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CHEMICAL MUTAGENESIS IN HEAD CABBAGE (*Brassica oleracea var. capitata* L.). II. EFFECT OF ETHYL METHANE SULPHONATE ON PHOTOSYNTHETIC APPARATUS EFFICIENCY

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ABSTRACT: Chlorophyll (Chl) fluorescence parameters F_0, Fm, Fv and their ratios and the plastid pigments content were used to characterize physiological response of head cabbage plants, cv. Ditmarsko, to treatments with chemical mutagen ethyl methane sulphonate. (EMS). For this purpose the seeds were treated with EMS in concentration 0,5%, 0,6% and 0,7%. The produced plants from M_1 generation at economic and generative phases were used for the study.

A certain stimulating effect of EMS on photosynthetic apparatus efficiency was found. It was more visible during the generative phase of the plants compared to the economic one. The total amount of synthesized plastid pigments in the M_1 generation plants from the treated variants exceeded the controlled plants by 22-46%. The values of the Chl fluorescence parameters indicated same or better physiological status of the plants in the EMS-variants in comparison with the controlled ones. No proportional dependence between the studied parameters and the used EMS concentrations was obtained.

Key words: *head cabbage, ethyl methane sulphonate, photosynthesis, pigments, chlorophyll fluorescence*

INTRODUCTION: The application of induced mutagenesis in plant selection requires studying of any promising mutation at the molecular, cellular and organism level, in the complexity of its interdependence with the entire spectrum of additional biological processes occurring in living organisms (Medrano et al., 1986; Park & Buttery, 1992; Svetleva & Kouzmova, 2003; Archana Patil et al., 2004).

It is known that the photosynthetic apparatus (PSA) is under dual control that of the plastome and that of the nuclear genome. The formation of pigment apparatus in the chloroplasts as well as photosynthetic activity are a functional manifestation of the genetic information under certain environmental conditions. Because of its high functional activity, the PSA is highly vulnerable to different stresses including the chemical stress (Srivastava & Strasser, 1995, 1996; Georgieva & Lichtenthaler, 1999; Jarvis et al., 1999; Lichtenthaler, 1988, 1996, 2005; Hirata et al., 2000; Yamane et al. 2000; Bae et al., 2005).

Chlorophyll (Chl) fluorescence is one of the forms, under which the energy excess, unused in the photosynthesis, is dissipated. The ***in vivo*** Chl fluorescence intensity in plants depends mainly on the state of the photosystem II (PSII) reaction centres (RC) (Govindjee, 1995). Chl fluorescence analysis is a non-invasive tool to study the effects of many biotic and abiotic factors on photosynthesis in plants ***in vivo*** (Schreiber et al., 1994; Yordanov et al., 1997; Pastenesm&Horton, 1999; Yamane et al. 2000; Lichtenthaler, 2005).

Originalni naučni rad (Original scientific paper)

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Our physiological studies are a part of the breeding programme in the Maritsa Vegetable Crops Research Institute for inducing, stabilisation and multiplication lines of head cabbage (***Brassica oleracea var. capitata*** L.) with male sterility and valuable agro-economic properties, employing the methods of induced mutagenesis.

The objective of this investigation was to determine the influence that the treatment with ethyl methane sulphonate (EMS) of head cabbage seeds has on PSA efficiency of the produced plants in M_1 generation (economic and generative phases).

Materials and methods

The experiments were conducted in the Maritsa Vegetable Crops Research Institute during the period 2004-2005 years.

Plant material: Seeds of head cabbage, cv. ***Ditmarsko***, were treated with 0.5 %, 0.6 % and 0.7 % v/v EMS for 18 hrs. The produced plants in M_1 generation (economic and generative phases) were used for analyses and measurements.

Chlorophyll fluorescence: Fast fluorescence induction kinetics of Chl a was used as a tool to evaluate the photosynthetic activity of M_1 generation plants. Measurements were taken in the field conditions in economic and generative phases, 5-times in each phase in 10 replications. Chl fluorescence parameters initial fluorescence (F_0), maximum (Fm), variable fluorescence (Fv) and their ratios were registered using a Plant Efficiency Analyser (Hansatech Instruments Ltd, UK). The fluorescence signal was always sampled at a standard position of the leaf, on the upper adaxial surface. The dark-adapted (30 min) intact leaves were illuminated with excitation actinic light (>650 nm) with a photon flux 3000 µmol m^{-2} sec^{-1} provided by an array of six ultra-bright light-emitting diodes, focused on a circle of 4 mm diameter of the sample surface (Strasser et al., 2000).

Photosynthetic pigment analysis: Photosynthetic pigments were analyzed two times during the economic stage of the plants development and twice during the generative phase. The leaf tissue was cleaned of any attach epiphytes, and the non-photosynthetic parts of the leaf were removed. Chl pigments were extracted in acetone according to Wettstein (1961). The amounts of the Chl ***a***, Chl ***b*** and carotenoides were determined by spectrophotometer VSU-2P.

The data were statistically processed by the common Excel software.

Results and discussion

The values from the analyses for the plastid pigments content in the plants in two phases of the M_1 generation are presented in Table 1.

Tab. 1. Mean values sd.(n= 4) photosynthetic pigment content in head cabbage plants, cv. Ditmarsko, produced from treated with EMS seeds, in M_1 generation

Variants	Chl ***a***		Chl ***b***		Carotenoides		Chl (***a***+***b***)		Chl (***a***+***b***)+ Carotenoides	
	mg g^{-1} FW	%	mg g^{-1} FW	%	mg g^{-1} FW	%	mg g^{-1} FW	%	mg g^{-1} FW	%
Economic phase										
Control	0.410±0.02	100.00	0.192±0.01	100.00	0.213±0.02	100.00	0.602±0.03	100.00	0.815±0.04	100.00
0.5 % EMS	0.494±0.03	120.48	0.243±0.02	126.56	0.269±0.02	126.29	0.737±0.03	122.42	1.006±0.05	123.43
0.6 % EMS	0.493±0.02	120.24	0.250±0.02	130.21	0.248±0.02	116.43	0.743±0.03	123.42	0.991±0.04	121.60
0.7 % EMS	0.624±0.03	152.20	0.269±0.02	140.10	0.246±0.02	115.49	0.893±0.03	148.34	1.139±0.05	139.75
Generative phase										
Control	0.420±0.02	100.00	0.213±0.02	100.00	0.184±0.01	100.00	0.633±0.03	100.00	0.817±0.04	100.00
0.5 % EMS	0.554±0.03	131.90	0.263±0.01	123.47	0.214±0.02	116.30	0.817±0.04	129.07	1.031±0.04	126.19
0.6 % EMS	0.634±0.03	150.95	0.320±0.02	150.23	0.235±0.02	127.71	0.954±0.04	150.71	1.189±0.04	145.53
0.7 % EMS	0.593±0.03	141.26	0.307±0.03	144.13	0.229±0.02	124.46	0.900±0.04	142.18	1.129±0.04	138.19

Chl ***a*** is directly associated with photosynthesis and represents almost the half of total quantity of green and yellow pigments. There was a significant increase of chl pigments content, during the economic phase, mainly Chl ***a***, to a smaller degree chl ***b*** and carotenoides.

A similar effect, but expressed more clearly, was found during the generative phase, when the increase in chl ***b*** was more significant. The Chl pigments content (***a+b***) in generative phase increase with up to 50 % compared to the control. The changes in carotenoids content are expressed in a smaller degree. They don't exceed the control levels with 26 % (in 0.5% EMS).

The ratioChl pigments / carotenoides (Fig.1) was lower during the economic phase compared with the generative one, which confirm the stimulating effect of EMS treatment on the Chl pigments synthesis.

Fig. 1. Changes in ratio chlorophylls/carotenoids after EMS treatment. The values are presented as a percent of the control plants

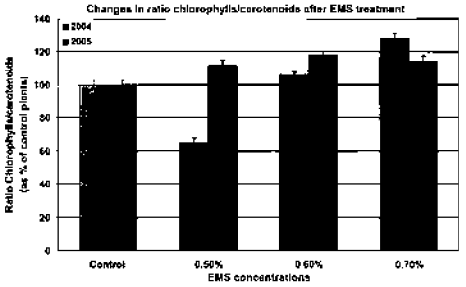
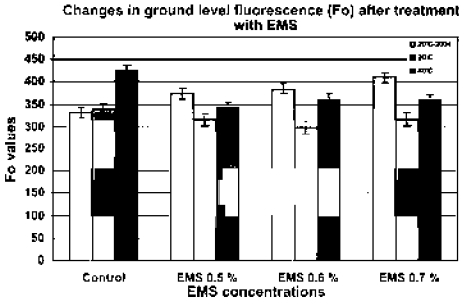


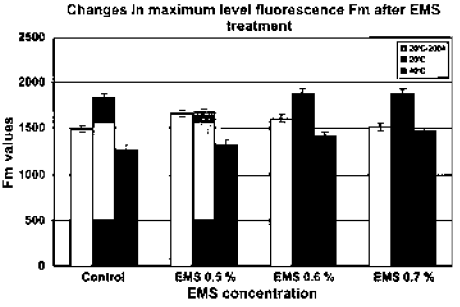
Fig. 2. Changes in the ground level fluorescence (F_0) after treatment with EMS. The samples were kept in darkness for 30 min before measurements. The first set of values represents the F_0 levels at economic stage of development (year 2004, 20°C) while the two other seta were measured at generative stage (year 2005, at 20°C and at 40°C respectively)



There have been reports in the scientific literature (Allen & Tresini, 2000) that the significant increase in Chl pigments synthesis is able to lead to photodynamic oxidative damages of the PSA. To verify if there is a similar effect of EMS treatment we made measurements of the Chl fluorescence parameters of the M_1 generation plants at the economic and generative phases.

The results obtained showed (Fig. 2) that the dark level fluorescence (F_0) at the economic phase indeed has higher values in treated variants according to the control, however not connected with a decrease at maximum fluorescence (Fm) (Fig.3).

Fig. 3. Changes in the maximum level fluorescence (Fm) after treatment with EMS. The samples were kept in darkness for 30 min before measurements. The first set of values represents the Fm levels at economic stage of development (year 2004, 20°C) while the two other seta were measured at generative stage (year 2005, at 20°C and at 40°C respectively)

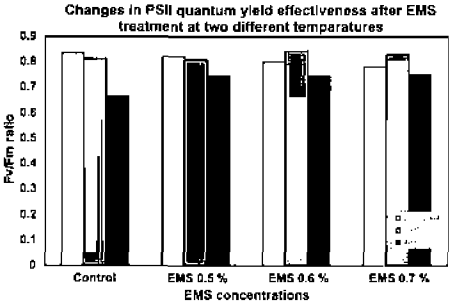


Fm values remained almost unchanged at 0.6% and 0.7 % EMS and were with 5 % higher at 0,5 % according to the control. Furthermore the damages in PSII will lead to a decrease of the maximum quantum yield (Fm/Fv) (Fig. 4).

We did not register such decreases in quantum effectiveness in the studied variants. Judging by the simultaneous increase of F_0 and Fm Chl fluorescence parameters we suppose that at economic stage there are not significant damages in PSA, but rather there is accumulation of small quantities of Chl ***a*** pigments, which are not integrated in the structures of the photosystems. Probably the action of the green pigments in this case as photosensebilizators was neutralized from the plants by increase of the synthesis of carotenoids, which, act as non-enzymatic antioxidants. We assume also, that at the generative phase the synthesized higher amounts of Chl

pigments were included in the structure of the photosystems as the possible adaptive plant response to EMS treatment. This was demonstrated by lower levels of the dark Chl fluorescence (Fo) at EMS variants compared to the control at the generative phase (Fig.2). This conclusion is confirm also from significant higher Fm levels at generative phase, which correlated with the increased synthesis of the Chl ***a*** and Chl ***b*** (Tabl.1).

Fig. 4 Changes in the maximum quantum yield effectiveness (Fv/Fm) after treatment with EMS. The samples were kept in darkness for 30 min before measurements. The first set of values represents the Fv/Fm levels at economic stage of development (year 2004, 20°C) while the two other seta were measured at generative stage (year 2005, at 20°C and at 40°C respectively)



In order to determine the EMS effect on the stability of PS2 of PSA we measured the Chl fluorescence parameters in the plants at the generative phase at two temperature regimes 20°C and 40°C. The high temperature is usually associated with damage of PSA and with reduction of the maximum quantum yield and electron-transport efficiency of the PS2 (Schreiber&Berry, 1977; Yamane et al., 1997). The results obtained showed (Fig. 2) that the F_0 has higher values in control plants than in EMS variants. Analogically, Fm values decrease in a greater extent in the control than in EMS variants, which reflects in the values in the ratio

Fv/Fm, which characterizes the quantum effectiveness of PS2. It is visible that the control plants in high temperature conditions have more serious damages in primary photochemistry than the plants from EMS variants.

Conclusions

A certain stimulating effect of EMS on PSA efficiency was found. It was more visible during the generative phase of the plants development compared to the economic one.

Plastid pigments content generally increased after EMS treatment. The total amount of synthesized plastid pigments in the M_1 generation plants exceeds the controlled plants by 22% - 46%.

The photosynthetic pigment analysis showed significant increase of Chl ***(a+b)*** pigment synthesis after EMS treatment in both phases. The ratio Chl pigments / carotenoids has lower values at economic stage compared with the generative one.

Experimental plants responded to the stimulated synthesis of the Chl pigments with accumulation of more carotenoids. We consider increased carotenoids accumulation to be adaptive reaction of the plants to the abundance of chlorophylls aimed to prevent formation of free radicals.

The overall effect of EMS treatment of the seeds was an increase of the stability of the PSII to high temperature stress at the generative phase of head cabbage plants.

No proportional dependence between the studied parameters and the used EMS concentrations was observed.

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HEMIJSKA MUTAGENEZA KUPUSA *Brassica oleracea var capitata* L. II. EFEKAT ETIL METAN SULFONATA NA EFIKASNOST FOTOSINTESKOG APARATA

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IZVOD

Parametri flourescencije hlorofila F_0, Fm, Fv i njihovih odnosa kao i sadržaj pigmenata plastida su korišćeni za karakterizaciju fiziološkog odgovora biljaka kupusa sorte Ditmarsko na tretman sa hemijskim mutagenom etil metane sulfonatom (EMS). Za ovu svrhu seme je tretirano sa EMS u koncentracijama 0,5, 0,6 i 0,7%. Biljke dobijene iz M_1 generacije u tehnološkoj i generativnoj fazi su korišćene u ovim istraživanjima.

Utvrđen je značajan stimulativni efekat EMS na efikasnost fotosintetskog aparata. Efekat je bio više vidljiv tokom generativne faze biljaka u poređenju sa fazom tehnološke zrelosti. Ukupna količina sintetisanih pigmenata plastida u M_1 generaciji biljaka dobijenih od tretiranih biljaka u odnosu na kontrolne biljke bila je veća za 22-46%. Vrednosti parametara fluorescencije hlorofila ukazuju na isto ili bolje fiziološko stanje u EMS varijantama biljaka u poređenju sa kontrolom. Nije dobijena srazmerna zavisnost između ispitivanih svojstava i koncentracije primenjenog EMS.