dm^{-3}$ potassium phosphate buffer (pH 7.5) and 0.2 cm^3 of buffer extract. The fluorescence yield was measured at an excitation of 315 nm and emission at 425 nm.

Dry matter

Samples (about 1 g) were dried at 60°C for 24 h.

Results and discussion

First experiment

After two weeks the surface of immature maize embryos (K103 × K85 and KOC 9431) cultured on medium MS and N6 became irregular, and embryos were transformed into a friable callus tissue. After 4 and 6 weeks of culture statistically significant genotypic differences in the increase of fresh matter were observed (Fig. 2). For K103 × K85 genotype the fresh matter was about 60–90 mg higher than for KOC 9431. Moreover, the MS medium was better for callus induction for both genotypes. The increase of K103 × K85 and fresh matter was accompanied by an increase of H_2O_2 content (Fig. 3). Generally, after 6 weeks of culture, the K103 × K85 genotype accumulated a higher amount of H_2O_2 in 1 g fresh matter than KOC 9431. The higher inorganic nitrogen level (MS) stimulated hydrogen peroxide production in K103 × K85 callus and inhibited in KOC 9431 callus.

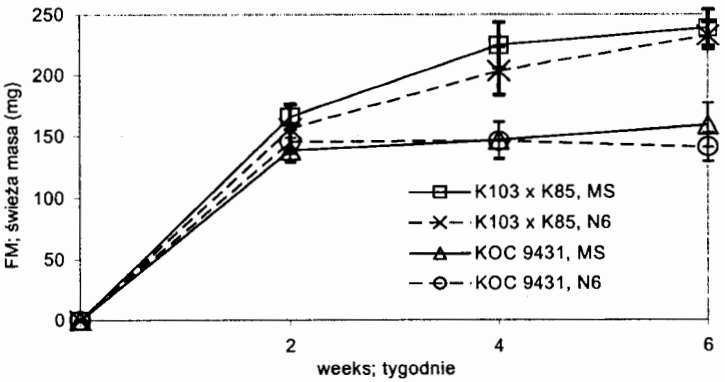

Fig. 2. Callus induction (fresh matter, mg) of maize lines K103 × K85 and KOC 9431 on MS and N6 medium

Rys. 2. Indukcja kalusa (świeża masa, mg) linii kukurydzy K103 × K85 i KOC 9431 na pożywkach: MS i N6

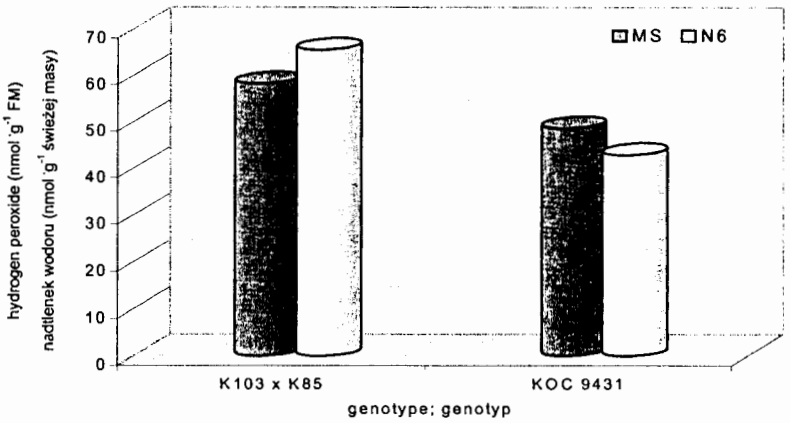

Fig. 3. H_2O_2 accumulation (nmol) in callus of maize lines K103 × K85 and KOC 9431 on MS and N6 medium after 6 weeks of culture

Rys. 3. Akumulacja H_2O_2 (nmol) w kalusie kukurydzy linii K103 × K85 i KOC 9431 na pożywkach MS i N6 po 6 tygodniach hodowli

Hydrogen peroxide is one of weaker oxidizing agents among ROS, owing to its long lifetime. The fact that it can be generated from a variety of cell types and that it has an ability of rapid cell membranes penetration, H_2O_2 is expected to play the most important role among ROS in cell functioning. Hydrogen peroxide also plays a significant role in transduction of the defence signal in plants. What is more, H_2O_2 can induce gene expression and protein synthesis [KAIRONG et al. 1999]. Our study shows that the chilling-sensitive genotype (K103 × K85) produced more hydrogen peroxide in 1 g fresh matter, and it was accompanied with higher tissue growth of this genotype. In *in vitro* culture, H_2O_2 level could be an indicator of the genotype ability to callus production. Genotypes more sensitive to stress conditions (chilling) are better to study oxidative stress in *in vitro* culture.

Second experiment

For this experiment maize line K103 × K85 was chosen as a more sensitive genotype to study light stress conditions. Blue-light-grown callus exhibited lower H_2O_2 level (86% control – white-light-grown callus), than dark- (91%) and red- (95%), (Tab. 1). The lower content of phenols was obtained in callus cultured under blue- (75% control) and red-light conditions (63% control).

The positive correlation between H_2O_2 content and the content of phenols in plant has been reported in many papers [SALAME and ZIESLIN 1994; WYŻGOLIK 1998]. One of metabolic path catalysed by H_2O_2 is the biosynthesis of lignin, a component increasing the mechanical strength of the cell walls [CATTESSON et al. 1986]. However, it has also been suggested that H_2O_2 is involved in the signalling of various stress factors [MORITA et al. 1999]. Somewhat higher hydrogen peroxide contents in red- and white-light conditions of culture are converged with influences of this light types on callus induction and plant regeneration [NI et al. 1987].

Table 1; Tabela 1

Hydrogen peroxide (% control) and phenols (% control) after 6 weeks *in vitro* culturing under white-, red-, blue-light conditions and darkness (control – white light conditions)

Nadtlenek wodoru (% kontroli) i fenole (% kontroli) po 6 tygodniach kultury *in vitro* w warunkach białego, czerwonego, niebieskiego światła i ciemności (kontrola – białe światło)

Light Światło	White Białe	Red Czerwone	Blue Niebieskie	Dark Ciemność
Hydrogen peroxide Nadtlenek wodoru	100	95	86	91
Phenols; Fenole	100	63	75	83

The observed decrease of hydrogen peroxide is accompanied by a lower phenols content in blue- and red-light conditions. It is suggested, the possibility to utilize blue or red light for quicker adaptation to *in vitro* conditions. Blue light might be used for decreasing the H_2O_2 content and red light for decreasing the phenol level. Those light conditions might be an alternative for *in vitro* culturing in the darkness.

Conclusions

The higher H_2O_2 level in callus obtained from chilling-sensitive maize line shows its bigger sensitivity to oxidative stress in *in vitro* conditions. The increase of fresh matter in that case might be a response to oxidative stress.

The MS medium was better than N6 for *in vitro* culturing of chilling resistant genotype (higher nitrogen content). For chilling-sensitive genotype MS medium induced H_2O_2 accumulation.

The red and blue light might be used to utilise for quicker adaptation to stress conditions and more effective callus induction.

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Key words: oxidative stress, different light conditions, *Zea mays*

Summary

The aim of the first experiment was to study the effect of medium composition (MS and N6) on intracellular H_2O_2 content in maize genotype (two single-cross maize hybrids: K103 × K85 and KOC 9431). After 4 and 6 weeks of culture statistically significant genotypic differences in the increase of fresh mater were observed. For K103 × K85 genotype, the fresh matter was higher than KOC 9431. The MS medium was better for callus induction for both genotypes. The increase of K103 × K85 and fresh matter was accompanying the increase of H_2O_2 content. After 6 weeks of culture the K103 × K85 genotype accumulated the higher amount of H_2O_2 in 1 g fresh matter than KOC 9431. The higher inorganic nitrogen level (MS) stimulated hydrogen peroxide production in K103 × K85 callus and inhibited in KOC 9431 callus.

In the second experiment we analysed the influence of light spectral on intracellular H_2O_2 level and the total content of phenols. K103 × K85 genotype was chosen as a more sensitive genotype to study light stress conditions. Blue-light-grown callus exhibited lower H_2O_2 level (86% control), than dark- (91%), red- (95%) and white-light-grown one (100%). The lower content of phenols was obtained in callus cultured under blue- (75% control) and red-light conditions (63% control).

WPŁYW WARUNKÓW ŚWIETLNYCH NA STRES OXYDACYJNY W KALUSIE KUKURYDZY

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Słowa kluczowe: stres oksydacyjny, różne warunki świetlne, kukurydza

Streszczenie

W pierwszym eksperymencie analizowano wpływ pożywki (MS i N6) na zawartość H_2O_2 w komórce dwóch genotypów kukurydzy: K103 × K85 i KOC 9431. Po 4 i 6 tygodniach zaobserwowano różnice w przyroście świeżej masy kalusów: dla genotypu K103 × K85 znacząco wyższą w porównaniu z genotypem KOC 9431. Skład pożywki MS był korzystniejszy dla obu genotypów niż skład pożywki N6. Wraz ze wzrostem masy akumulowany był nadtlenek wodoru. Oznaczany po 6 tygodniach w 1 g świeżej masy poziom H_2O_2 był znacznie wyższy dla K103 × K85.

W drugim eksperymencie przeprowadzono badania wpływu rodzaju światła na poziom nadtlenku wodoru i fenoli. Do badań wybrano wrażliwszy genotyp K103 × K85. Rosnący w niebieskim świetle kalus gromadził niższy poziom H_2O_2 (86% kontroli) niż rosnący w ciemności (91%), w świetle czerwonym (95%) i białym (100%). Niższy poziom fenoli obserwowano podczas kultury w warunkach światła niebieskiego (75% kontroli) i czerwonego (63% kontroli).

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