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Physiological Studies on Nuclear-Organelle Interaction of Cybrid Plants Containing *Solanum nigrum* Genome and *Solanum tuberosum* Plastome

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With 7 Figures

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Summary

HASSANEIN A. M., FAYEZ K. A. & AHMED A. M. 1998. Physiological studies on nuclear-organelle interaction of cybrid plants containing *Solanum nigrum* genome and *Solanum tuberosum* plastome. – Phyton (Horn, Austria) 37 (2), 167–179, 7 figures. – English with German summary.

Comparison between second generation of functional cybrid (containing *Solanum nigrum* genome and *Solanum tuberosum* plastome) and original (*S. nigrum*) plants was used to study the effect of nucleus/chloroplast interaction on the physiology of the respective cybrid. Percentage of seed germination, response of shoot apices to form adventitious shoots on B5 medium supplemented with 5% liquid coconut endosperm and 2.5 M BAP, and morphological characters of cybrid were similar to that of the original plant. The cybrid shoots were light green and bleached under atrazine stress while the original plant shoots were dark green and were not affected by atrazine. Fresh and dry matter as well as carbohydrate contents of cybrid shoots were lower than those of original plant, generally, these values were reduced under atrazine stress. Protein and sodium contents remained unaffected under chloroplast substitution and atrazine stress. Potassium content of cybrid was higher than those of corresponding original plant, but with a tendency to be lowered under atrazine stress. X-ray microanalysis data showed differences in ion contents between the cybrid and original plant on atrazine free medium or under atrazine stress.

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Zusammenfassung

HASSANEIN A. M., FAYEZ K. A. & AHMED A. M. 1998. Physiologische Untersuchungen über Kern-Organelleninteraktionen von cybriden Pflanzen mit dem Genom von *Solanum nigrum* und dem Plastom von *Solanum tuberosum*. – Phyton (Horn, Austria) 37 (2): 167–179, 7 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Der Vergleich zwischen der zweiten Generation von Cybriden (mit dem Genom von *Solanum nigrum* und dem Plastom von *Solanum tuberosum*) und den ursprünglichen Pflanzen (*Sinigrum nigrum*) wurde angestellt, um den Effekt von Kern/Chloroplastenwechselwirkungen auf die Physiologie des betreffenden Cybriden zu untersuchen. Der Prozentsatz an Samenkeimung, die Bereitschaft des Vegetationspunktes Nebensprosse auf den B5Medium, welches mit 5%iger Kokosmilch und 2,5 M BAP versetzt war, zu bilden, sowie die morphologische Ausbildung der Cybriden waren ähnlich wie bei den Originalpflanzen. Die Sprosse der Cybriden waren hellgrün und blichen unter Atrazin-Streß aus, während die ursprünglichen Pflanzensprosse dunkelgrün waren und nicht durch Atrazin beeinflußt wurden. Die Frisch- und Trockenmasse sowie der Gehalt an Kohlenhydraten von Sprossen der Cybride waren geringer als die der ursprünglichen Pflanzen und diese Werte wurden im allgemeinen auch unter Atrazinstreß vermindert. Die Gehalte an Protein und Natrium blieben nach Chloroplastenaustausch und Atrazinstreß unbeeinflußt. Der Kaliumgehalt von Cybriden war höher als der der ursprünglichen Pflanzen, aber mit einem Trend zur Verminderung unter Atrazinstreß. Die Ergebnisse der Röntgenmikroanalyse zeigten Unterschiede in den Ionengehalten zwischen Cybriden und ursprünglichen Pflanzen sowohl auf atrazinfreiem Medium als auch unter Atrazinstreß.

Introduction

Intraspecific and interspecific transfer of cytoplasmic genes in sexual crossing requires repeated back-cross to exchange the chromosomes of the cytoplasmic donor. The cytoplasmic genes are still unaltered during backcrossing repeats because of its uniparental inheritance in most higher plants.

Moreover, sexual incompatibility barriers strongly restrict gene transfer between different species. Recently, somatic hybrid or cybrid (cytoplasmic hybrids) between species that are sexually incompatible can be obtained in one step by fusion of plant protoplasts (e.g. SCHIEDER 1977, EHLENFELDT & HELGESON 1987, BINDING & al. 1988, JOURDAN & al. 1989, CHRISTEY & al. 1991, SAKOMOTO & TAGUCHI 1991, HASSANEIN & al. 1993 WANG & BINDING 1994). Transfer of cytoplasmic genes after protoplast fusion is the result of independent segregation of nuclei and cytoplasmic organelles in the primary protoplast fusion product (NEHLS & al. 1986).

A concerted intracellular interaction between nuclei and transferred chloroplasts is required to maintain the functionality of the cybrid plants (KUSHNIR & al. 1987, PERL & al. 1990, 1991). It is well known that fraction-1-protein is the most abundant protein in the plant leaves (40% of the soluble protein). It has both carboxylase and oxygenase activities in photo-

synthesis and photorespiration. This protein contains small and large subunits, they are coded for by nuclear and chloroplast genes respectively. Therefore, fraction-1-protein in cybrid plant contains fractions from the two parents. The catalytic action of this recombinant protein may be more or less active than those of the original plant.

Atrazine (2-Ethylamino-4-chloro-isopropylamino-1.3.5-triazine) is one of the most widespread herbicides used in weed control of many crops. BESTER & al. 1995 studied the effect of atrazine on marine phytoplankton, they found that it reduced photosynthesis, which was accompanied by lower chlorophyll contents, reduced primary production and higher concentrations of water soluble amino acids. The resistance mutation is translated into a decrease in plant yield (STOWE & HOLTS 1988) and is suggested to be responsible for the fitness difference observed between triazine susceptible and triazine resistant plants (HOLT & al. 1981, ALI & al. 1986).

The material used in this study is cybrid plant which contains nucleus and mitochondria of *Solanum nigrum* (black nightshade) cells with *Solanum tuberosum* (potato) chloroplasts (HASSANEIN & al. 1993). Whereas, atrazine resistance is presumed to be on the chloroplast DNA, the substitution of *S. nigrum* chloroplasts by others of *S. tuberosum* resulted in sensitive cybrid plant. Consequently, the same plant was propagated in two forms: The original was atrazine resistant but the created one (cybrid) was atrazine sensitive.

The physiological aspects of the cellular interaction in created somatic hybrid and cybrid plants are rarely studied especially for the next generations. Therefore, the first goal of our approach is to investigate the effect of the incompatibility between the nucleus and the transferred chloroplasts on some physiological processes of the second generation of respective cybrid in comparison to original plant. The second is the use of cybrid and the original *S. nigrum* plants to study the role of atrazine on the growth and some chemical composition of sensitive and resistant lines.

Materials and Methods

Plant materials

The somatic cybrid plant (C-18-1) was originally obtained (HASSANEIN & al. 1993) by fusion of albino plastid mutant of *Solanum nigrum* (Sn-F-W2) and diploid clone St-H^2 258 of *Solanum tuberosum L.* (BINDING & al. 1978, BINDING & al. 1987).

Seeds of cybrid and original plant (*S. nigrum*) were disinfected by dipping in 5% chlorox solution for 5 min followed by 5 min dip in 75% ethanol. Seeds were germinated on modified hormone free B5 medium (GAMBORG & al. 1968). Percentage of seed germination was estimated. Shoot sections with shoot apices (0.5–0.8 cm) of the germinated seeds were placed on modified B5 agar medium (B5C medium) containing 5% liquid coconut endosperm and 2.5 µM BAP (benzyl amino purine). After 40 days many shoots were initiated on each section. The shoots formed from each

section were propagated as single subline on B5 medium supplemented with 2.5 μM BAP. These sublines were subcultured at shorter period (two weeks to obtain high multiplication rates). Three sublines of cybrid as well as original plant were used as replicates to study the effect of chloroplast substitution on the different parameters. All shoot cultures were maintained in 16 h daily light (100 μmol $m^{-2}s^{-1}$ at $25 \pm 1°C$ without humidity control.

Atrazine test

About 30 shoots of each sublines were planted on B5 medium with sucrose concentration reduced to 1% (w/v) and with 10^{-4} M atrazine (BINDING & al. 1982). The response to atrazine required 20 days to detect significant bleaching of the sensitive cybrid shoots.

Chemical analysis

Shoots of the cybrid and original plant were harvested three times at 7 days intervals and assayed for growth and other analysis, in the presence or absence of herbicide.

A known fresh weight of shoots was homogenized in 85% (V/V) aqueous acetone. The pigment contents (Chl a, b and carotenoids) were determined using spectrophotometeric method recomended by METZNER & al.1965.

Carbohydrate contents were determined in aqueous solution (soluble carbohydrate) and in acid solution (total carbohydrate) with anthrone sulphuric acid reagent according to FALES 1951 and BADOUR 1959 using glucose as a standard. A spekol Carl-Zeiss spectro-colorimeter was used and the developed blue colour was measured at 620 nm.

The contents of protein were determined according to LOWRY & al. 1951. Flame photometer (M7D) was used to determine the concentration of sodium and potassium in the shoot of plants.

X-ray microanalysis

Dry shoots were grinded to fine powder and a thin layer of powder was adhered to specimen disc of scanning electron microscope. X-ray micro analysis was performed on Jeol 5300 microscopes combined with HNU X-ray system 5000 standardless ZAF analysis. Complete spectrum from 0.0 to 20 KeV 10 eV/ch was analysed. Spectra were recorded and counts were made at live time 200s.

Results and Discussion

Protoplast fusion can result in novel nucleus/organelle combination. The compatibility between nucleus and the transferred organelles in the produced cybrid should determine their functionality. The functional cybrid plant is therefore suitable material to study the physiological aspects of the novel nucleus/organelle interactions.

The seeds of cybrid and original plant were germinated in vitro for two weeks. At that time, the seedlings were 0.5–3 cm long. The percentage of seed germination of both plants was the same (75%). This indicated that

Fig. 1. Original (*S. nigrum*) and cybrid shoots grown on atrazine free medium after three weeks in culture

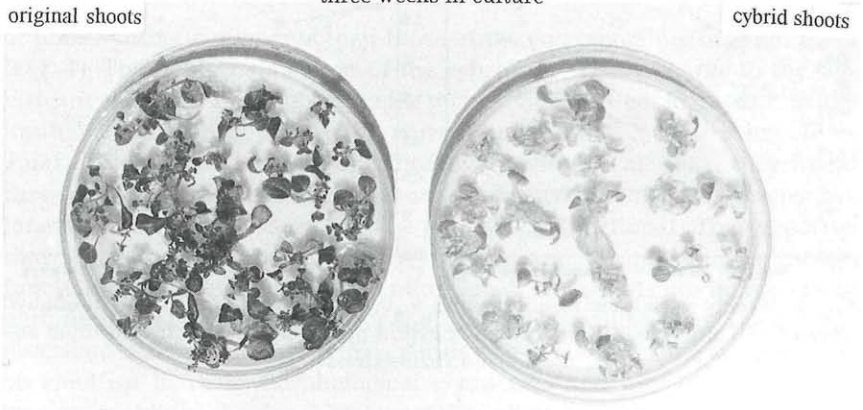

Fig. 2. Original (*S. nigrum*) and cybrid shoots on atrazine medium after three weeks in culture.

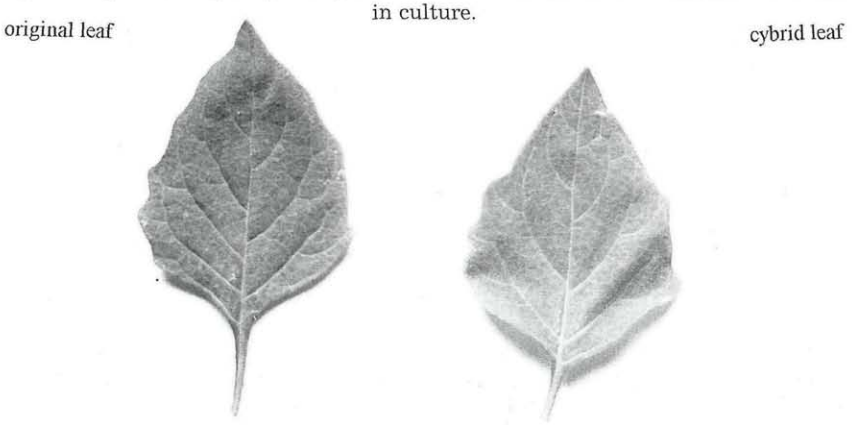

Fig. 3. Original (*S. nigrum*) and cybrid plant leaves grown under green house conditions.

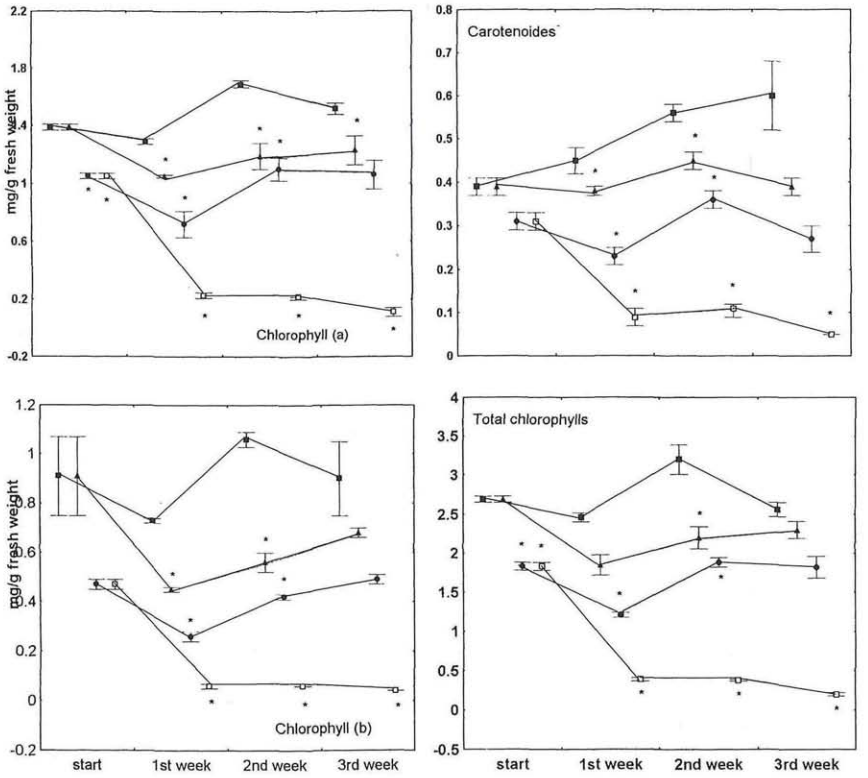

Fig. 4. Weekly variations in chlorophyll a, b, carotenoides and total chlorophyll (Mean (SD) of original (*S. nigrum)* and cybrid plants on atrazine free medium and under atrazine stress.

the chloroplast substitution in the respective cybrid exhibited normal pollination and embryo formation. NEHLS & al. 1986 reported that incompatibilities in sexual cycle act in the course of pollination and zygote formation, embryo and plant development, meiosis, and gametogenesis.

The response of cybrid shoot apices was similar to that of original plant in the formation of adventitious shoots on B5C medium. Ten to thirty shoots formed on each segment, were cut, and transferred to B5 medium supplemented with 2.5 µM BAP for further subculture. Our intensive observation showed that *S. tuberosum* chloroplasts could be transferred to *S. nigrum* without causing any morphological changes in the respective cybrid under shoot culture condition (Fig. 1) or in green house, e.g. plant leaves (Fig. 3). On the other side, the original plant shoots were dark green but the cybrid shoots were light green. Light green pigmentation was found and used for selection of cybrids after protoplast fusion (BINDING & al. 1982, HASSANEIN & al. 1993). The cybrid shoots exhibited lower values

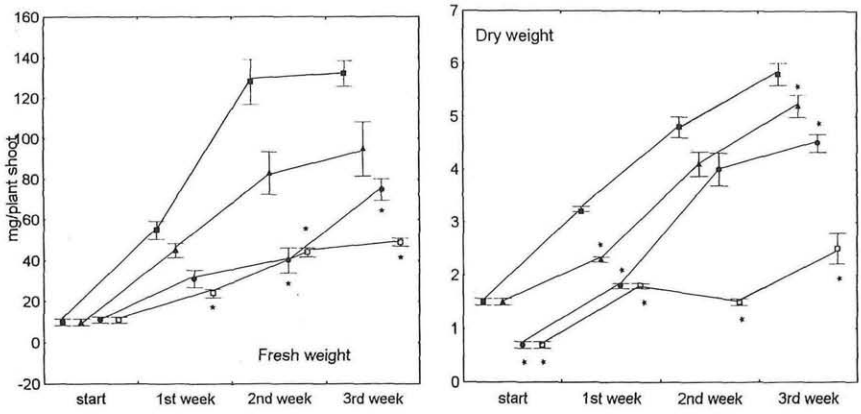

Fig. 5. Weekly variations in fresh and dry weight (Mean (SD) of original (*S. nigrum*) and cybrid plants on atrazine free medium and under atrazine stress.

of photosynthetic pigments than those of the corresponding original plant (Fig. 4). The light green colour of the cybrid plant may be due to the significant decrease in photosynthetic pigments which could be due to the limited compatibility between *S. nigrum* nucleus and *S. tuberosum* chloraplast. Similar results were obtained by KUSHNIR & al. 1987, they found that fertile cybrids containing genome and plastome of plant species belonging to different genera may be genetically functional. They reported that the co-operation between the genome and the plastome, although functional, is not perfect. On the other hand, our results are in agreement with data of many workers (AVIV & al. 1984, KUMASHIRO & al. 1988). They described different interspecific cybrids of *Nicotiana* which were similar or identical in their morphological characters and pigmentation to the original partners. Also, in this respect, PERL & al. 1990, 1991 found that the chloroplasts could be transferred without causing any morphological changes in the cybrid when the transferred chloroplasts are very similar to that of the original plant. Abnormal pigmentation was found only when the species of the chloroplast donor has a considerable phylogenetic distance from the original plant (recipient species).

Shoots of cybrid sublines carrying atrazine sensitive chloroplasts turned yellow or pale green under atrazine stress but the leaves of the original plant shoots were not affected (Fig. 2). The difference between the original and cybrid shoots treated with atrazine resulted from plastome transfer. Young leaves of cybrid shoots were more sensitive than the old leaves. They required 10 days only to detect bleaching. Previous characterization of the chloroplasts in respective cybrid was done by analysis of chloroplast DNA (HASSANEIN & al. 1993). Co-existence of atrazine resistant and atrazine sensitive chloroplasts in the second generation was excluded.

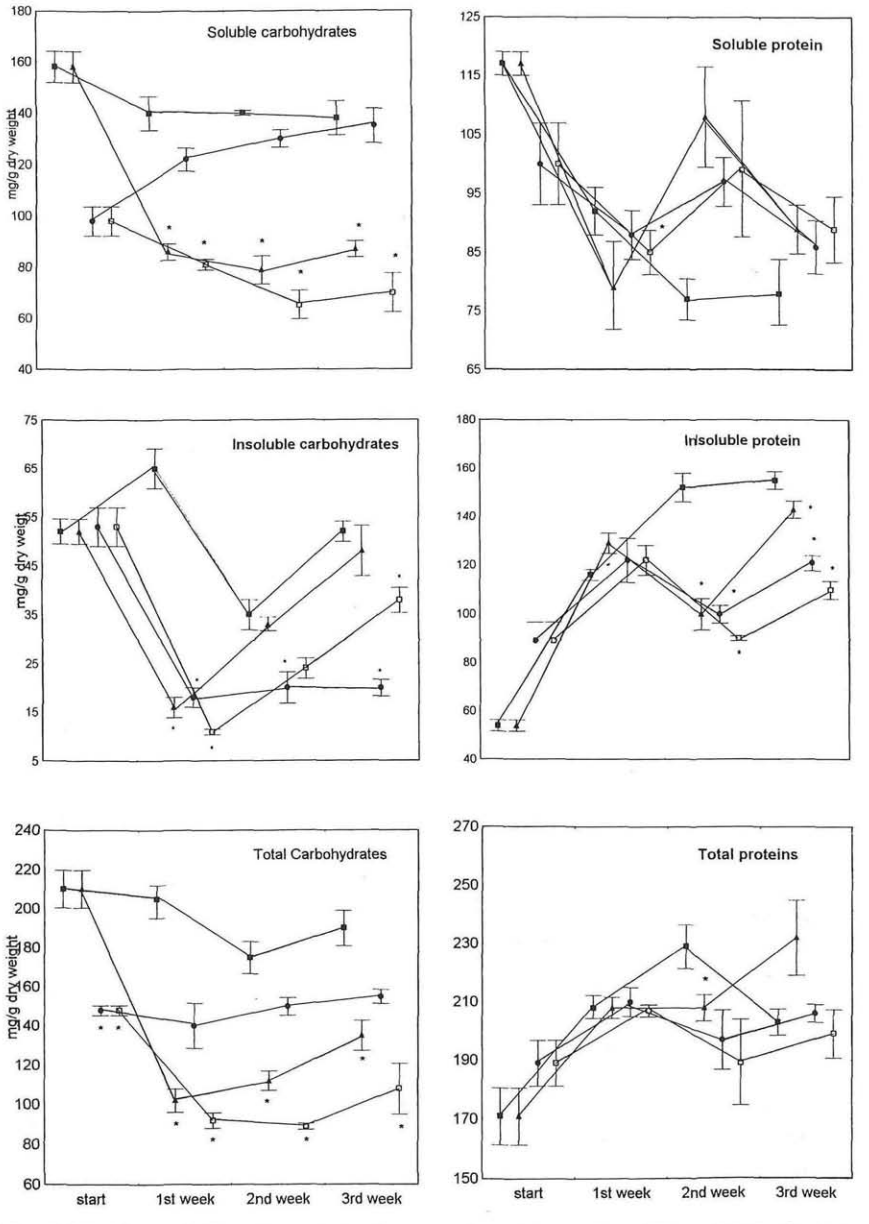

Fig. 6. Weekly variations in carbohydrates and proteins values (Mean (SD) of original (*S. nigrum*) and cybrid plants on atrazine free medium and under atrazine stress.

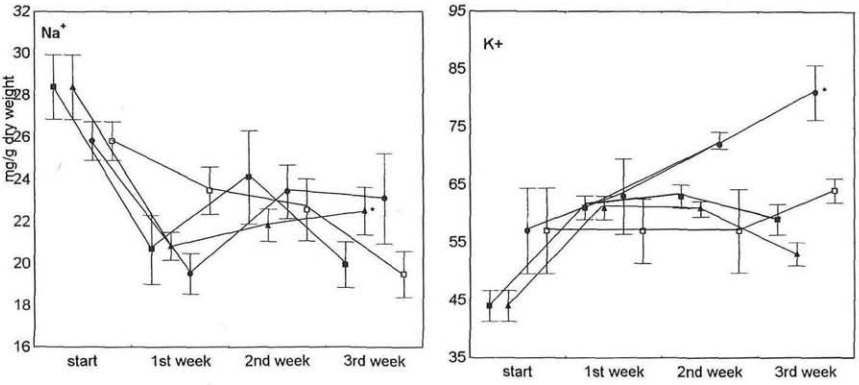

Fig. 7. Weekly variations in sodium and potassium values (Mean (SD) of original (*S. nigrum*) and cybrid plants on atrazine free medium and under atrazine stress.
■ Original plant on atrazine free medium.
▲ Original plant under atrazine stress.
● Cybrid plant on atrazine free medium.
○ Cybrid plant under atrazine stress.
⁕ Means significantly different (t-test) from original plants on atrazine free medium at $P < 0.05$

Consequently, all the used cybrid sublines were bleached under atrazine stress. Original and cybrid shoots under atrazine stress exhibited lower values of pigment contents than those of the corresponding plant shoots on atrazine free medium (Fig. 4).

The previous conclusions could be used to explain the effect of chloroplasts transfer on fresh and dry matter (Fig. 5) as well as carbohydrate contents (Fig. 6). A progressive increase in the values of fresh and dry matter with time was recorded. Generally, these values of the cybrid plant on atrazine free medium were lower than those of original plant. This significant decrease in fresh and dry matter may be due to the corresponding decrease in photosynthesis and/or other incompatibility factors.

Fresh and dry matter were reduced under atrazine stress in both the cybrid and original plant (Fig. 5). Similar results were also obtained by other authors using other plants under atrazine and other stress types (e. g. AHMED & al. 1976, HOLT & al. 1981, ALI & al. 1986, ABDEL-RAHMAN & HASSANEIN 1986, STOWE & HOLTS 1988).

Insoluble, and total carbohydrate contents were reduced significantly in somatic cybrid compared to those of the original plant (Fig. 6). This decrease in carbohydrate values may be due to the corresponding decrease in photosynthesis, which could be due to the decrease in pigment contents. Other reasons may be responsible for the photosynthesis reduction, e.g. the formation of recombinant fraction-1-protein in cybrid cells as well as the decrease of some important protein in the photosystem II (KUSHNIR & al. 1987).

A significant reduction in carbohydrate contents of both original and cybrid plant shoots was recorded after being subjected to atrazine stress (Fig. 6). BOWS & al. 1980 and ALI & al. 1986 reported that atrazine inhibits photosynthesis because the rate of electron transfer from primary electron acceptor of PS II to the secondary acceptor is decreased.

The total proteins (Fig. 6) remained more or less unaffected under the influences of chloroplast transfer or atrazine stress. After one week under atrazine stress, the insoluble protein was reduced significantly in both original and cybrid plant shoots. In this respect, KUSHNIR & al. 1987 found that the content of at least one of the major thylakoid membrane polypeptides was drastically reduced in the cybrid. Other workers found that higher concentrations of water soluble amino acids were found after subjected marine phytoplankton to atrazine (BESTER & al. 1995).

Sodium contents (Fig. 7) of the plant shoots remained unaffected under chloroplast substitution or atrazine stress. On the other side, potassium contents (Fig. 7) of the cybrid shoots were higher than those of the corresponding original plant. These values were reduced under atrazine stress.

X-ray microanalysis data (Table 1) showed differences in ion contents between the cybrid and original plant on atrazine free medium or under atrazine stress. Analysis of dry shoots of plants exhibited that K ion is the dominant ion in all tested plants. The percentages of each of K , P , (Fe) or S ion to the rest of total ions in cybrid were higher than those of the original plant shoots. On the other hand , the cybrid exhibited lower values of Ca, Na, Cl, Mg, or Mn than those of the original plant shoots. It can be said

Table 1

The percentage of each of K, Ca, Na, P, Fe, Mg, S, or Mn, ion to the rest of total ions in original (*S. nigrum*) and cybrid shoots on atrazine (Atr) free medium and under atrazine stress (after 21 days in culture), as resulted from X-ray microanalysis.

Ions	Original plant (Atr-resistant)		Cybrid plant (Atr-sensitive)	
	On atrazine free medium	Under atrazine stress	On atrazine free medium	Under atrazine stress
K	70.88	56.65	73.60	68.82
Ca	12.72	12.23	9.52	9.28
Na	7.22	12.26	5.10	5.84
Cl	3.24	8.95	2.05	7.42
P	1.68	0.79	3.68	3.45
Fe	1.54	1.44	1.60	1.28
Mg	1.14	4.06	0.64	0.49
S	1.06	2.06	3.38	2.92
Mn	0.53	0.86	0.04	0.30

that the effect of atrazine on the percentages of ions was not the same in both cybrid and original plant shoots. Concerning the ion composition of the original plant shoots, atrazine treatment was found to induce some considerable changes in the percentages of studied ions. The percentages of some ions (e. g. Na , Cl , Mg , S , and Mn) were increased but others such as K , (Ca) , P , and (Fe) were decreased under atrazine stress. On the other side, after subjecting the cybrid shoots to atrazine the contents of some ions (K, Ca. P, Fe, Mg, and S) were lower, but others (Na, Cl, and Mn) were higher than those of the corresponding shoots on atrazine free medium.

Finally, it can be concluded that the X-ray microanalysis and other analysis presented in this study indicated that the physiology of the plants was affected by changing the chloroplasts after protoplast fusion. The cybrid plants need more investigations to give more information about the physiology of the cybrid plants.

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Recensio

CONERT Hans Joachim 1996 [eingelangt 11.4.1997]. ***Gramineae*/Echte Gräser oder Süßgräser.** – Lief. 8/9. – Lex. 8°, Seiten 561–736, Abb. 245–303, Tafel 36–38; brosch. – In: CONERT H.J. & al. (Eds.): Gustav Hegi, Illustrierte Flora von Mitteleuropa, Band 1, Teil 3; 3. völlig neu bearbeitete Auflage. – Parey Buchverlag im Blackwell Wissenschafts-Verlag GmbH, Berlin. – DM 98,–. ISBN 3-8263-3078-1.

In der vorliegenden Lieferung [zuletzt besprochen Lief. 6 in Phyton 33 (2): 230] wird mit den Arten 8. – 54. der größte Teil der Gattung *Festuca* behandelt. Daß diese immer wieder Schwierigkeiten bereitende Gattung nun komplett in einer neuen Bearbeitung vorliegt, in der die Forschungsergebnisse der letzten Jahre berücksichtigt sind, ist besonders erfreulich. Von ×*Festucololium* werden fünf, von ×*Festulpia*

Abb. 1. *Poa sylvicola*. Unterirdische Kriechsprosse, z.T. mit voll entwickelten Speicherinternodien; Topfkultur. Material von der Insel Pag, phot. 21.5.1974 (vgl. Phyton 20:51, 53–58).