ÚZPI

ÚSTAV ZEMĚDĚLSKÝCH A POTRAVINÁŘSKÝCH INFORMACÍ

ROSTLINNÁ VÝROBA

Plant Production

ČESKÁ AKADEMIE ZEMĚDĚLSKÝCH VĚD

6

VOLUME 42 (LXIX)
PRAHA
ČERVEN 1996
CS ISSN 0370-663X

ROSTLINNÁ VÝROBA

Mezinárodní vědecký časopis vydávaný z pověření České akademie zemědělských věd a s podporou Ministerstva zemědělství České republiky

PLANT PRODUCTION

An international journal published by the Czech Academy of Agricultural Sciences and with the promotion of the Ministry of Agriculture of the Czech Republic

Cíl a odborná náplň: Časopis publikuje původní vědecké práce, výsledky výkumu a studie z oborů rostlinná výroba, půdoznalství, meliorace a z navazujících disciplín.
Časopis je citován v bibliografickém časopise Current Contents – Agriculture, Biology and Environmental Sciences. Abstrakty z časopisu jsou zahrnuty v těchto databázích: Agricola, Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Environmental Sciences, Czech Agricultural Bibliography, Toxline Plus, WLAS.

Periodicita: Časopis vychází měsíčně (12x ročně), ročník 42 vychází v roce 1996.

Přijímání rukopisů: Rukopisy ve dvou vyhotoveních je třeba zaslat na adresu redakce: RNDr. Eva Stříbrná, vedoucí redaktorka, Ústav zemědělských a potravinářských informací, Slezská 7, 120 56 Praha 2, tel.: 02/25 25 41, fax: 02/25 70 90, e-mail: braun@uzpi.agrec.cz. Den doručení rukopisu do redakce je publikován jako datum přijetí k publikaci.

Informace o předplatném: Objednávky na předplatné jsou přijímány pouze na celý rok (leden–prosinec) a měly by být zaslány na adresu: Ústav zemědělských a potravinářských informací, vydavatelské oddělení, Slezská 7, 120 56 Praha 2. Cena předplatného pro rok 1996 je 588 Kč.

Aims and scope: The journal publishes scientific papers, results of research and studies of the branches plant production, pedology, amelioration and related disciplines.
The journal is cited in the bibliographical journal Current Contents – Agriculture, Biology and Environmental Sciences. Abstracts from the journal are comprised in the databases: Agricola, Agris, CAB Abstracts, Current Contents on Diskette – Agriculture, Biology and Environmental Sciences, Czech Agricultural Bibliography, Toxline Plus, WLAS.

Periodicity: The journal is published monthly (12 issues per year), Volume 42 appearing in 1996.

Acceptance of manuscripts: Two copies of manuscript should be addressed to: RNDr. Eva Stříbrná, editor-in-chief, Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, tel.: 02/25 25 41, fax: 02/25 70 90, e-mail: braun@uzpi.agrec.cz. The day the manuscript reaches the editor for the first time is given upon publication as the date of reception.

Subscription information: Subscription orders can be entered only by calendar year (January–December) and should be sent to: Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2. Subscription price for 1996 is 148 USD (Europe), 154 USD (overseas).

F 4883

POTENTIAL USE OF RAPD MARKERS IN VERIFICATION OF CUCUMBER HYBRIDS

MOŽNOST POUŽITÍ RAPD MARKERŮ PŘI VERIFIKACI HYBRIDŮ OKUREK

M. Truksa, S. Procházka

Mendel University of Agriculture and Forestry, Brno, Czech Republic

ABSTRACT: DNA from three new lines used for the production of hybrid cucumber seeds has been extracted and amplified by PCR with 60 different random oligonucleotides as primers and with different thermostable DNA polymerases. Amplified fragments were then analyzed on the agarose gel. Obtained RAPD markers differed depending on the DNA polymerase used and had relatively low degree of polymorphism. The possible use of RAPD markers for cucumber hybrid seeds verification is discussed.

Cucumis sativus L.; RAPD; PCR; hybrid cucumber; hybrid seed production; molecular markers

ABSTRAKT: DNA připravená ze tří nových linií používaných pro výrobu hybridů okurek byla analyzována PCR amplifikací s použitím 60 různých oligonukleotidů jako primerů a s různými termostabilními DNA polymerázami. Amplifikované produkty byly potom rozděleny agarózovou elektroforézou. Získané RAPD markery se lišily v závislosti na použité DNA polymeráze a vyznačovaly se relativně nízkým stupněm polymorfizmu. Dále je diskutována možnost využití RAPD markerů pro verifikaci hybridního charakteru okurkového osiva.

Cucumis sativus L.; RAPD; PCR; hybridní okurky; výroba hybridního osiva; molekulární markery

INTRODUCTION

The use of hybrid varieties of vegetable crops has increased dramatically during recent years (Tigchelaar, 1985; Kalloo, 1993; Tatlioglu, 1993; Chloupek, 1995). Technology of hybrid seed production, necessary to support this development, requires a fast, efficient and reliable method of quality control that would prove the hybrid character of the seeds.

The traditional methods used at present in the cucumber seeds production are based on morphological and phenological markers and have some serious drawbacks. They are time and labour consuming, blurred by environmental effects and often lack the resolving power to undoubtedly identify the individual genotypes (Wang et al., 1994). However, the most important drawback of this kind of tests is, that they usually require the whole vegetative season to complete and the produced seeds are available for the market only a year later (Prášil, personal communication).

One of the possible solutions is the use of DNA-fingerprinting methods based on Polymerase Chain Reaction (PCR) amplification. PCR based methods have, compared to more traditional Restriction Fragment Length Polymorphisms (RFLP), many methodological advantages. Firstly, only a minute amount of DNA is necessary for analysis, secondly, there is no need for radioactive labelling of probes and the whole process of setting up a PCR reaction is much simpler and amenable to automation. Method of Random Amplified Polymorphic DNA (RAPD) (Williams et al., 1990) acquired large popularity among plant geneticists and breeders especially for the ease of the method and the possibility to screen a large number of markers with relatively simple laboratory set-up (Rafalski, Tingey, 1993). RAPD can be also a suitable method for the verification of hybrids (Wang et al., 1994; Marshall et al., 1994). For this purpose, we need a set of RAPD markers represented only in the male line and present, in an unchanged form, also in the F_1 generation.

We have tested a possibility to use RAPD in characterization of cucumber genotypes and their hybrids.

MATERIAL AND METHODS

Plant material

The seeds of cucumber genotypes used in the experiment were kindly provided by the breeding company SEMO Smržice (Czech Republic) and represent new promising breeding lines, that are expected to be used

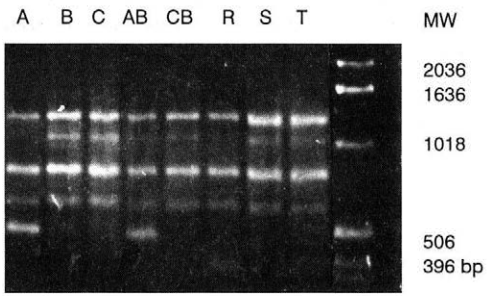
in the commercial production of cucumber hybrids. Line 6022 was for the purpose of this study labelled as A, line 6514 as B, line 6020 as C. The hybrids used in the experiment were 6022 x 6514 (labelled AB) and 6020 x 6514 (labelled CB). The germplasm collection items R (SM-5001-S1, Pi 114 339, Manchuko Wonder, Japan), S (SM-5005-S1, Pi 163 217, Khira, India), and T (SM-5018, Pi 288 238, Yomaki Plant II, Egypt) were used for comparison in case of some markers.

DNA preparation

The total genomic plant DNA was prepared from 5 g of two weeks old seedlings (i.e. 10 to 15 plantlets) using method of Dellaporta et al. (1983) published also elsewhere (Herrera, Simpson, 1988) followed by phenol-chloroform extraction. DNA prepared in this way represents a pooled sample from several individual plants suitable for screening and selection of random primers.

RAPD protocol

The PCR amplifications were carried out in the 96--well format thermal plates in total volume of 25 µl and the reaction mixture consisted of filter sterilized deionized water, 10x appropriate commercial reaction buffer (supplied with each enzyme), 3.0 µM $MgCl_2$, 200 µM of each nucleotide, 20 pmol of primer (Operon, Alameda, USA), 25 ng of genomic template DNA, and 0.2 to 1.0 unit of thermostable DNA polymerase. The reaction mixture was overlaid with two drops of light mineral oil (Sigma, USA). To optimize the RAPD protocol we tested thermostable DNA polymerases of different origin and from different suppliers. Taq DNA polymerases tested were from Promega (Madison, USA), ExBio (Prague, Czech Republic) BioVendor (Brno, Czech Republic) and Tbr DNA polymerase PrimeZyme from Biometra (Göttingen, Germany). The thermal cycler PHC-3 Techne (Cambridge, U. K.) was programmed as follows. Initial denaturation step 4 minutes at 95 °C followed by 50 cycles: denaturation 1 minute at 94 °C; annealing 1 minute at 35 °C and primer extension 2 minutes at 72 °C; followed by 10 minutes extension of unfinished strands at 72 °C after which the samples were cooled to 4 °C until recovered and analyzed on the gel. The fastest temperature transition rates (ramping 0) were used in all steps except the transition from the denaturing to annealing temperature, where ramping setting was 15 (15 °C per minute). Amplified fragments were separated on 1.5% agarose gel, stained with ethidium bromide (0.5 mg/l) and viewed on UV transilluminator.



1. Agarose gel electrophoresis of PCR products obtained by amplification of the genomic DNA from three cucumber lines A, B, C and their hybrids and genotypes R, S, T with the use of OPI-18 primer; molecular weight marker (MW) was 1-kb DNA ladder (Stratagene)

RESULTS

Optimization of RAPD protocol

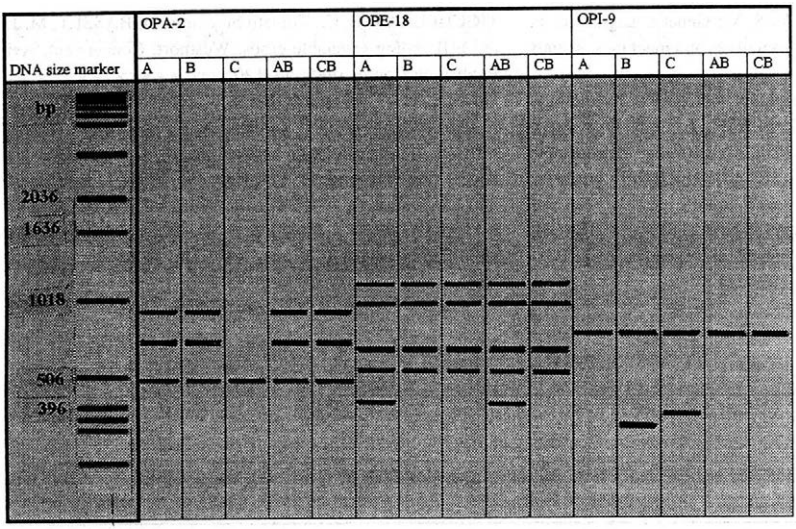
The spectrum of amplification products was changing with different DNA polymerases used (data not shown). The best results and reproducibility were achieved with Tbr polymerase PrimeZyme. Due to high efficiency of this enzyme, caused probably by the higher thermal stability, 0.4 units in 25 µl reaction were sufficient to achieve amplification comparable with 1 to 2 units of Taq polymerase. Further improvement in reproducibility was achieved by the change of ramping time during the transition from denaturation to annealing temperature as indicated.

RAPD markers

The 60 random 10-mer primers used in the experiment were from Operon kits A, E, and I. The number of amplified fragments was between 0 to 6 per primer and the size was typically between 0.2 kb and 2.5 kb. Out of 108 markers screened, 14 were polymorphic. It represents 8.6% degree of polymorphism between genotypes A/B, 7% between B/C and 11.4% between A/C. However, most of the detected polymorphic markers were not unique for certain genotype and were present also in the third genotype used in the experiment. The only unique marker detected for the line A was a 0.3 kb fragment amplified with the primer OPE-18 and not present in genotypes B and C, nor in the germplasm accessions R, S and T (Fig. 1). Examples of polymorphic markers potentially useful in typing of cucumber hybrids are presented in the diagram (Fig. 2).

DISCUSSION

The fact that different Taq polymerases give different results with RAPD is known and well documented (Schierwater, Ender, 1993; Bassam et al., 1992; Samec, 1993). It only stresses the need for proper optimization of a RAPD protocol in case of potential routine application. The Tbr polymerase PrimeZyme appears to be a suitable enzyme, not only for its



2. Diagram of RAPD markers obtained by amplification with primers OPA-2, OPE-18, and OPI-9

high efficiency and consequently economical assay, but also it has higher tolerance to contaminants, very often present in plant DNA preparations, than Taq polymerase (Truksa, unpublished). The requirement for a robust and highly reproducible assay is, unfortunately, in divergence with the need for maximum of amplified markers. The optimal balance between these two requirements is essential, especially in cucumber, where narrow genetic base (Lebeda, Křístková, 1993; Kennard et al., 1994; Tatlioglu, 1993) and relatively small genome size (Kennard, 1993) with high percentage of repetitive satellite regions (Ganal et al., 1986) are limiting factors of the usable marker number. Despite of the above-mentioned procedure with possibly reduced capability to detect variability between used genotypes, the achieved degree of polymorphism is comparable to the data published by Kennard et al. (1994) where this value is 9.3% with total number of screened markers 1076.

From the data published and discussed here we could conclude that RAPD can reveal only low level of polymorphism in cucumber and is not suitable for hybrid seed testing in its standard form. The possible solution is in an extensive screening of a large number of primers or use of less robust and more laborious assays for primary screening with later conversion of RAPD marker into more reproducible specific PCR test. This kind of assay would also make easier testing of individual plantlets following very simple DNA minipreparation.

Acknowledgement

This work was supported by Grant Agency of the Czech Republic (grant No. 506/93/0515)

REFERENCES

BASSAM, B. J. – CAETANO-ANOLLÉS, G. – GRESSHOFF, P. M.: DNA amplification fingerprinting of bacteria. Appl. Microbiol., *38*, 1992: 70–76.

CHLOUPEK, O.: Genetická diverzita, šlechtění a semenářství. Praha, Academia 1995. 71 p.

DELLAPORTA, S. L. – WOOD, J. – HICKS, J. B.: A plant DNA minipreparation: version II. Pl. Mol. Biol. Rep., *1*, 1983: 19–21.

GANAL, M. – RIEDE, I. – HEMLEBEN, V.: Organization and sequence analysis of two related satellite DNAs in cucumber (*Cucumis sativus* L.). J. Mol. Evol., *23*, 1986: 23–30.

HERRERA-ESTRELLA, L. – SIMPSON, J.: Foreign gene expression in plants. In: SHAW, C. H. (ed.): Plant molecular biology – a practical approach. Oxford, IRL Press 1988. 147 p.

KALLOO, G.: Tomato. In: KALLOO, G. – BERGH, B. O. (eds): Genetic improvement of vegetable crops. Oxford, Pergamon Press 1993: 56–112.

KENNARD, W. C.: Construction and application of a genetic linkage map towards the detection and estimation of effects of genes conditioning fruit-quality in cucumber (*Cucumis sativus* L.). Univ. Wisconsin, Madison, PhD Thesis 1993.

KENNARD, W. C. – POETTER, K. – DIJKHUIZEN, A. – MEGLIC, V. – STAUB, J. E. – HAVE, M. J.: Linkages among RFLP, RAPD, isozyme, disease resistance, and morphological markers in narrow and wide crosses of cucumber. Theor. Appl. Genet., *89*, 1994: 42–48.

LEBEDA, A. – KŘÍSTKOVÁ, E.: Genetická variabilita rodu *Cucumis* a její využití ve šlechtění. Genet. a Šlecht., *29*, 1993 (1): 59–66.

MARSHALL, P. – MARCHAND, M. C. – LISIECZKO, Z. – LANDRY, B. S.: A simple method to estimate the percentage of hybridity in canola (*Brassica napus*) F_1 hybrids. Theor. Appl. Genet., *89*, 1994: 853–858.

RAFALSKI, J. A. – TINGEY, S. V.: Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends Genet., *9*, 1993: 275–280.
SAMEC, P.: DNA polymorphism and RAPD technology. Genet. a Šlecht., *29*, 1993: 291–320.
SCHIERWATER, B. – ENDER, A.: Different thermostable DNA polymerases may amplify different RAPD products. Nucl. Acids Res., *21*, 1993: 4647–4648.
TATLIOGLU, T.: Cucumber. In: KALLOO, G. – BERGH, B. O. (eds): Genetic improvement of vegetable crops. Oxford, Pergamon Press 1993: 197–234.
TIGCHELAAR, E. C.: Tomato breeding. In: BASSET, M. J. (ed.): Breeding vegetable crops. Westport, Connecticut, Avi Publishing Comp. 1985: 54–120.
WANG, G. – CASTIGLIONE, S. – ZHANG, J. – FU, R. – MA, J. – LI, W. – SUN, Y. – SALA, F.: Hybrid rice (*Oryza sativa* L.): identification and parentage determination by RAPD fingerprinting. Pl. Cell Rep., *14*, 1994: 112–115.
WILLIAMS, J. G. – KUBELIK, A. R. – LIVAK, K. J. – RAFALSKI, J. A. – TINGEY, S. V.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., *18*, 1990: 6531–653.

Received on January 17, 1996

Contact Address:

Ing. Martin Truksa, Mendelova zemědělská a lesnická univerzita, Zemědělská 1, 613 00, Brno, Česká republika, tel.: 05/45 13 30 21, fax: 05/45 13 30 25

FORMATION OF ETHYLENE, ETHANE AND ABSCISIC ACID CONTENT IN RELATION TO DORMANCY OF SPRING BARLEY (*HORDEUM VULGARE* L.) KERNELS

PRODUKCE ETYLENU, ETANU A ABSCISOVÉ KYSELINY VE VZTAHU K DORMANCI OBILEK JARNÍHO JEČMENE (*HORDEUM VULGARE* L.)

H. Fišerová, J. Hradilík, S. Procházka, M. Klemš, A. Ráčilová

Mendel University of Agriculture and Forestry, Brno, Czech Republic

ABSTRACT: Gas hydrocarbons (ethylene and ethane) produced by kernels and abscisic acid influence the process of barley kernel germination and have effect on malting quality. Production of hydrocarbons was highest during the milk maturity in all three tested varieties and decreased gradually. Higher content of abscisic acid increases production of both hydrocarbons, but positive regression dependence was not confirmed. Variety Alexis had the highest level of abscisic acid, the highest production of ethylene and ethane and the lowest growth activity of excised embryos.

spring barley; kernels; ethylene; ethane; abscisic acid

ABSTRAKT: V práci byla sledována produkce etylenu a etanu ve vztahu k obsahu abscisové kyseliny u obilek jarního ječmene s různou hloubkou dormance v období 1,5; 3 a 4 měsíce po sklizni, v mléčné a voskové zralosti, v období sklizně, 2 a 4 týdny po sklizni. Obilky odrůdy Rubín produkují nejméně etylenu, etanu a mají nejnižší obsah abscisové kyseliny z námi sledovaných odrůd ječmene. Izolovaná embrya této odrůdy rostou v kultuře *in vitro* nejintenzivněji. Obilky odrůdy Alexis mají nejvyšší obsah abscisové kyseliny a růst izolovaných embryí v kultuře *in vitro* je nejpomalejší. Produkce uhlovodíků (etylenu a etanu) a obsah abscisové kyseliny v obilkách spolu souvisejí. Zvýšení hladiny abscisové kyseliny předchází zvýšená produkce etylenu a etanu. Průkazná závislost mezi etylenem či etanem a abscisovou kyselinou však nebyla statisticky prokázána, pouze u odrůdy Akcent se korelační koeficient blíží průkaznosti. U všech sledovaných odrůd je vztah etylenu k etanu průkazný a vysoce průkazný.

jarní ječmen; obilky; etylen; etan; kyselina abscisová

INTRODUCTION

Uneven kernel germination during the postharvest ripening of barley has a negative effect on malt quality. Many authors had paid attention to the hormonal control of barley dormancy (Balkema-Boomstra, Asperen-Gebala, 1983; Doran, Briggs, 1993), however, some questions remain unsolved. Ethylene decreases deepness of dormancy in kernels by increasing the level of gibberellins and decrease of endogenous auxin. Changes in ethylene levels were studied by Lalonde, Saini (1992).

Abscisic acid (ABA) inhibits the synthesis of α-amylase in kernels and is considered to be an antagonist of gibberellins. ABA causes endogenous dormancy of seeds, tubers and buds and accelerates dormancy of the whole plant. It also inhibits other growth processes, protein synthesis, nucleic acids synthesis and has effect on seed germination and cold resistance (Murelli et al., 1995). The link between deepness of kernel dormancy, abscisic acid and the state of embryo was described by Beckum et al. (1993) and by Wang et al. (1995).

We studied the changes in hydrocarbon production and ABA content during the period of kernel maturation and dormancy and their relationships.

MATERIAL AND METHODS

Three varieties of malt spring barley (Akcent, Alexis, and Rubín) were chosen for the experiments in the years 1993 and 1994. The kernel analyses were done in eight terms (Tab. I).

ABA radio-immuno assay

1 g of barley kernels was overlaid by liquid N_2 closed in the small glass bottle and kept at –24 °C till the processing of the sample according to the method

of Quarrie, Calfre (1985). The monoclonal antibody MAC 252 (MAC, Cambridge, UK) (+)-S-ABA and ^{3}H-ABA (Amersham) were used in the RIA analysis. Radioactivity was measured on scintillation counter Packard 2000 CA.

Ethylene and ethane analysis

3 g of barley kernels were placed on wet filter paper and closed in glass bottles. After 60 minutes' incubation the gas environment was analyzed. The bottles were then opened and placed in dark at 13 °C. After 24 hours 2 ml of distilled water were added, bottles closed again and after 60 minutes of incubation the gas environment was analyzed again on gas chromatograph by the method described by Fišerová, Hradilík (1994). Three gas samples were taken from each bottle and each combination was repeated five times. The values used in the statistical evaluation were calculated on 1 g of plant material and 1 ml of closed space.

Embryo growth

The growth activity of excised embryos was studied on the medium agar-sucrose as described by Ráčilová (1996).

RESULTS AND DISCUSSION

The average values of hydrocarbon production and level of endogenous ABA at the time of starting the experiment and after 24 hours' incubation are interpreted graphically in Figs 1, 2, and 3. We tried to find relations among production of ethylene, ethane and ABA level, the results are in the common graphs in arbitrary units of the integrator. Regressions between ethylene : ethane, ABA : ethylene and ABA : ethane are in the Fig. 4. The growth activity of excised embryos is presented in Figs 1 to 3.

Kernels produce more ethane than ethylene. The highest level of hydrocarbons is produced by the varieties Akcent and Alexis. The production of ethylene and ethane is highest during the milk maturity period (Figs 1 to 3), what is in agreement with the results of Mladý (1994), who studied the dynamics of ethylene in the ears of spring barley from the phase of stem elongation till the beginning of wax maturity. He measured the maximum during the stem elongation. The ethylene production is lowest during flowering but begins to raise again at the end of this period. This increase can be linked with the process of intensive cell division during early stages of seed development and is concomitant with various hormonal changes. Maximum of this increase is reported during the milk maturity and can be linked with the increase of auxin level reported by Procházka et al. (1982) and Dundelová (1989).

I. Terms of sample analysis in the years 1993 and 1994

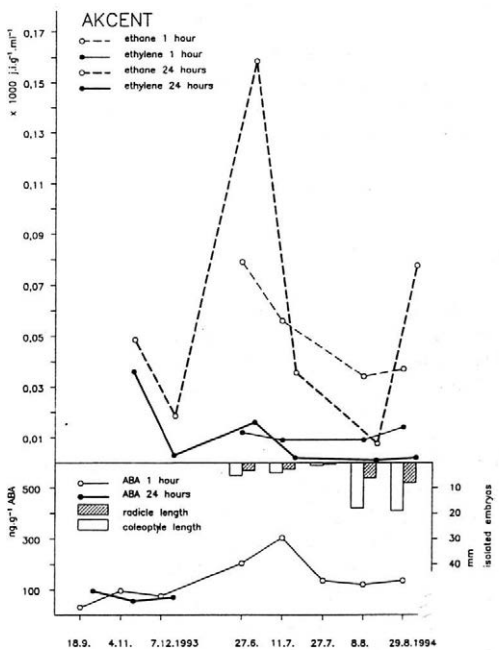
Term		
I.	18. 9. 1993	
II.	4. 11. 1993	
III.	7. 12. 1993	
IV.	27. 6. 1994	milk maturity
V.	11. 7. 1994	wax maturity
VI.	27. 7. 1994	harvest
VII.	8. 8. 1994	14 days after harvest
VIII.	29. 8. 1994	30 days after harvest

The hydrocarbon production is decreasing till the harvest. Four months after the harvest it begins to raise again and during four months following the harvest decreases gradually (Figs 1 to 3; year 1993).

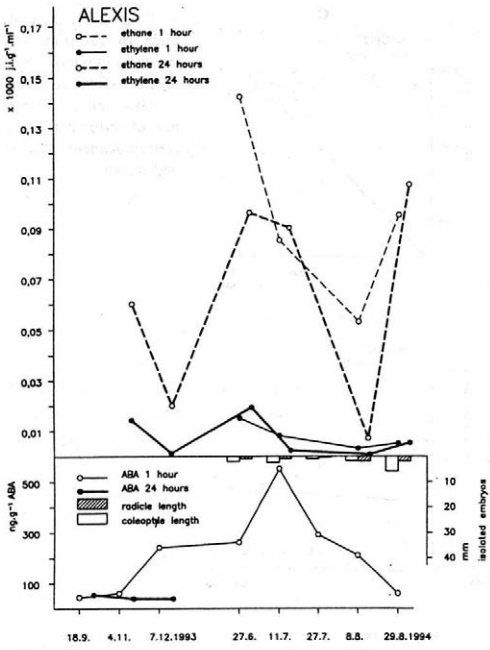
The hydrocarbon production per hour is higher in kernels after 60 minutes of soaking compared to the levels measured after 24 hours (Figs 1 to 3).

Regressions of ethylene and ethane (Fig. 4) are significant and highly significant with positive correlation coefficients.

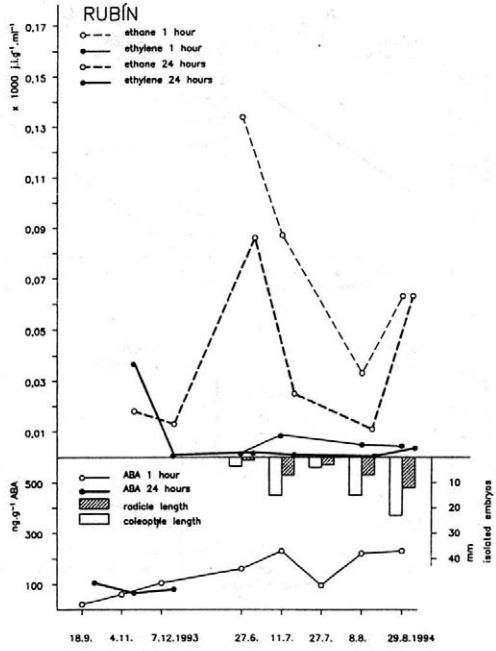
The ABA content reaches the maximum during wax maturity – the highest values are with Alexis – and in case of Alexis and Akcent decreases till the fifth term of sampling. On the contrary, in Rubín the level of ABA is raising after the harvest (Figs 1 to 3). Hudeová et



1. Production of ethylene, ethane and ABA content in kernels after 1 hour and 24 hours of soaking in sampling terms of years 1993 and 1994 and the coleoptyle and root length of excised barley embryos in Akcent variety



2. Production of ethylene, ethane and ABA content in kernels after 1 hour and 24 hours of soaking in sampling terms of years 1993 and 1994 and the coleoptyle and root length of excised barley embryos in Alexis variety



3. Production of ethylene, ethane and ABA content in kernels after 1 hour and 24 hours of soaking in sampling terms of years 1993 and 1994 and the coleoptyle and root length of excised barley embryos in Rubín variety

al. (1995) found the lowest germination rate in the same terms of sampling, which can be caused by higher level of ABA.

More obvious dependence on abscisic acid appears in case of excised embryos (Ráčilová, 1996) which is presented in Figs 1 to 3. Alexis with the highest content of endogenous ABA has the lowest growth rate of excised embryos and, on contrary, Akcent and Rubín with lower ABA content grow more.

It is obvious from the graphs that higher level of abscisic acid corresponds with higher production of ethylene and ethane. These results are in accordance with studies of Abeles (1973) who describe the function of ABA as a promotor in production of ethylene in fruits and also in leaves. Nowak, Veen (1982) observed the autocatalytic increase of ethylene production after exogenous application of ABA.

Gamborg, LaRue (1971) observed the inhibitory effect of ABA on ethylene production in suspension cell cultures. The different results have to be put into relation with used ABA concentrations, because the production of ethylene depends on the ABA concentration (Desouky et al., 1987).

Although the curves for ethylene levels and especially for ethane and ABA are obviously similar, the regression dependence is not significant and only in case of Akcent the correlation coefficient has the value 0.89 (Fig. 4).

Acknowledgements

This work was supported by Grant Agency of the Czech Republic (grant No. 501/93/0828).

REFERENCES

ABELES, F. B.: Ethylene in plant biology. New York, London, Acad. Press 1973.

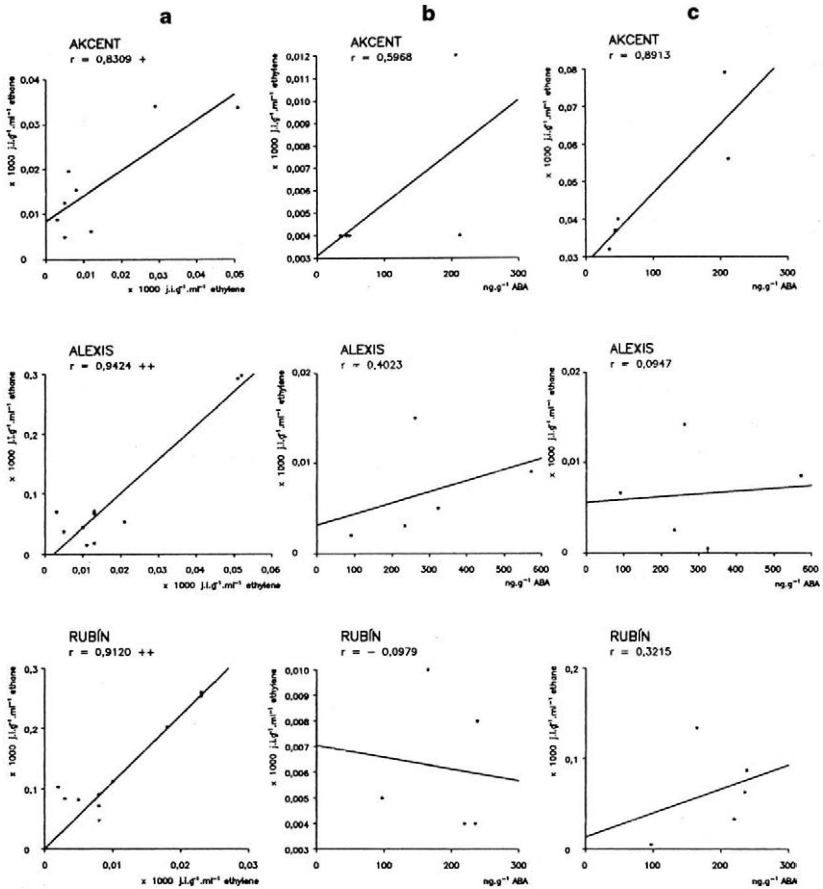
BALKEMA-BOOMSTRA, A. G. – ASPEREN-GEBALA, B. VAN: Dormancy in barley: a review of literature. Bier, 1983. 41 p.

BECKUM, J. M. M. VAN et al.: Abscisic acid and gibberellic acid-regulated responses of embryos and aleurone layers isolated from dormant and nondormant barley grains. Physiol. Plant., *89*, 1993 (3): 483–489.

DESOUKY, S. A. – HRADILÍK, J. – FIŠEROVÁ, H.: Nitrogenase activity in *Azolla pinata* as affected by different growth regulators. Acta Univ. Agric. (Brno), Fac. Agron., *35*, 1987 (3–4): 17–21.

DORAN, P. J. – BRIGGS, D. E.: The use of chemical agents to overcome dormancy in malting barley. J. Inst. Brew. (London), *99*, 1993: 85–89.

DUNDELOVÁ, M.: Aktivita fytohormonů v průběhu tvorby obilek u pšenice ozimé a ječmene jarního. [Kandidátská dizertace.] Brno, 1989. – VŠZ.



4. Regression dependence of relations between a) ethylene : ethane, b) ABA : ethylene, c) ABA : ethane in kernels of spring barley varieties Akcent, Alexis and Rubín

FIŠEROVÁ, H. – HRADILÍK, J.: Produkce etylenu a etanu při tvorbě adventivních kořenů na stonkových segmentech révy vinné. Rostl. Výr., *40*, 1994 (8): 755–762.

GAMBORG, O. L. – LA RUE, T. A. G.: Ethylene production by plant cell cultures. Pl. Physiol., *46*, 1971: 399.

HUDEOVÁ, M. – PSOTA, V. – VÍTKOVÁ, H.: Změny aktivity giberelinů a amylázy v průběhu klíčení obilek ječmene jarního. In: Sbor. VII. Dni fyziológie rastlín, Nitra, 1995.

LALONDE, S. – SAINI, H. S.: Comparative requirement for endogenous ethylene during seed germination. Ann. Bot., *69*, 1992 (5): 423–428.

MLADÝ, I.: Endogénny etylén v ontogenéze klasu vybraných obilnín a jeho vzťah k produkčnému procesu. [Kandidátska dizertácia.] Nitra, 1994: 1–100. – VŠP.

MURELLI, C. et al.: Metabolic changes associated with cold-acclimation in contrasting cultivars of barley. Physiol. Plant., *94*, 1995 (1): 87–93.

NOWAK, J. – VEEN, H.: Effect of silver thiosulphate on abscisic acid content in cut carnations as related to flower senescence. J. Pl. Growth Regul., *1*, 1982: 153–159.

PROCHÁZKA, S. – BLAŽKOVÁ, J. – DUNDELOVÁ, M.: Aktivita cytokininů v období tvorby obilek u ječmene jarního (*Hordeum vulgare* L.). Rostl. Výr., *28*, 1982 (4): 439–443.

QUARRIE, S. A. – GALFRE, G.: RIA protocol. Anal. Biochem., *151*, 1985: 389–399.

RÁČILOVÁ, A: Studium dormance embryí jarního ječmene v podmínkách *in vitro*. [Diplomová práce.] Brno, 1996. – MZLU.

WANG, M. et al.: Modulation of germination of embryos isolated from dormant and nondormant barley grains by manipulation of endogenous abscisic acid. Planta, *195*, 1995 (4): 586–592.

Received on January 17, 1996

Contact Address:

Ing. Helena Fišerová, Mendelova zemědělská a lesnická univerzita, Zemědělská 1, 613 00 Brno, Česká republika, tel.: 05/45 13 30 15, fax: 05/45 21 11 28

SOME FACTORS AFFECTING ANTHER CULTURE IN *LINUM USITATISSIMUM* L.

NĚKTERÉ FAKTORY OVLIVŇUJÍCÍ PRAŠNÍKOVOU KULTURU *LINUM USITATISSIMUM* L.

E. Tejklová

AGRITEC, Research, Breeding and Services, Ltd., Šumperk, Czech Republic

ABSTRACT: Influence of genotype, culture conditions of donor plants and culture conditions of isolated anthers on callogenesis in anthers and organogenesis in anther calli as well were studied in *Linum usitatissimum* L. There were differences in anther callogenesis among genotypes. Donor plant culture conditions, especially temperature, influence on callogenesis in anthers. 2,4-D, NAA and picloram induced anther callogenesis in higher frequencies than benzolinon, dicamba, IAA, IBA or TIBA. Callogenesis was higher in dark than in 16-h' day. Cold pretreatment of isolated anthers influenced negatively anther callogenesis. Transfer of calli from induction medium with lower level of some nutrient elements, especially nitrogen, and with higher content of NAA and BAP onto medium with higher level of nutrient elements without auxin and with lower cytokinin level induced bud development in anther calli. Method of production of dihaploid plants in *Linum usitatissimum* L. through the anther culture was proposed.

anther culture; flax; linseed; *Linum usitatissimum* L; temperature influence; growth regulators

ABSTRAKT: Byl studován vliv genotypu, kultivačních podmínek donorových rostlin a kultivačních podmínek izolovaných prašníků lnu (*Linum usitatissimum* L.) na kalogenezi v prašnících a organogenezi v kalusech získaných z prašníků. Byly prokázány rozdíly v kalogenezi v prašnících mezi jednotlivými genotypy lnu přadného i olejného a jejich hybridy. Z testovaných genotypů vykazoval nejvyšší kalogenezi Viking. Kalogeneze v prašnících je silně ovlivněna podmínkami, při kterých se vyvíjejí prašníky na rostlině. Responzivitu prašníků ovlivňuje pozitivně nižší teplota, při které jsou pěstovány donorové rostliny v posledních cca 4 dnech před izolací prašníků (pod 10 °C v noci a pod 20 °C ve dne). Velmi významný vliv na kalogenezi v prašnících měl také obsah růstových regulátorů v iniciačním médiu. 2,4-D, NAA a picloram v koncentraci 1 $mg.l^{-1}$ a v kombinaci s 2 $mg.l^{-1}$ BAP indukovaly kalogenezi ve vyšší frekvenci než benzolinon, dicamba, IAA, IBA nebo TIBA. Kalogeneze byla vyšší při kultivaci prašníků ve tmě než při 16h fotoperiodě. Chladové předpůsobení na prašníky (1 až 5 dnů při 4 °C ve tmě po izolaci na indukční média) snižovalo kalogenezi v prašnících. Frekvence kalogeneze byla vyšší u prašníků položených na povrch agarového média ve srovnání s prašníky vnořenými do stejného média. Snížení nebo vynechání auxinu a snížení obsahu cytokininu v následném médiu (regeneračním) vyvolá v kalusech tvorbu pupenů. Frekvence organogeneze může být také ovlivněna změnou obsahu některých makro- a mikroelementů v regeneračním médiu. Indukce pupenů byla vyšší při přenesení kalusů z média se sníženým obsahem N na médium bohatší na N, B, Mn a Zn ve srovnání s variantou, kde zůstával obsah makro- a mikroelementů v iniciačním i regeneračním médiu nezměněný. Regenerované pupeny mohou být udržovány na médiu pro dlouhodobou kulturu shluků pupenů (Tejklová, 1992). Prýty zakořeňovaly na MS médiu s 0,019 $mg.l^{-1}$ NAA. Regenerované rostliny byly diploidní. Byla navržena metodika pro produkci dihaploidních linií lnu prašníkovou kulturou. Jako iniciační médium pro indukci kalogeneze v prašnících je doporučeno médium AA22 (A22 sterilované autoklávováním), jako regenerační BI médium (tab. I), neboť tato média byla otestována na více genotypech olejného i přadného lnu a při dodržení zásad pro předpěstování donorových rostlin (teploty pod 10 °C v noci a pod 20 °C ve dne) dávala dobré výsledky.

prašníková kultura; len přadný; len olejný; *Linum usitatissimum* L; vliv kultivační teploty; růstové regulátory

INTRODUCTION

The production of haploid or dihaploid plants from immature pollen grains through microspore or anther cultures allows a rapid development of homozygous lines for breeding of new crop cultivars. Several papers are concerned with this problem in flax (Sun, Fu, 1981; Nichterlein et al., 1989, 1991; Poliakov, 1991; Nichterlein, Friedt, 1993; Tejklová, 1992b, 1994; Poliakov et al., 1994; Rutkowska-Krause et al., 1995). Complete haploid or dihaploid plants have been obtained through the microspore callogenesis in anthers, organogenesis in calli and rhizogenesis in shoots. The efficiency of this

method is, however, low and it cannot be included into flax breeding systems yet. Many unknown factors influence a process of plant regeneration in such system and this paper is trying to find out some of them. Effect of genotype, culture conditions of donor plants, cold pretreatment of anthers, basal medium, sucrose and growth regulators content in culture medium and donor plant fertilization on callogenesis in anthers and organogenesis in anther calli were studied as well.

MATERIAL AND METHODS

We started with the anther culture methods of Sun, Fu (1981), Nichterlein et al. (1989) and Nichterlein et al. (1991). Anthers with late uninuclear microspores (flower buds 3.5–4.5 mm in length) were treated in experiments (Fig. 9a, b). Sterilization of buds was as follows: rinsing in 70% ethanol, 25 min in 7% calcium chloride (Chlorové vápno, Zach, Plzeň), three times rinsing in sterile water. Culture media were autoclaved for 15 min at 121 °C. Anthers were cultured in Petri dishes (10 cm in diameter, 20 ml of medium in each, max. 50 anthers per Petri dish) in culture room in 16-h' photoperiod, 25–29/20–25 °C, day/night and callogenesis in them was evaluated after 4-weeks' culture on induction media, unless indicated otherwise. Calli were transferred on regeneration media and shoots on rooting ones (5 pieces in 100 ml Erlenmeyer's flasks with 25 ml of medium). Composition of culture media used in experiments are described in Tab. I.

Experiment 1

Flax, linseed and their hybrids were used in experiments (see Results). Donor plants were grown in the greenhouse (anthers were harvested on 13–28 May), in the vegetative hall (21 June–15 July) or in the field (9–16 July 1991). Anthers were cultured on media MS 5/1, A (medium for good green callus induction in hypocotyl segments followed by shoot bud regeneration in calli according to Mathews, Narayanaswamy, 1976), SF, N and N6. Anthers were cultured either in culture room (28/23 °C, 16/8 h, day/night, fluorescent light 14 days 1000 lx, next 14 days 4000 lx) or in laboratory (24/18 °C, day light).

Experiment 2

Donor plants of flax, linseed and their hybrids (some of genotypes tested in Exp. 1, and several new genotypes, see Results) were grown in greenhouse and in the field. Anthers were harvested at three intervals: 18–31 May (greenhouse), 28 June–9 July (field), 16–24 July 1992 (the same plants in the field). Anthers were cultured on media A3, A22, SI or P.

Experiment 3

Donor plants of linseed Areco were cultivated in the field, anthers were harvested on 22–28 July 1993 and cultured on media A22 (filter sterilization), AA22 (A22 autoclaved medium), B and C, 100 anthers per variant. Evaluation after 5-weeks' culture. Calli were transferred on medium BI.

Experiment 4

Anthers from Areco plants grown in the field were cultured on media AA22 and C with 6 or 10% sucrose (AA22/60, AA22/100, C/60, C/100). Calli were collected during 4-weeks' anther culture and transferred on BI medium. Bud regeneration was evaluated after 4-weeks' culture on BI medium.

Experiment 5

Donor plants of flax, linseed and their hybrids (as in Exp. 2) were grown in the field. Anthers were cultured on AA22, B and C media (see Exp. 3). We used the week mean values of temperature.

Experiment 6

Areco plants were grown in pots in greenhouse and at the beginning of flowering period were transferred into culture box with 16-h' photoperiod, 14/8 °C, day/night for 1–6-days' intervals. Then pots were transferred to the greenhouse again. Anthers were isolated every day since the 2 days before the 1st pot was transferred into growth chamber to the 4th day after transferring into greenhouse, and cultured on media AA22/60, AA22/100, C/60, C/100 (as in Exp. 4).

Experiment 7

Areco plants were grown in greenhouse (higher temperature, see Results) and at the same time in vegetative hall (lower temperature). On 2 May plants from greenhouse were transferred out in the open area. The temperature was recorded. Isolated anthers were cultured on the same media as in Exp. 6.

Experiment 8

Areco plants were grown in vegetative hall, anthers were cultured in three places: culture room (16-h' photoperiod, 29–30/25–26 °C, day/night), thermostat I (dark, 21 °C), thermostat II (dark, 30 °C) on the same media as in Exp. 6.

Experiment 9

Anthers collected from Areco plants in greenhouse or vegetative hall were isolated on media as in Exp. 4

I. Composition of culture media used in experiments

Components	MS 5/1	A	SF (Sun, Fu, 1981, mod.)	N	N6	A3 (Nichterlein et al., 1989, mod.)	A22 (Nichterlein et al., 1991, mod.)	SI	P (Poliakov et al., 1994, personal communication)	B	C	Bl (P20 medium Nichterlein et al., 1991, mod.)
Macroelements	MS (Murashige, Skoog, 1962)	1/2 MS	B5 (Gamborg et al., 1968)	MS	N6 (Chu et al., 1978)	MS but 165 mg.l^{-1} NH_4NO_3	MS but 165 mg.l^{-1} NH_4NO_3	MS	MS	MS but 165 mg.l^{-1} NH_4NO_3	MS but 165 mg.l^{-1} NH_4NO_3	N6
Microelements	MS	1/2 MS	B5	MS	N6	MS	MS	MS	B5 (Gamborg et al., 1968)	MS	MS	N6
Vitamins	MS	MS	B5	MS	MS but 1 mg.l^{-1} of thiamine	MS but 1 mg.l^{-1} of thiamine	MS but 1 mg.l^{-1} of thiamine	MS	B5	MS but 1 mg.l^{-1} of thiamine	MS but 1 mg.l^{-1} of thiamine	MS but 1 mg.l^{-1} of thiamine
Yeast extract								300 mg.l^{-1}				
m-Inositol	100 mg.l^{-1}	100 mg.l^{-1}	100 mg.l^{-1}	100 mg.l^{-1}		100 mg.l^{-1}	100 mg.l^{-1}		100 mg.l^{-1}	100 mg.l^{-1}	100 mg.l^{-1}	1 001 mg.l^{-1}
Glycine	2 mg.l^{-1}	2 mg.l^{-1}	2 mg.l^{-1}	2 mg.l^{-1}	10 mg.l^{-1}							2 mg.l^{-1}
Glutamine						750 mg.l^{-1}	750 mg.l^{-1}			750 mg.l^{-1}	750 mg.l^{-1}	375 mg.l^{-1}
NAA			0.1 mg.l^{-1}	2 mg.l^{-1}		2 mg.l^{-1}	1 mg.l^{-1}	1 mg.l^{-1}	0.05 mg.l^{-1}	10 mg.l^{-1}		
2,4-D	1.105 mg.l^{-1}										1 mg.l^{-1}	
IBA		2 g.l^{-1}										
BAP				1 mg.l^{-1}		1 mg.l^{-1}	2 mg.l^{-1}	1 mg.l^{-1}	1 mg.l^{-1}	20 mg.l^{-1}	2 mg.l^{-1}	1 mg.l^{-1}
Kinetin	0.215 mg.l^{-1}	1 g.l^{-1}	1 mg.l^{-1}									
Sucrose	30 g.l^{-1}	30 g.l^{-1}	20 g.l^{-1}	60 g.l^{-1}	80 g.l^{-1}	60 g.l^{-1}	60 g.l^{-1}	60 g.l^{-1}	50 g.l^{-1}	60 g.l^{-1}	60 g.l^{-1}	30 g.l^{-1}
Agar	8 g.l^{-1}	8 g.l^{-1}	8 g.l^{-1}	8 g.l^{-1}	8 g.l^{-1}	8 g.l^{-1}	8 g.l^{-1}	8 g.l^{-1}	8 g.l^{-1}	8 g.l^{-1}	8 g.l^{-1}	8 g.l^{-1}
pH	5.5	5.8	5.5	5.5	5.8	5.8	5.8	5.5	5.5	5.8	5.8	5.5

and placed into refrigerator for 1, 3 or 5 days at 4 °C. Then Petri dishes with anthers were transferred into culture room at 30/25 °C, 16/8 h, day/night.

Experiment 10

To find the most convenient auxin for callus induction in flax anthers eight growth regulators in induction medium were tested. Basal medium AA22 with 2 $mg.l^{-1}$ BAP was supplemented with 1 $mg.l^{-1}$ of 2,4-D, benzolinon (BEN), dicamba (DIC), IAA, IBA, NAA, picloram (PIC) or TIBA. Anthers from Areco (linseed) and Texa (flax) plants, grown in greenhouse, were isolated during October and November. Callogenesis was evaluated after 8-weeks' culture.

Experiment 11

Areco, Texa and Su 1527 flax line donor plants were grown in the field, anthers were collected at the beginning of July, Areco and Texa twice (Areco I, Areco II, Texa I, Texa II). Anthers were put on surface of culture medium or they were dipped into medium. Anther callogenesis was evaluated after 8-weeks' culture.

Experiment 12

Areco and Texa were grown in vegetative hall in pots. Plants were fertilized three times in 7-days' intervals with Floran (full combined fertilizer, 0.5 g per pot) or NH_4NO_3 (0.2 g per pot). Control plants were only watered. Temperature in vegetative hall was recorded. To determine nutrient elements or growth regulators influence on regeneration, callogenesis and bud regeneration were induced on following sequences of media: AA 22 ➡ BI, A 22 with growth regulators P ➡ BI, P ➡ P and P with growth regulators A 22 ➡ BI as well.

Callogenesis in anthers was evaluated after 8-weeks' culture. Organogenesis in anther calli was evaluated during four subcultures on the same organogenic medium (BI or P, resp.). Mean temperature was calculated as an arithmetic mean of minimal and maximal day temperatures of the last five days before anther isolation.

RESULTS AND DISCUSSION

Influence of genotype

Tab. II shows an anther callogenesis in different genotypes on media MS 5/1, A, 2, N and N6 (Exp. 1). Some differences were observed in callus induction among genotypes, flax and linseed types or their hybrids. Viking seems to be the most responsive genotype, Duferin did not produce callus in anthers on any culture medium. The frequencies of callogenesis in different types (flax, linseed, hybrids) were similar. Exp. 2 gave similar results (Tab. III). Callogenesis in Viking was the highest, Duferin showed the lowest frequency of anther callogenesis. Frequencies of the anther callogenesis in Exp. 2 were higher in comparison with those in Exp. 1.

Results of Exp. 11 confirmed differences in callus induction capability between Areco and Texa on media with different auxins (Tab. X). Areco reached higher

II. Influence of genotype on callogenesis in anthers of flax, linseed and hybrids flax x linseed (Exp. 1)

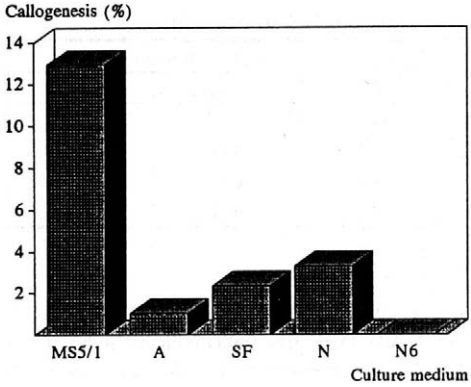
Genotype	No. anthers isolated	Callogenesis (%)
Antares^{L}	900	3.4
Areco^{L}	350	2.0
Ariane^{L}	650	5.9
Belinka^{F}	400	1.3
Duferin^{L}	300	0.0
Laura^{F}	850	7.7
Linda^{F}	800	2.6
Marine^{F}	950	5.1
SL 1585^{F}	800	2.4
SU 161/83/16^{H}	250	3.6
SU 284/82^{H}	400	2.0
SU 45/85^{F}	700	5.1
SU 670/80^{F}	400	1.5
Te 93/13^{F}	850	3.8
Texa^{F}	750	2.0
Viking^{F}	750	10.0
Walsh^{L}	350	3.7
Flax	7 250	4.4
Linseed	2 550	3.5
Hybrids	650	2.6

L – linseed, F – flax, H – hybrid L x F

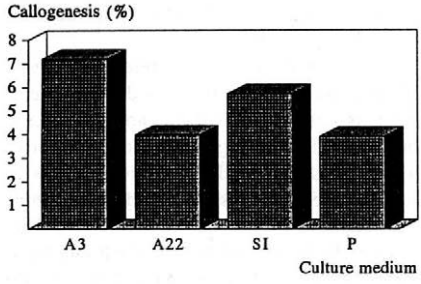
III. Influence of genotype on callogenesis in anthers (Exp. 2)

Genotype	No. anthers isolated	Callogenesis (%)
Areco^{L}	1 585	5.43
Duferin^{L}	1 778	1.80
Laura^{F}	1 547	4.33
Linda^{F}	1 667	4.56
Redvood x V12^{H}	2 242	5.98
SU 45/85^{F}	1 950	8.41
Texa^{F}	1 845	1.46
Tverca x (Red. x Tv.)^{H}	1 982	5.55
(Tv. x 1015) x V12^{H}	1 886	6.15
Viking^{F}	1 590	11.51
Flax	8 599	6.01
Linseed	3 363	3.51
Hybrids	6 110	5.89

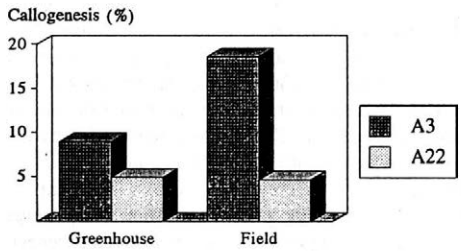
L – linseed, F – flax, H – hybrid L x F



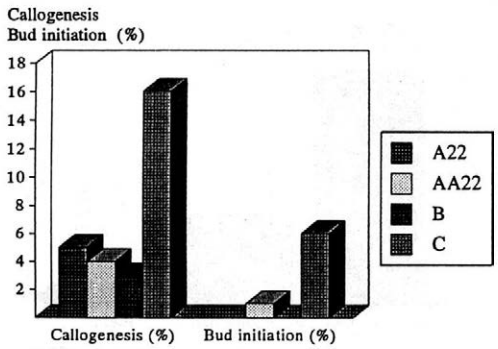
1. Influence of culture medium on callogenesis in anthers (Exp. 1)



2. Influence of culture medium on anther callogenesis (Exp. 2)



3. Callogenesis in anhers in connection to anther culture medium and place of donor plant cultivation (Exp. 2)



4. Callogenesis and bud initiation per anther isolated on media differing in way of sterilization and auxin content (Exp. 3)

frequencies of callogenesis on all media except medium with IBA.

Influence of anther and callus culture media

Differences in anther callogenesis on media used in Exp. 1 shows Fig. 1. The highest callogenesis was observed on medium MS with 1.105 mg.l^{-1} 2,4-D and 0.215 mg.l^{-1} kinetin. The callogenesis on this medium was even higher than on medium N (modified medium A3 according to Nichterlein et al., 1989). There was no callogenesis on medium N6 (medium for anther culture of cereal crops, Chu, 1978).

Differences in anther callogenesis on next four culture media (Exp. 2) are described in Fig. 2. Media A3 and A22 are different only in growth regulators contents. A3 contains 2 mg.l^{-1} NAA and 1 mg.l^{-1} BAP, medium A22 on the contrary, 1 mg.l^{-1} NAA and 2 mg.l^{-1} BAP. When we compare callogenesis only in anthers from plants grown in greenhouse and in anthers harvested from plants in the field (the 1st collection) differences between these two media are seen clearly (Fig. 3).

Medium A22 is filter sterilized (Nichterlein et al., 1991). Results of Exp. 3 demonstrate that there are no differences in anther callogenesis between filter sterilized and autoclaved media. Higher frequency was obtained on medium C with 2,4-D (Fig. 4). This finding corresponds with the results in Exp. 1 (Fig. 1), where the highest callogenesis was obtained on medium with 2,4-D, too, although other factors could be effective there. Bud regeneration was obtained on N6 modified (BI) medium in calli from AA22 and C media.

IV. Callogenesis in anthers harvested from plants grown in different conditions (Exp. 2)

Donor plant cultivation place	No. anthers isolated	Callogenesis (%)
Greenhouse	7 077	5.8
Field I	5 524	9.7
Field II	5 471	0.8

Field I – 1st isolation of anthers
Field II – 2nd isolation of anthers

Exp. 4 did not show any significant differences in anther callogenesis between media with 6 or 10% sucrose in basal AA22 medium. There were no differences in bud regeneration from calli developed either on 6 or 10% sucrose media, too (Tab. V). The highest callogenesis was recorded on media with 2,4-D and 10% sucrose (Tab. V). Exp. 4 and 9 showed interaction between anther callogenesis and 2,4-D and sucrose content in induction medium. Bud regeneration was higher in calli from media with 2,4-D (Tab. V). These results were fully confirmed in experiment next year when anthers were cultured in three light and temperature conditions (see Exp. 8).

V. Influence of auxins and sucrose on callogenesis and bud development in anther culture of linseed Areco (Exp. 4)

Medium	Auxin (1 mg.l^{-1})	Sucrose (g.l^{-1})	No. isolated anthers	Callogenesis (%)	Bud development in calli (%)	
					per anther	per callus
AA22/60	NAA	60	1 545	11.8	0.13	1.10
AA22/100	NAA	100	1 545	11.7	0.13	1.11
C60	2,4-D	60	1 545	8.8	0.26	2.94
C100	2,4-D	100	1 545	14.2	0.32	2.28

Replacement of NAA in AA22 induction medium by other auxins or auxin-like substances (Exp. 10) gave the following results: The lowest callogenesis was recorded on medium with IAA or TIBA, the highest one on 2,4-D, NAA or PIC (Tab. X). The frequencies of callogenesis are relatively low because anthers from old donor plants were included.

High content of growth regulators can lead to low organogenesis in callus. Callogenesis on medium AA22 and organogenesis in calli on BI medium were compared with callogenesis and organogenesis on medium Poliakov (1994). The results of Exp. 12 suggest that the highest callogenesis was on media with higher NAA and BAP contents (Tab. XI, systems 1 and 4). Bud regeneration in calli was the highest in system 2 where bud-induction medium contained higher levels of nitrogen, boron, manganese, zinc and vitamins and lower glutamine and sucrose levels in comparison with callus-induction medium. The lowest callogenesis and bud regeneration were in system 3 in which anthers as well as induced calli were maintained on the same medium.

Influence of donor plant growing conditions

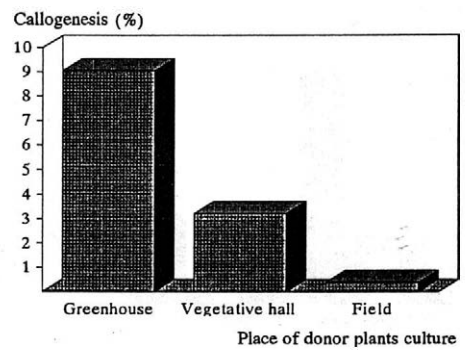
Anthers from plants from various culture places differed in frequencies of callogenesis (Fig. 5). The best results were obtained in anthers from plants grown in greenhouse. Very low callogenesis was recorded in anthers from the field (Exp. 1). Results from the next experiment (Exp. 2) were a bit different (Tab. IV). The highest callogenesis was obtained in anthers harvested at the beginning of July. Anthers collected two weeks later from the same plants were much less responsive.

We can see different reactions of anthers from donor plants cultured in different culture places in two vegetative periods. In Exp. 1, the highest callogenesis was obtained in anthers from donor plants cultured in greenhouse at low spring temperature. In July the temperature reached values about 30 °C in the field and callogenesis was low. In the next year (Exp. 2), there was a warm spring (time of donor plant cultivation and anther harvesting in greenhouse), a cold end of June and a beginning of July (the 1st anther harvesting from plants cultured in the field) and a hot second half of July (the time of the 2nd collecting of anthers in the field).

Exp. 5 demonstrates certain relation between temperature in which anthers are developing on donor plants and callogenesis in isolated anthers in successive days (Tab. VI). Dark grey fields indicate higher donor plants cultivation temperatures in the last days before anther isolation and very low or zero callogenesis in isolated anthers. Light grey fields indicate, by analogy, lower donor plants cultivation temperatures in the last week before anther isolation and higher frequencies of callogenesis in isolated anthers.

Nichterlein et al. (1991) recommended to grow donor plants in a 16-h' day at 14/8 °C, day/night temperature but it is not known if the donor plants need this temperature regime through the whole vegetative period. Possibilities of breeders to grow plants in growth chambers are limited, so we tried to determine the necessary length of 14/8 °C period before the anther isolation. Exp. 6, however, did not carry out any regularity because callogenesis was not induced in any variant. Results of previous experiment suggest that lowering of donor plants cultivation temperature during the week before anther isolation increases callogenesis in isolated anthers. However, 2–6-days' cultivation of donor plants in 14/8 °C, day/night temperature regime in growth chamber did not increase callogenesis in anthers. There were probably some additional factors influencing callogenesis (low light intensity in growth chamber, physiological state of plants, etc.).

The light and dark grey fields in Tab. VII (Exp. 7) suggest that low donor plant cultivation temperature induces and higher temperature suppresses callogenesis in anthers isolated in about the 4th day after temperature effect. The experiment was in progress in both



5. Influence of donor plant cultivation place on callogenesis in anthers (Exp. 1)

VI. Influence of cultivation temperature of donor plants on callogenesis in anthers (Exp. 5)

Week		Mean day temperature (°C)	Max. day temperature (°C)	Min. night temperature (°C)	Morning ground--level temperature (°C)	Anthers isolation	Callogenesis induction
from	to						
28. 6.	4. 7.	17.0	31.0	8.5	7.1		
5. 7.	11. 7.	16.8	28.0	9.7	6.6		
12. 7.	18. 7.	13.4	22.3	6.1	4.5	I	–
18. 7.	25. 7.	17.0	27.0	10.7	8.4	I	C
26. 7.	1. 8.	18.5	30.0	9.2	7.2	I	C
2. 8.	8. 8.	19.7	33.0	7.5	7.0	I	(C)
9. 8.	15. 8.	19.1	32.1	7.9	7.8	I	–
16. 8.	22. 8.	19.0	32.5	6.7	1.1		
23. 8.	29. 8.	13.5	23.1	5.6	5.2	I	C
30. 8.	5. 8.	11.1	20.0	3.6	3.1	I	C

I – isolation of anthers, – no callogenesis, C – callogenesis, (C) – weak callogenesis

places at the same time, in the same light conditions. When we compare anther callogenesis in the mid-April from both places and in the period from the 9th to the 13th May we can see the differences which can be explained as a reaction to temperature, not to the length of light period or the light intensity.

Fig. 8 shows again certain tendency of higher callogenesis in anthers isolated from plants cultivated in low temperature in the last 5 days before anther isolation. Minimal day temperature above 15 °C and maximal day temperature above 30 °C stopped fully callogenesis in anthers. Extremely low temperatures (about 5 °C) supported, probably, callus induction (data not shown).

Experiment 12 did not give an evidence about influence of fertilization of donor plants on callogenesis in isolated anthers.

Influence of anther culture conditions

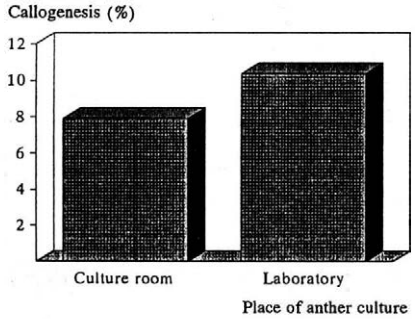
Differences between callogenesis of anthers cultured in culture room and in laboratory were observed (Exp. 1, Fig. 6). Day light supported, probably, callogenesis in anthers. Anther culture in different light and temperature conditions (Exp. 8) shows the following results: Callogenesis in the dark was higher, on the average, than in the 16-h' photoperiod and it was higher on the average at 21 °C than at 30 °C, as well (Tab. VIII).

The cold pretreatment of isolated anthers inhibited anther callogenesis as Exp. 9 demonstrates (Tab. IX). Storage of flower buds for 2–8 days at 4 °C before anther isolation (data not shown) did not increase anther callogenesis in linseed or flax.

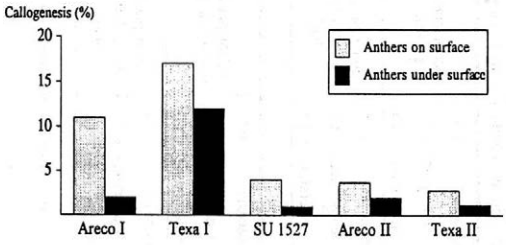
Position of explants in the medium can influence gas exchange or medium component uptake and it can influence callogenesis or regeneration in explants. Anthers immersed into agar culture medium (Exp. 11) showed lower callus production than anthers placed on medium surface (Fig. 7).

Calli developed in anthers originate probably in single microspores as it is possible to see under the microscope (Fig. 9c). Sometimes multiple callogenesis in anther was recorded (Fig. 9d). Callogenesis does not often start at the same time in all anthers or callus development is not of the same intensity. Fig. 9e documents anther culture after 4 weeks on A 22 medium. There are calli of different size.

Bud regeneration was obtained on medium BI or on medium Poliakov (1994). Calli regenerating were compact yellowish or light or dark green (Fig. 9f). Bud



6. Influence of culture place on callogenesis in anthers (Exp. 1)



7. Influence of anther position in culture medium on callogenesis (Exp. 11)

VII. Callogenesis in anthers from donor plants cultivated in two places with different temperature (Exp. 7)

Callogenesis (%)	donor plants in greenhouse																							0.8
Max. temperature (°C)																20	22	21	24	31	26	25	28	25
Min. temperature (°C)																6	16	16	20	19	18	17	16	18
Callogenesis (%)	donor plants in vegetative hall								12.1		20.0				12.8			18.5				7.0		21.0
Max. temperature (°C)		14	24	23	24	15	18	17	22	16	19	21	21	11	21	12	13	20	20	22	22	21	15	18
Min. temperature (°C)		9	10	10	11	10	10	7	9	9	9	9	9	8	8	7	8	8	8	11	10	9	8	9
Date		29. 3.	30. 3.	31. 3.	1. 4.	2. 4.	3. 4.	4. 4.	5. 4.	6. 4.	7. 4.	8. 4.	9. 4.	10. 4.	11. 4.	12. 4.	13. 4.	14. 4.	15. 4.	16. 4.	17. 4.	18. 4.	19. 4.	20. 4.
Callogenesis (%)	donor plants in greenhouse	0.6					1.0		4.7				6.0		5.0					34.3		14.0	38.0	
Max. temperature (°C)		15	29	30	29	30	31	31	31	32	29	16	27	28	29	20	21	31	32	33	19	30	29	30
Min. temperature (°C)		15	14	16	16	13	14	15	13	10	17	12	7	7	13	10	8	7	8	12	10	12	10	10
Callogenesis (%)	donor plants in vegetative hall	34.2							19.5							24.0	12.5	15.0	8.7	0.0	7.3	0.0	0.0	0.0
Max. temperature (°C)		21	30	25	25	26	29	29	30	31	32	24	21	26	25	27	31	32	29	33	23	32	30	32
Min. temperature (°C)		10	11	10	10	10	13	13	8	10	13	9	2	1	10	12	12	12	12	17	14	16	15	14
Date		21. 4.	22. 4.	23. 4.	24. 4.	25. 4.	26. 4.	27. 4.	28. 4.	29. 4.	30. 4.	1. 5.	2. 5.	3. 5.	4. 5.	5. 5.	6. 5.	7. 5.	8. 5.	9. 5.	10. 5.	11. 5.	12. 5.	13. 5.

VIII. Callogenesis in anthers cultured in the light or in the dark (Exp. 8)

Date of anther isolation	Anther culture conditions			No. anthers cultured	Callogenesis (%)
	place	light	temperature (°C)		
7. 4.	culture room	16 h	30/25	140.0	20.0
7. 4.	thermostat I	dark	21	280.0	16.4
11. 4.	culture room	16 h	30/25	320.0	12.8
11. 4.	thermostat I	dark	21	280.0	15.0
14. 4.	culture room	16 h	30/25	135.0	18.5
14. 4.	thermostat I	dark	21	270.0	30.4
18. 4.	culture room	16 h	29/25	550.0	0.7
18. 4.	thermostat I	dark	21	400.0	1.0
26. 4.	culture room	16 h	29/26	200.0	0.0
26. 4.	thermostat II	dark	30	400.0	0.0
28. 4.	culture room	16 h	30/26	200.0	19.5
28. 4.	thermostat II	dark	30	200.0	31.0
4. 5.	culture room	16 h	30/26	100.0	6.0
4. 5.	thermostat II	dark	30	100.0	12.0
Total culture room	culture room	16 h	29–30/25–26	1 645.0	8.7
Total thermostat	thermostat	dark	21 or 30	1 930.0	12.8
Total thermostat 21 °C	thermostat I	dark	21.0	1 230.0	14.1
Total thermostat 30 °C	thermostat II	dark	30.0	700.0	10.6

IX. Influence of pretreatment on anther callogenesis (Exp. 9)

Pretreatment on anthers			Callogenesis in anthers (%) on media			
Place	temperature (°C)	period	AA22/60	AA22/100	C/60	C/100
Without pretreatment			13	15	11	18
Refrigerator	4	1 day	0	0	0	0
Refrigerator	4	3 days	0	0	0	0
Refrigerator	4	5 days	0	0	0	0

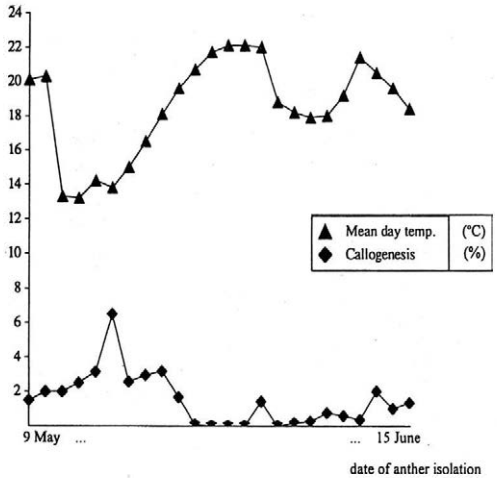
X. Callogenesis in anthers cultured on media with different auxins (Exp. 10)

Genotype	No. anthers isolated								Callogenesis (%)							
	2,4-D	BEN	DIC	IAA	IBA	NAA	PIC	TIBA	2,4-D	BEN	DIC	IAA	IBA	NAA	PIC	TIBA
Areco	1 050	1 035	935	785	635	950	885	535	4.3	2.9	1.5	0.9	0.3	5.2	6.6	0.4
Texa	400	250	350	350	350	350	350	200	2.5	0.4	1.1	0.0	1.1	2.6	2.3	0.0

XI. Role of basal medium and level of growth regulators in anther callogenesis and organogenesis in anther calli (Exp. 12)

System of regeneration			No. isolated anthers	Callogenesis (%)	Organogenesis (%)	
Callus induction medium ➡ bud induction medium	No.	changed factors			related to calli	related to anthers
A 22 ➡ BI	1	N, auxin, cytokinin, pH	783	4.2	21.2	0.9
A 22/growth regulator P ➡ BI	2	N, (auxin), pH	725	2.5	44.4	1.1
P ➡ P	3	–	685	1.0	14.3	0.1
P/growth regulator A22 ➡ BI	4	auxin, cytokinin, pH	684	4.7	18.8	0.9

N – higher macro- and micronutrient (N, B, Mn, Zn, predominantly) levels in bud induction media compared to callus induction ones



8. Influence of cultivation temperature of donor plants on anther callogenesis (Exp. 12)

regeneration was lower in green friable calli, which developed in high culture temperature (above 30 °C).

Regenerated buds can be transferred on MS 0.00095 mg.l^{-1} NAA/0.0225 mg.l^{-1} BAP medium (Tejklová, 1992a, b) for long-term shoot-tip culture (Fig. 9g). Shoots rooted on MS medium with 0.019 mg.l^{-1} NAA (Fig. 9h).

Regenerated plants can be transferred into soil and harvested after maturation. All our regenerants were fertile dihaploids (except of one regenerant which was sterile; however, cytoflowmetric method documented it was diploid, too), though Nichterlein et al. (1991) described both haploid and dihaploid regenerants.

CONCLUSIONS

The critical factor for callus formation from anthers is the temperature in which donor plants are cultivated. It seems that other studied factors (composition of culture media, culture regime) cannot eliminate the negative influence of higher donor plants cultivation temperature on the process of callogenesis.

Method of anther culture is proposed for dihaploid genotype production in flax and linseed. The good results can be reached when the following items are kept:

- The donor plants are cultivated in min. day temperature below 10 °C and max. day temperature below 20 °C.
- The buds isolation and sterilization and anther extirpation is quick (data not shown).
- The bud about 5 mm (according to genotype, Texa: 4–5 mm) in length are sterilized as follows: rinsing in 70% ethanole, 20 min. in 7% calcium chloride, three times rinsing in sterile water.
- The extirpated anthers are transferred on surface of induction agar medium in Petri dishes (max. 50 anthers in Petri dish of 10 cm in diameter with 20 ml of medium).
- The induction medium contains: macro-microelements MS except of ammonium nitrate (165 mg.l^{-1}), MS vitamins, m-inositol 100 mg.l^{-1}, l-glutamine 750 mg.l^{-1}, NAA 1 mg.l^{-1}, BAP 2 mg.l^{-1}, sucrose 60–100 g.l^{-1}. Medium is solidified with agar, pH is 5.5.
- The culture of anthers in a 16-h' day, 25–29/20–24, °C, day/night or at 21 °C in the dark.
- The calli about 2 mm in diameter are transferred on regeneration medium (5 pieces in 100 ml Erlenmeyer's flasks with 25 ml of medium) and cultured in a 16-h' day, 25–29/20–24 °C, day/night.
- Regeneration medium is N6 macro-micronutrients and vitamins, m-inositol 100 mg.l^{-1}, l-glutamine 375 mg.l^{-1}, BAP 1 mg.l^{-1}, sucrose 30 g.l^{-1}. Medium is solidified with agar, pH is 5.8.
- Buds developing in calli are transferred on long--term shoot-tip culture medium (MS 0.00095 mg.l^{-1} NAA/0.0225 mg.l^{-1} BAP; 5 pieces in 100 ml Erlenmeyer's flasks with 25 ml of medium) in 16-h' day, 25/20 °C, day/night.
- Shoots 2 cm in length growing up are cut with a sharp scalpel and rooted in MS medium with 0.02 mg.l^{-1} NAA (5 pieces in 100 ml Erlenmeyer's flasks with 25 ml of medium) in 16-h' day, 25/20 °C, day/night.
- Rooted shoots are rinsed in 0.15% Previcur, then they are planted in pots with soil (autoclaved 30 min in 121 °C) and covered with glass or similar cap. The cap is removed after several days.

Acknowledgements

The author would like to thank J. Vychodilová for her excellent technical assistance and M. Griga for photodocumentation.

Abbreviations

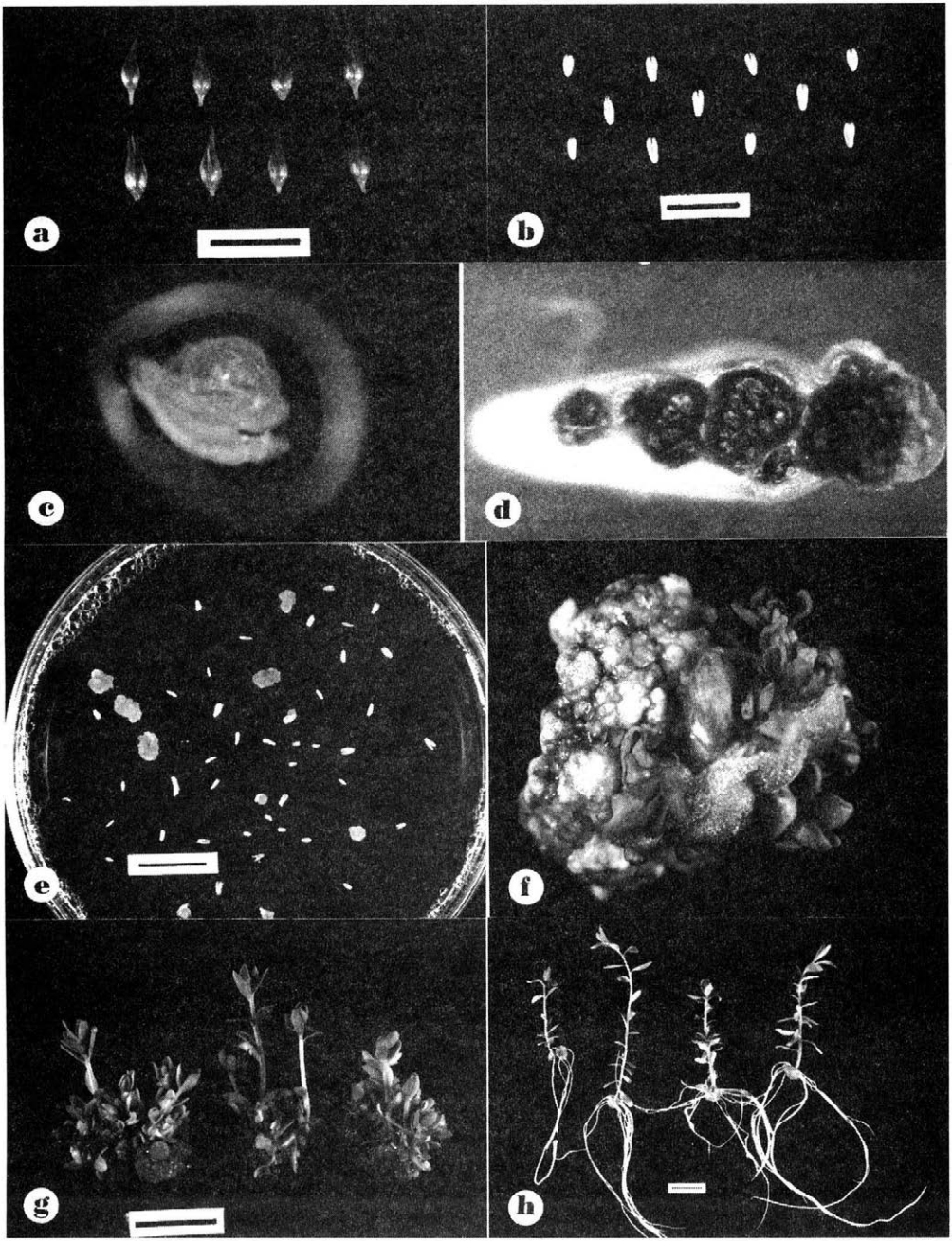
2,4-D = 2,4-dichlorophenoxyacetic acid, IAA = b-indolylacetic acid, IBA = b-indolylbutyric acid, NAA = α-naphthaleneacetic acid, TIBA = 2,3,5-triiodobenzoic acid, BAP = 6-benzylaminopurine

REFERENCES

CHU, C. C.: The medium N6 and its applications for anther culture of cereal crops. Proc. Symp. Pl. Tiss. Cult., Beijing, Sci. Press, 1978: 43–50.

MATHEWS, V. H. – NARAYANASWAMY, S.: Phytohormone control of regeneration in cultured tissues of flax. Z. Pfl.-Physiol., *80*, 1976: 436–442.

NICHTERLEIN, K. – FRIEDT, W.: Plant regeneration from isolated microspores of linseed (*Linum usitatissimum* L.). Pl. Cell Rep., *12*, 1993: 426–430.



9. Anther culture in *Linum usitatissimum* L. a) buds of Areco available for isolation of anthers, bar = 1 cm; b) anthers of Areco with ± late uninuclear microspores, bar = 5 mm; c) anther of Red x (Red x Tv) hybrid in early time of callogenesis on C medium; d) multiple callogenesis in Areco on AA22/100 culture medium; e) callogenesis in anther culture of Texa after four weeks on AA22/100 medium; f) bud regeneration of Texa, system of regeneration No. 2; g) shoot-tip culture of Areco from buds regenerated in anther culture, anthers on C60 medium in the dark, callus on BI medium, bar = 1 cm; h) rooted shoots of Areco regenerated in anther culture, after two weeks on MS medium with 0.019 $mg.l^{-1}$ NAA, bar = 1 cm

NICHTERLEIN, K. – UMBACH, H. – FRIEDT, E.: Investigations on androgenesis in breeding of linseed (*Linum usitatissimum*). Vortr. Pfl.-Zücht., *15*, 1989: 25–13.

NICHTERLEIN, K. – UMBACH, H. – FRIEDT, E.: Genotypic and exogenous factors affecting shoot regeneration from anther callus of linseed (*Linum usitatissimum* L.). Euphytica, *58*, 1991: 157–164.

POLIAKOV, A. V.: The utilization of haploidy and tissue culture technique for obtaining new breeding material of fibre flax. Proc. 2nd European Regional Workshop on Flax, Brno, 1991: 54.

POLIAKOV, A. V. – LOSHAKOVA, N. I. – KRYLOVA, T. V. – RUTKOWSKA-KRAUSE, I. – TROUVE, J. P.: Perspectives of haploids use for flax improvement (*Linum usitatissimum* L.). Rep. Flax Genetic Resources Workshop, Brno, 1994: 38–44.

RUTKOWSKA-KRAUSE, I. – MANKOWSKA, G. – POLIAKOV, A. V. – PROLIOTOVA, N. V.: Plant regeneration through anther culture of flax (*Linum usitatissimum* L.). Proc. 3rd Meet. Int. Flax Breeding Res. Group, Saint-Valery-en-Caux, France, 7–8 November 1995. (In press.)

SUN, H. T. – FU, W. D.: Induction of haploid plants of flax (*Linum usitatissimum* L.) by anther culture and preliminary observations on their progeny. Acta Genet. Sin., *8*, 1981: 369–374.

TEJKLOVÁ, E.: Investigation of explant culture methods in flax. [Final report.] Šumperk, VÚTPL 1990. 74 p. (In Czech.)

TEJKLOVÁ, E.: Long-term *in vitro* shoot-tip culture and plant regeneration in flax. Rostl. Výr., *38*, 1992a: 1009–1022. (In Czech.)

TEJKLOVÁ, E.: The first results with anther culture of *Linum usitatissimum*. Proc. Biotechnology in Central European Initiative Countries, Graz, 1992b: 51.

TEJKLOVÁ, E.: Anther culture in flax (*Linum usitatissimum* L.). Proc. II Semin. Projects R 329-103 a Z 660, Průhonice, 19–20 January 1994: 63-66. (In Czech.)

Received on January 17, 1996

Contact Address:

RNDr. Eva Tejklová, AGRITEC, výzkum, šlechtění a služby, s. s r. o., Zemědělská 16, 787 01 Šumperk, Česká republika, tel.: 0649/38 21 11, fax: 0649/38 29 99

ACCUMULATION OF ALUMINIUM, CALCIUM AND MAGNESIUM IN MAIZE AND SUNFLOWER PLANTS GROWN IN ACIDIFIED SOLUTIONS WITH ALUMINIUM

AKUMULACE HLINÍKU, VÁPNÍKU A HOŘČÍKU V ROSTLINÁCH KUKUŘICE A SLUNEČNICE POD ZÁTĚŽÍ VYSOKÉ ACIDITY A KONCENTRACE

M. Vicherková

Masaryk University, Faculty of Science, Brno, Czech Republic

ABSTRACT: In the present paper the problems of accumulation and distribution of Al are solved in two plant species – the monocotyledonous maize (*Zea mays* L., cv. CE 200) and the dicotyledonous sunflower (*Helianthus annuus* L., cv. Albena) – at different acidity and Al effect on the growth of shoots and roots and on the accumulation of divalent cations Ca^{2+} and Mg^{2+} in those organs. The experiments were made in nutrient solutions with controlled acidity and reduced salt concentration (Al concentration 0.6 mM) under constant conditions of temperature and illumination. Maize and sunflower react differently to the given experimental conditions by growth parameters, Al accumulation as well as by the inhibition intensity of accumulation of both divalent cations. Al toxicity evoked the reduction of elongation and dry matter production of roots as well as shoots, the reduction of the leaf area and the decrease of root branching with disorders of gravitropism. In maize the formation of adventive roots was observed. All symptoms of Al toxicity were more pronounced in sunflower than in maize. The same holds for the effect of high acidity. Al accumulation in plant organs prevails in the roots, where the concentration is ten times higher than in the shoots. Sunflower accumulated three times as much Al than maize. The two species accumulate also divalent cations differently due to the interaction of Al and high acidity. In the whole maize plant the accumulation of Mg^{2+} is conspicuously inhibited, the inhibition of Ca^{2+} accumulation being only low. The Al toxicity of maize plants is rather connected with Mg than Ca. In sunflower a marked reduction of the accumulation of both Ca and Mg in the shoots occurs. The accumulation of Ca^{2+} in the roots is non-standard. All observed growth and divalent cation accumulation changes in the experimental plants lead to the conclusion that maize (the employed variety) belongs to Al tolerant species. Sunflower is much more sensitive towards Al and H^+ toxicities.

phytotoxicity; high acidity – Al; accumulation; aluminium; calcium; magnesium; maize; sunflower

ABSTRAKT: V práci je řešena problematika akumulace a distribuce Al u dvou rostlinných druhů, jednoděložné kukuřice (*Zea mays* L., hybrid CE 200) a dvouděložné slunečnice (*Helianthus annuus* L., odrůda Albena), při rozdílné aciditě a účinku Al na růst nadzemních orgánů a kořenů a na akumulaci divalentních kationtů Ca^{2+} a Mg^{2+} v těchto orgánech. Pokusy probíhaly v živných roztocích s udržovanou aciditou a sníženou koncentrací solí (koncentrace Al 0,6 mM) v konstantních podmínkách teploty a osvětlení. Kukuřice a slunečnice reagovaly rozdílně na dané pokusné podmínky růstovými parametry, akumulací Al i intenzitou inhibice akumulace obou divalentních kationtů. Toxicita Al vyvolávala redukci elongace a tvorby sušiny kořenů i nadzemních částí, redukci listové plochy, redukci větvení kořenů s poruchami gravitropismu. U kukuřice byla pozorována tvorba adventivních kořenů. Všechny symptomy Al toxicity byly výraznější u slunečnice než u kukuřice. Totéž platí o vlivu vysoké acidity. Akumulace Al v rostlinných orgánech převažuje v kořenech, kde je koncentrace řádově desetkrát větší než v nadzemní části. Slunečnice akumulovala třikrát více Al než kukuřice. Oba druhy akumulují rozdílně vlivem interakce Al a vysoké acidity i divalentní kationty. V celé rostlině kukuřice je výrazně inhibována akumulace Mg^{2+}, inhibice Ca^{2+} je jen nízká. Al toxicita u kukuřice je spíše spojena s Mg než Ca. U slunečnice dochází k výrazné redukci akumulace Ca i Mg v nadzemních částech, akumulace Ca^{2+} v kořenech je atypická. Pozorované změny růstu i změny akumulace divalentních kationtů u pokusných rostlin prokazují, že kukuřici (použitou odrůdu) lze přiřadit k Al tolerantním druhům. Slunečnice je mnohem citlivější vůči Al i H^+ toxicitě.

fytotoxicita; vysoká acidita – Al; akumulace; hliník; vápník; hořčík; kukuřice; slunečnice

INTRODUCTION

The prevailing part of Al in neutral to weakly acid soils is bound in the sorption complex and it does not affect plants unfavourably. As a consequence of anthropogenic activities, due to acid depositions there is a progressive acidification of the environment (above all water and soil), which results in the subsequent release of a great amount of Al into these mediums. Those in turn have an inhibitory to toxic effect on the growth and development of plants (e.g. Foy, 1984; Rengel, 1992). The limit of toxic effect of Al is about pH 5.0, but also pH 5.5 is often mentioned. The overall amount of Al present does not, however, represent an absolutely effective dose affecting plants. Biologically active are only monomeric species of Al, above all Al^{3+} and the series of Al-hydroxide ions from $Al(OH)^{2+}$ up to $Al(OH)_3$ (Alva et al., 1986) with a decrease of activity from Al^{3+} to $Al(OH)_3^0$ (Fageria et al., 1989). On acid, agronomically utilized soils, these Al cations significantly reduce the production of agricultural crops. From the external environment Al is taken up by plants and accumulated in plant organs. It inhibits above all the cell division and the root growth. The growth inhibition is connected with the effect of Al on the whole series of metabolic and physiological processes of plants (Taylor, 1988). It is very difficult to differentiate the primary effects of Al operation from secondary symptoms. Plant species and varieties differ conspicuously in the reaction to increased Al concentration. The tolerance and/or the intolerance of species and varieties is genetically controlled and conditioned by a number of protective mechanisms of different type (Foy, 1984; Horst, Göppel, 1986; Kinraide, Parker, 1987; Spehar, 1994).

One of the most distinct physiological changes evoked by Al are disturbances of mineral nutrition resulting in the loss of nutrient balance and conspicuous nutrient deficiency in the plant. Particularly the sorption of divalent cations and P is affected, but also that of further cations. Changes in the uptake capacity of nutrients and in the mineral composition of plant tissues due to Al effect often appear even before changes in plant growth (Bengtsson et al., 1988). The possibilities of affecting mineral nutrition of plants by Al are characterized by a considerable variability. The mechanism of Al effect in this sense is very complicated and it can be quite different in specific plant groups.

The aim of the present paper was to compare – under different acidity – the accumulation and distribution of Al in two plant species (maize and sunflower) and to investigate its effect on the accumulation of Ca and Mg in the individual organs of the experimental plants.

MATERIAL AND METHODS

The experiments were made with maize plants (*Zea mays* L., cv. CE 200) and sunflower plants (*Helianthus annuus* L., cv. Albena), cultured in a diluted modified Richter nutrient solution containing (in mM): 1.50 $Ca(NO_3)_2.4\,H_2O$, 0.99 KNO_3, 0.73 KH_2PO_4, 2.02 $MgSO_4.7\,H_2O$ and 0.5 $FeCl_3.6\,H_2O$ in the form of EDTA. Into half of the vessels $Al_2(SO_4)_3.18\,H_2O$ was added in the concentration of 0.6 mM Al. The plants were cultivated in all variants of nutrient solutions after a four-day pregermination for the period of 15 days at the temperature of 25 ± 2 °C and 16 h illumination of intensity 560 $\mu mol.m^{-2}.s^{-1}$.

The acidity of the solutions was kept at pH 3.5 and 4.5 by a daily adjustment by means of H_2SO_4 and NaOH and by the exchange of solutions after 5 days of cultivation. For the sake of comparison, another variant was made with pH kept at the value of 5.5. The experiment included following variants for both plant species: pH 5.5, pH 4.5/–Al, pH 4.5/+Al, pH 3.5/–Al and pH 3.5/+Al.

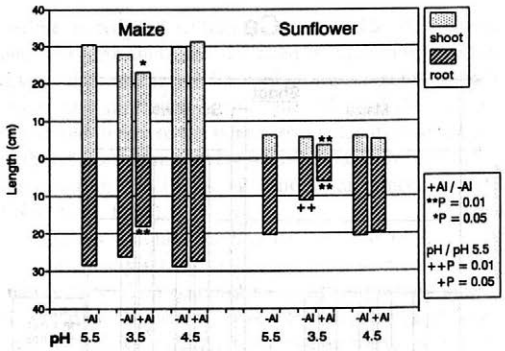
The plant growth characteristics and the content of mineral elements Al, Ca and Mg by the emission spectrometry by means of induction coupled plasma (Sommer, 1990; Otruba, Navrátil, 1990) were determined. All samplings and all measurements were carried out in triplicate. Statistical evaluation was performed by the *t*-test by means of the set of statistical programs Statgraphics.

RESULTS

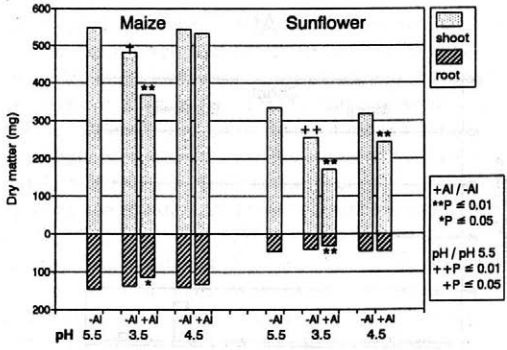
Employed Al concentrations in the nutrient solution in interaction with high acidity (pH 3.5) negatively affect the growth of roots and shoots of both experimental plant species, also accompanied by morphological changes in roots and partly also in leaves. The effect of Al is conspicuously rhizotoxic what is reflected more in length and branching of the root than in the production of dry matter. The roots are short, hypertrophic, laterally unbranched with brownish root tips. The leaves, particularly in the sunflower, exhibit visual symptoms of Ca, Mg and/or P deficiency, they are thickened and have a strongly reduced area. The intensity of damage of both plant species is different. The above changes are more conspicuous in the sunflower.

The elongated growth (Fig. 1) of roots in both species at pH 3.5 is reduced most conspicuously, in sunflower the longitudinal inhibition is stronger. The length of shoots is affected less than the length of roots, in sunflower statistically highly significantly. The high acidity alone affects maize and sunflower differently. The comparison with treatment pH 5.5 shows that the length of the roots as well as of the shoots of maize reacts only by a mild insignificant reduction, whereas the roots of sunflower show a rapid decrease of the longitudinal growth by as much as 45%.

The dry matter (Fig. 2) of the roots of both plant species is affected by Al at pH 3.5 less than the length. The difference of the reduction between dry matter and length of the roots in sunflower reaches about 20%, in



1. Shoot and root lengths [cm/1 plant] of maize and sunflower plants grown in nutrient solutions of different acidity with (+Al) or without (–Al) aluminium



2. Shoot and root dry matter [mg/1 plant] of maize and sunflower plants grown in nutrient solutions of different acidity with (+Al) or without (–Al) aluminium

maize 10%. More than roots shoot dry matter is affected by Al (in sunflower the inhibition reaches about 35%, in maize 25%). The sunflower shoot is inhibited by 25% even in milder acidity.

The acidity alone without Al (comparison with pH 5.5) inhibited only the elongation of sunflower roots and at pH 3.5 the dry matter of the shoots of both two plant species, with greater intensity in sunflower.

The degree of root damage due to Al and low pH was characterized by a lowered specific root length towards the respective control (SRL = the length related to dry weight root unit, Tab. I). The lowering of this value in maize by Al damage reaches 15%, whereas the low pH alone has a minimum effect. In sunflower the SRL reacts to Al and low pH in both cases by a much greater lowering – by 32 and 38%, respectively. Also relative dry matter production of the overground organs (Tab. I) is inhibited by these two factors more intensively in sunflower than in maize.

In both, maize and sunflower, Al accumulation (Fig. 3) is the greatest in the roots. Its distribution in plants is not homogenous, since only a small part is transported to the shoots. At high acidity, this part is roughly ten times lower in sunflower plants and six times lower in maize plants than in the roots. In the solution with milder acidity it is again lower, in sunflower seven times and in maize six times.

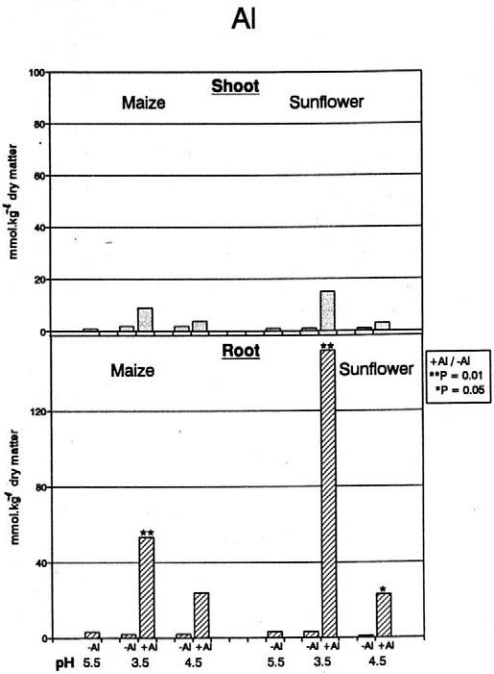
The interaction of Al with low pH induces different changes in the content of divalent cations Ca^{2+} and Mg^{2+} in both experimental species. The Ca content (Fig. 4) in maize is significantly lower only in the roots, in the shoots the slight decrease of Ca content is insignificant. In sunflower plants, an increased Ca accumulation in the roots appears but, it does not reach statistical significance although the increase approaches closely the limit of significance. Statistically significant is, however, the increased Ca content under the effect of acidity without Al (ratio 3.5/5.5). In sunflower shoots from the Al variants Ca content is highly significantly lowered at high as well as mild acidity, the lowering reaching 35% in both cases.

The Mg accumulation (Fig. 5) in the two organs of both the sunflower and maize conspicuously lowered due to Al in combination with high acidity, most in the maize roots (by 60%) and in the sunflower shoots (by 55%). In milder acidity the Mg content is lowered also in maize roots and in the sunflower shoots (by 45%).

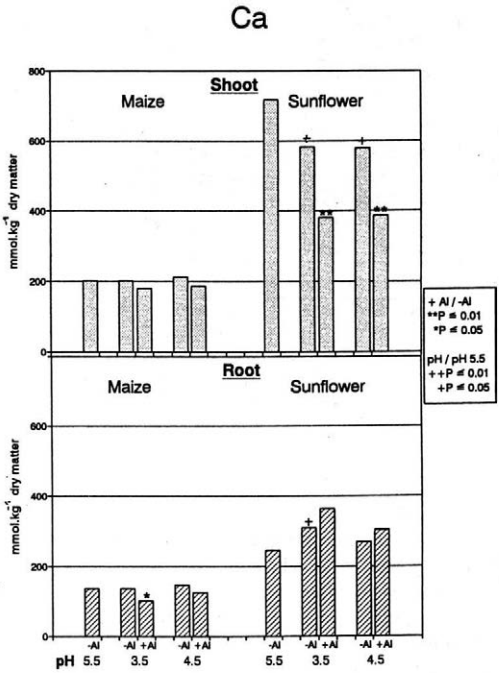
The change in the solution acidity alone without Al participation has no effect on the Ca content in maize. In sunflower the lowering of pH without Al evokes the increase in Ca accumulation in the roots and its decrease in the shoots. The Mg content is lowered in both maize organs with the lowering of pH to 3.5, milder acidity 4.5 pH does no longer change its content in

I. Relative specific root length and relative shoot dry weight (+Al/–Al or pH/pH 5.5) of maize and sunflower plants grown at different pH with or without aluminium in nutrient solution

	Ratio of variants	Maize		Sunflower	
		pH		pH	
		3.5	4.5	3.5	4.5
Relative specific root length	+Al/–Al	0.84	1.00	0.68	0.96
	pH/pH 5.5	0.95	1.05	0.62	1.02
Relative shoot dry weight	+Al/–Al	0.76	0.98	0.67	0.76
	pH/pH 5.5	0.88	0.99	0.76	0.95



3. Aluminium concentration [mmol.kg^{-1}dry matter] in maize and sunflower shoots and roots grown in nutrient solutions of different acidity with (+Al) or without (–Al) aluminium

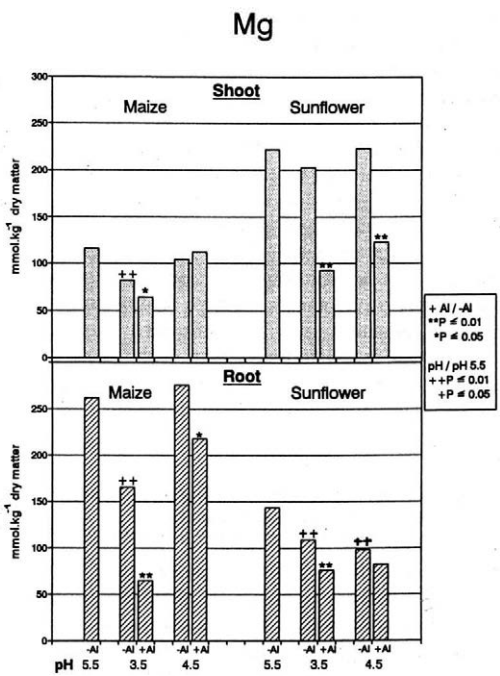


4. Calcium concentration [mmol.kg^{-1}dry matter] in maize and sunflower shoots and roots grown in nutrient solutions of different acidity with (+Al) or without (–Al) aluminium

comparison with the control. In sunflower, decreased pH lowers the Mg content only in the roots, this trend being kept even at milder acidity.

DISCUSSION

In general, plant roots are characterized by the fact that under certain conditions they can regulate pH of external nutrient or soil solutions to their optimum. That is why they can quickly and effectively change original acidity of the medium. In view of this fact our experiments were carried out in nutrient solutions with the exchange and daily correction of pH, so as to adjust the original acidity. The binding of toxic forms of Al and buffer properties of the nutrient solution were partly limited by lowering salt concentrations used in the nutrient solutions and the choice of a sufficiently high Al concentration as well as the acidity of the solutions. The intensity of Al phytotoxicity in nutrient solutions is conditioned by the presence of biologically active monomeric species of Al, dependent on pH and also on the concentration and composition of nutrient solution (Alva et al., 1986; Kinraide, 1991; Foy et al., 1992; Spehar, 1994). The lower the acidity and the higher ionic strength the higher level of Al is required to produce a given effect in the plants. The employed pH 4.5 and particularly 5.5 in our ex-



5. Magnesium concentration [mmol.kg^{-1}dry matter] in maize and sunflower shoots and roots grown in nutrient solutions of different acidity with (+Al) or without (–Al) aluminium

periments reduced Al toxicity to a considerable extent, and therefore there was no need of including into the results the variant pH 5.5 containing Al, since in both maize and sunflower species the effect of Al was not registered in any of the parameters studied. In solutions with pH near neutral pH effective soluble Al is present only within the limits of nanomolar concentrations (Martin, 1986).

Progress in understanding mechanisms of Al toxicity and closely related Al tolerance in plants may ultimately depend on comprehending events which underlie the plants' reaction to Al. The physiological basis for Al toxicity and Al tolerance remains uncertain. However, a number of phytotoxic responses of plants to Al involved is known: inhibition of cell division and elongation in the roots, nutrient disorders, binding to membranes and biologically important molecules, disorders in key regulatory processes and root growth control mechanisms (Foy, 1988; Wagatsuma, 1983; Bennet, Breen, 1989; Kochian, Shaff, 1991). The highest inhibition intensity is observed in the region of the root tip and the root cap (Marschner, 1991).

The growth changes in roots established in our experiments confirm fully the above information about Al inhibition. That is documented not only by the reduction of elongation and dry matter production of the roots, but also by the reduction of root branching, disorders in gravitropism and the damage of root tips. All these symptoms of Al toxicity were more distinct in sunflower than in maize. Of interest was the finding that maize plants started to form adventive roots towards the end of cultivation in variant pH 3.5/+Al. The strategy of the initiation of forming adventive or lateral roots in some plants is included among Al induced changes in the development of roots which can be connected with greater Al tolerance or a better adaptation ability of those plants towards Al toxicity (Bennet, Breen, 1989; Marschner, 1991). The mutual ratios of changes in length and dry matter of the roots due to Al were very well demonstrated by the calculated value of specific root length.

Symptoms of Al toxicity were also observed on plant shoots. Particularly sunflower leaves are thickened with a conspicuously reduced area, on leaves of maize these symptoms were not visible. Shoot length and dry matter of both two species reacted to Al by a considerable reduction, again more indentifiable in sunflower. The reduction of shoots and the visual symptoms can be well accounted by the changes in carbon dioxide assimilation rate, chlorophyll content and the activity of numerous regulatory enzymes found for Al toxicity (Haugh, Shi, 1991; Simon et al., 1994), besides changes in supply of mineral nutrients which will be mentioned lower.

All observed growth changes in experimental plants lead to the conclusion that maize (the employed cultivar) belongs to Al tolerant species. Sunflower is much more sensitive towards Al toxicity and the limit of Al toxic effect lies near higher pH. In the mildly acid solution sunflower had highly significantly reduced shoot dry matter, whereas maize did no longer exhibit any signs of Al toxicity. Further changes described for the interaction of high acidity and Al did not appear in the mildly acid mediums which confirmed the fact that in this acidity the most toxic forms of Al are released only to a lesser extent, Al toxicity disappearing (Alva et al., 1986; Foy et al., 1992). In sunflower, at milder acidity of the solution a more toxic reaction to $AlOH^{2+}$ and further monomeric hydroxide Al ions than Al^{3+} could assert itself. This reaction was registered with some dicots (Kinraide, Parker, 1990).

Different is also the reaction of maize and sunflower growth to acidity alone. Root and shoot growth of sunflower is sensitive not only to Al concentration but also to high concentration of H^+. Maize reacts only by a mild inhibition of the shoot growth.

Phytotoxically acting fractions of soluble „free" Al comprised in external solution enters the cell walls of the root cells where it is bound with a relatively high affinity to many biological compounds. Metabolically and physiologically more important than the interaction of Al and its accumulation in the apoplast is Al bond in membrane structures and in the cytoplasm. From the agronomical and, above all, nutrition point of view the total Al content in plant organs is important. The mobility of Al in plant cells, tissues and plant organs is low, which coincides with the chemical analyses of plants which demonstrate that Al is retained preferentially in the roots of most plants (Foy, 1988). Maize and sunflower in our experiments accumulated Al in roots at high acidity in six and ten times greater amounts than in the shoots. Our sensitive sunflower accumulated in the roots three times as much and in the shoots twice as much Al than maize (cf. Delhaize et al., 1993). In the shoots of sunflower most of the Al was located in cotyledons, and in leaves it did not even reach the value of 3 $mmol.kg^{-1}$ of the dry matter (Vicherková, unpublished).

The interaction of Al with low pH altered nutrient uptake capacity and mineral composition of plant tissues. In our previous papers (Vicherková, Minář, 1987; Minář, Vicherková, 1990; Vicherková, Kramářová, 1995), changes were found in all main macronutrients in pea and maize plants under the effect of a different Al concentration and different acidity. However, little attention was paid to Ca and Mg, divalent cations playing a distinct role in Al phytotoxicity. According to the results of many research projects there is an assumption that Al evokes a disorder of the homeostasis of cytosolic Ca^{2+} and changes the character of Ca^{2+} transport across membranes (Bennet, Breen, 1991; Ryan et al., 1994), changing the whole metabolism of Ca in cells with a subsequent influence on many further processes. The knowledge of the recent period also changes the views of many preceding hypotheses about the function of Ca in Al toxicity (e.g. Ryan et al., 1994). So far there is

a little information about the mechanism of Al interaction with Mg.

Under our experimental conditions the two experimental species react differently also in the accumulation of divalent cations. In maize the reduction of accumulation of Ca by Al and high acidity is almost absent, with only a mild reduction in the roots, which does not correlate much with the reduced growth of roots and shoots. The inhibition of Ca deposition in the roots may have been compensated by Al induced formation of adventive roots, which does not hold for Mg. In a conspicuous manner is reduced the accumulation of Mg^{2+} in the shoots, but especially in the roots, both by Al concentration and by high acidity. The action of Al is more intensive in the roots and lowered Mg^{2+} accumulation still appears at pH 4.5, where the growth was no longer reduced. It seems that in maize plants under our experimental conditions Al phytotoxicity is rather connected with Mg than Ca.

In sunflower there is a conspicuous reduction of the accumulation of both Ca and Mg in the shoots in both acidities. The accumulation of Mg is also inhibited in sunflower roots, a greater effect being that of the lowered pH than the presence of Al. The reduction of sunflower growth thus correlates with those parameters. In sunflower roots the accumulation of Ca was reduced neither by Al concentration nor by high acidity. Although the results did not reach statistical significance (besides the effect of 3.5 pH without Al), the obtained values were in all cases higher for Al and non-Al plants. This increase can be conditioned by a stronger bond of Ca in the apoplast than the bond of Mg. However, this increase does not reflect higher efficiency in nutrient uptake but is mainly caused by slow growth rates of plants. At the same time the Ca translocation into the shoots is limited by Al, but also by low pH, because not even an increased amount of Ca in the roots did not limit the reduction of the accumulation in shoots.

Genotypical differences of two plant species – the monocotyledonous maize and the dicotyledonous sunflower – were documented by our experiments also for Al and H^+ toxicities reflected in growth changes of both shoot and root organs and in changes in accumulation and distribution of divalent cations Ca^{2+} and Mg^{2+}.

Acknowledgements

This work was supported by Grant Agency of the Czech Republic (grant No. 501/2139/1993).

REFERENCES

ALVA, A. K. – EDWARDS, D. C. – ASHER, C. J. – BLAMEY, F. P. C.: Effects of phosphorus/aluminium molar ratio and calcium concentrations on plant response to aluminium toxicity. Soil Sci. Soc. Amer. J., *50*, 1986: 133–137.

BENGTSSON, B. – ASP, H. – JENSEN, P. – BERGGREN, D.: Influence of aluminium on phosphate and calcium uptake in beech (*Fagus sylvatica*) grown in nutrient solution and soil solution. Physiol. Plant., *74*, 1988: 299–305.

BENNET, R. J. – BREEN, C. M.: Towards understanding root growth responses to environmental signals: The effect of aluminium on maize. S. Afr. J. Sci., *85*, 1989: 9–12.

BENNET, R. J. – BREEN, C. M.: The aluminium signal: New dimensions to mechanisms of aluminium tolerance. In: WRIGHT, J. R. et al. (eds): Plant – soil interactions at low pH. Dordrecht, The Netherlands, Kluwer Acad. Publ. 1991: 703–716.

DELHAIZE, E. – CRAIG, S. – BEATON, C. D. – BENNET, R. J. – JAGADISH, V. C. – RANDAL, P. J.: Aluminium tolerance in wheat (*Triticum aestivum*) L. I. Uptake and distribution of aluminium in root apices. Pl. Physiol., *103*, 1993: 685–693.

FAGERIA, N. K. – BALIGAR, V. C. – WRIGHT, R. J.: The effects of aluminium on growth and uptake of Al and P by rice. Pesq. Agropec. Bras. (Brasilia), *24*, 1989: 677–682.

FOY, C. D.: Physiological effects of hydrogen, aluminium and manganese toxicities in acid soil. In: ADAMS, F. (ed.): Soil acidity and liming. Amer. Soc. Agron. (Madison, USA), 1984: 57–97.

FOY, C. D.: Plant adaptation to acid, aluminium – toxic soils. Commun. Soil Sci. Pl. Anal., *19*, 1988: 959–987.

FOY, C. D. – DUKE, J. A. – DEVINE, T. E.: Tolerance of soybean germplasm to an acid Tatum subsoil. J. Pl. Nutr., *15*, 1992: 527–547.

HAUGH, A. – SHI, B.: Biochemical basis of aluminium tolerance in plant cells. In: WRIGHT, R. J. et al. (eds): Plant – soil interactions at low pH. Dordrecht, The Netherlands, Kluwer Acad. Publ. 1991: 839–850.

HORST, W. J. – GÖPPEL, H.: Aluminium Toleranz von Ackerbohne (*Vicia faba*), Lupine (*Lupinus luteus*), Gerste (*Hordeum vulgare*) und Roggen (*Secale cereale*). II. Mineralstoffgehalte in Spross und Wurzeln in Abhängigkeit vom Aluminium-Angebot. Z. Pfl.-Ernähr. Bodenkde, *149*, 1986: 94–109.

KINRAIDE, T. B.: Identity of the rhizotoxic aluminium species. Pl. Soil, *134*, 1991: 167–178.

KINRAIDE, T. B. – PARKER, D. R.: Cation amelioration of aluminium toxicity in wheat. Pl. Physiol., *83*, 1987: 546–551.

KINRAIDE, T. B. – PARKER, D. R.: Apparent phytotoxicity of mononuclear hydroxy-aluminium to four dicotyledonous species. Physiol. Plant., *79*, 1990: 283–288.

KOCHIAN, L. V. – SHAFF, J. E.: Investigating the relationship between aluminium toxicity, root growth and root-generated ion currents. In: WRIGHT, R. J. et al. (eds): Plant – soil interaction at low pH. Dordrecht, The Netherlands, Kluwer Acad. Publ. 1991: 769–778.

MARSCHNER, H.: Mechanism of adaptation of plants to acid soils. Pl. Soil, *134*, 1991: 1–20.

MARTIN, R. B.: The chemistry of aluminium as related to biology and medicine. Clin. Chem., *32*, 1986: 1797–1806.

MINÁŘ, J. – VICHERKOVÁ, M.: Effect of aluminium on the growth and mineral nutrient contents in pea under different nutrient solution acidity. In: Physiological aspects of plant mineral nutrition VI, Brno, 1990: 168–171. (In Czech.)

OTRUBA, V. – NAVRÁTIL, D.: Laboratory apparate and manuals. Chem. Listy, *84*, 1990: 862–870. (In Czech.)
RENGEL, Z.: The role of calcium in aluminium toxicity. New Phytol., *121*, 1992: 499–513.
RYAN, P. R. – KINRAIDE, T. B. – KOCHIAN, L. V.: Al^{3+}–Ca^{2+} interactions in aluminium rhizotoxicity. I. Inhibition of root growth is not caused by reduction of calcium uptake. Planta, *192*, 1994: 98–103.
SIMON, L. – KIEGER, M. – SUNG, S. S. – SMALLEY, T. J.: Aluminium toxicity in tomato. 2. Leaf gas exchange, chlorophyll content, and invertase activity. J. Pl. Nutr., *17*, 1994 (2, 3): 307–317.
SOMMER, L.: Bases of analytical chemistry III. Praha, SPN, Brno, MU 1990.
SPEHAR, C. R.: Aluminium tolerance of soya bean genotypes in short term experiments. Euphytica, *76*, 1994: 73–80.
TAYLOR, G. J.: The physiology of aluminium phytotoxicity. In: SIEGEL, H. – SIEGEL, A. (eds): Metal ions in biological systems. Vol. *24*. Aluminium and its role in biology. New York, M. Dekker 1988: 123–163.
VICHERKOVÁ, M. – KRAMÁŘOVÁ, E.: Mineral nutrient distribution in plant organs of maize under higher acidity and aluminium concentrations. In: Plant nutrition and fertilization, Brno, MZLU 1995: 133–136. (In Czech.)
VICHERKOVÁ, M. – MINÁŘ, J.: Aluminium induced changes in the growth and mineral nutrient content of maize (*Zea mays* L.). Scripta Fac. Sci. Nat. Univ. Purk. Brno, Biologia, *17*, 1987 (3–4): 215–228.
WAGATSUMA, T.: Effect of non-metabolic conditions on the uptake of aluminium by plant roots. Soil Sci. Pl. Nutr., *29*, 1983: 323–333.

Received on January 17, 1996

Contact Address:

Doc. RNDr. Miroslava Vicherková, CSc., Masarykova univerzita, Přírodovědecká fakulta, Kotlářská 2, 611 37 Brno, Česká republika, tel.: 05/41 12 95 51, fax: 05/41 21 12 14

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION
Slezská 7, 120 56 Praha 2, Czech Republic
Fax: (00422) 25 70 90

In this institute scientific journals dealing with the problems of agriculture and related sciences are published on behalf of the Czech Academy of Agricultural Sciences. The periodicals are published in the Czech or Slovak languages with long summaries in English or in English language with summaries in Czech or Slovak.

Subscription to these journals should be sent to the above-mentioned address.

Periodical	Number of issues per year
Rostlinná výroba (Plant Production)	12
Živočišná výroba (Animal Production)	12
Veterinární medicína (Veterinary Medicine – Czech)	12
Zemědělská ekonomika (Agricultural Economics)	12
Lesnictví – Forestry	12
Zemědělská technika (Agricultural Engineering)	4
Ochrana rostlin (Plant Protection)	4
Genetika a šlechtění (Genetics and Plant Breeding)	4
Zahradnictví (Horticultural Science)	4
Potravinářské vědy (Food Sciencies)	6

EFFICIENCY OF NITRATE AND AMMONIUM NUTRITION IN MAIZE PLANTS AT DIFFERENT IRRADIANCES

EFEKTIVITA NITRÁTOVÉ A AMONNÉ VÝŽIVY U KUKUŘICE PŘI RŮZNÉ OZÁŘENOSTI

F. Plhák

Masaryk University, Faculty of Science, Brno, Czech Republic

ABSTRACT: Maize plants were grown 10 days in a greenhouse in sand culture with 7mM NO_3^-, NH_4^+ or mixed NO_3^-/NH_4^+ N nutrition at 1 200, 800 and 400 $\mu mol.m^{-2}.s^{-1}$ PPFD irradiance established by using shade net. Biomass production decreased in all N treatments with irradiance decrease. Plant with NH_4^+ nutrition produced less biomass significantly in overground parts at full irradiance and more biomass in roots under light reduction in comparison with NO_3^- nutrition. It was remarkable also on root-shoot relation which increased by NH_4^+ nutrition and decreased less intensively as influenced by shade. Leaf area as well as SLA increased with shade and both leaf characteristics were diminished by NH_4^+ nutrition at full irradiance. Mixed NO_3^-/NH_4^+ nutrition caused positive effects on biomass production at full irradiance and intermediate effects at low irradiance. Biomass production related to irradiation unit showed increasing radiation use efficiency (RUE) with light decrease. For NH_4^+ fed plants RUE was lower at full irradiance and higher at lower irradiance in comparison with NO_3^- nutrition. The irradiance about 900 µmol PPFD showed comparable RUE for both N forms. Lower irradiance appeared to be insufficient for NO_3^- metabolisation which was accompanied by remarkable NO_3^- increase in maize tissues. Nitrate reduction seems to be more dependent on light irradiance and therefore a limiting factor of its metabolisation. In NH_4^+ fed plants higher soluble protein and total N-red contents were determined under conditions of lower irradiance which demonstrated also lower energetic input for NH_4^+ metabolisation in comparison with NO_3^-.

maize; nitrate nutrition; ammonium nutrition; efficiency; radiation use

ABSTRAKT: Dusík je významný živinný prvek přijímaný rostlinami jak ve formě aniontu NO_3^-, tak i kationtu NH_4^+. Produkce biomasy je však u mnoha rostlin živených NH_4^+ nižší než při NO_3^- výživě. Kukuřice vykazuje rovněž růstovou inhibici vůči samotné NH_4^+ výživě, i když je zde patrná jistá větší tolerance ve srovnání s jinými rostlinami (Cramer, Lewis, 1993a, b). Negativní reakce některých rostlin vůči NH_4^+ výživě značně závisí na světelné intenzitě (Magalhaes, Wilcox, 1984; Plhák, 1994). Práce je věnována srovnání reakce rostlin kukuřice na nitrátovou a amonnou výživu při třech hladinách světelné intenzity. K pokusům byl použit hybrid CE 205. Rostliny kukuřice byly pěstovány 10 dnů v pískové kultuře se 7 mM NO_3^-, NH_4^+ nebo se směsnou NO_3^-/NH_4^+ výživou při 1 200, 800 a 400 $\mu mol.m^{-2}.s^{-1}$ PPFD ve skleníku na přirozeném světle. Uvedené hladiny světelné ozářenosti byly nastaveny pomocí jednoduché a dvojité stínicí drátěné sítě při plné sluneční radiaci. Relace výživy a změny pH v pískových kulturách byly kontrolovány v roztocích proteklých jednotlivými pokusnými nádobami (tab. I). Pro redukci nitrifikace u NH_4^+ variant byl použit inhibitor DIDIN v koncentraci 5 $mg.l^{-1}$. Produkce biomasy klesala u všech tří N variant s poklesem světelné intenzity. Rostliny s NH_4^+ výživou produkovaly méně biomasy průkazně u nadzemních částí při plné ozářenosti a více biomasy u kořenů při redukci světla ve srovnání s NO_3^- výživou (obr. 1). To bylo patrné též z poměru kořen/nadzemní část, který vzrůstal při NH_4^+ výživě a klesal méně intenzivně se sníženou světelnou radiací. Listová plocha i specifická listová plocha se zvyšovaly se zastíněním a obě listové charakteristiky měly menší hodnoty při NH_4^+ výživě a plné ozářenosti (tab. II). Směsná NO_3^-/NH_4^+ výživa vykazovala pozitivní účinek na tvorbu biomasy při plné ozářenosti a intermediální účinek při zastínění. Produkce biomasy vztažená na jednotku ozářenosti zaznamenala vzrůstající efektivnost využití radiace s klesající intenzitou světla. Při NH_4^+ výživě byla efektivnost využití ozářenosti nižší na plném světle a naopak vyšší při nízké intenzitě světla ve srovnání s NO_3^- výživou (obr. 2). Ozářenost kolem 900 µmol PPFD vykazovala srovnatelnou hodnotu efektivnosti využití světelné radiace u obou srovnávaných N forem. Nižší ozářenost se ukazuje jako nedostatečná pro metabolizaci NO_3^-, což bylo patrné na výrazném zvyšování obsahu NO_3^- v tkáních kukuřice (tab. III, obr. 2). Redukce nitrátů se zdá být více závislá na světelné radiaci, a proto i limitujícím faktorem jejich metabolizace. Rostliny kukuřice s NH_4^+ výživou měly vyšší obsah rozpustné N-red frakce, bílkovinné frakce i celkového N-red za podmínek nižší ozářenosti, což rovněž svědčilo o nižší energetické spotřebě pro metabolizaci NH_4^+ ve srovnání s NO_3^- (tab. IV, V). Současné výsledky i výsledky dosažené v předchozí práci se slunečnicí (Plhák, 1994) prokazují určitou preferenci NH_4^+ výživy u NH_4^+ tolerantních rostlin v podmínkách snížené ozářenosti, která přichází v úvahu v nižších listových patrech rostlinných porostů.

kukuřice; nitrátová výživa; amonná výživa; účinnost; radiační využití

INTRODUCTION

Nitrogen is the nutrient element taken up by plants both in the anion NO_3^- and cation NH_4^+ forms. Biomass production is smaller, however, in many plants grown on NH_4^+ than on NO_3^- nutrition. Some physiological disturbances leading up to NH_4^+ toxicity are species and concentration specific, their biochemical causes are not always clear and are in the centre of interest. These negative effects occurring in plants growing on NH_4^+ nutrition as the only source of N are connected with several direct and indirect ways of action. Ammonium directly inhibits cation absorption (Mengel, Kirkby, 1967) and disrupts transmembrane proton gradient in chloroplasts which inhibits ATP synthesis (Lilley et al., 1975) and some other metabolic processes. Many indirect actions are connected with increase of pH value in vacuole after NH_4^+ uptake (Roberts, Pang, 1992) and with increased consumption of carbohydrates for NH_4^+ assimilation especially in roots (Lewis et al., 1989; Cramer, Lewis, 1993a).

Differences in plant tolerance to NH_4^+ nutrition are documented. Commonly calcifobic plants living in acid soils where nitrification does not occur are adapted or tolerant to NH_4^+ nutrition (Ingestad, 1976; Haynes, Goh, 1978). Such adaptation occurs also in some crop plants from which rice (Ta et al., 1981) and blueberry (Spiers, 1978) are referred to grow better with ammonium than nitrate. Maize was found more tolerant to NH_4^+ as compared with wheat (Cramer, Lewis, 1993a, b). On the other hand, ammonium toxicity in radish (Goyal et al., 1982), tomato (Magalhaes, Wilcox, 1984), and pea (Plhák, 1996, in press) was particularly documented.

Negative reaction to NH_4^+ nutrition may be connected with light intensity. For instance, under conditions of high irradiance tomato plants showed remarkable symptoms of NH_4^+ toxicity while under light reduction the growth was normal (Magalhaes, Wilcox, 1984). Some stimulation effects of NH_4^+ nutrition on biomass production under low irradiance in comparison with NO_3^- were noticed in sunflower plants (Plhák, 1994).

The present paper deals with the reaction of maize plants on NO_3^- and NH_4^+ nutrition at different level of light irradiance. The reaction of maize plants was evaluated by growth characteristics, including leaf area, specific leaf area, root-shoot relation, by radiation use efficiency for biomass production, as well as NO_3^-, NH_4^+, soluble N-red, protein N and total N determination.

MATERIAL AND METHODS

Maize hybrid CE 205 was used for experiments. Caryopses were placed 24 h in aerated water, then were germinated for 3 days on moistured filter paper. Seedlings with 2 cm radicles were planted in sand cultures. Mitscherlich's pots of 6 l volume filled with river sand were used. The sand in pots was washed by distilled water up to the NO_3^- content in eluate was lowered to zero and pH changed from 8 to 7. The pH of the sand was originally moderate alkaline. Fifteen maize plants were planted in each pot. Nutrient solution was then added in the amount which fully replaced the volume of distilled water. The nutrient solutions were supplied at 500 ml per pot every day and the eluates were analyzed for pH and NO_3^- contents. Nutrient solution with following composition was used: KH_2PO_4 3.6 mM, $CaCl_2.6\ H_2O$ 1.7 mM, $MgSO_4$ 2.1 mM, KCl 3.5 mM, Fe-chelaton 170 μM; following microelements were added: H_3BO_3 46 μM, $MnCl_2.4\ H_2O$ 7.4 μM, $ZnSO_4.7\ H_2O$ 0.5 μM, $CuSO_4$ 0.23 μM and $H_2MoO_4.H_2O$ 0.11 μM. The N treatments were 7 mM N added in the form of KNO_3, $(NH_4)_2SO_4$ and in the mixture half and half of both NO_3^-/NH_4^+. Kalium nitrate was replaced with kalium chloride in the treatment with NH_4^+-N. The content of $MgSO_4$ was lowered to one half in the treatment with NH_4^+-N. In all treatments with NH_4^+ nitrification inhibitor DIDIN in concentration 5 $mg.l^{-1}$ of nutrient solution was added (Peuke, Jeschke, 1993).

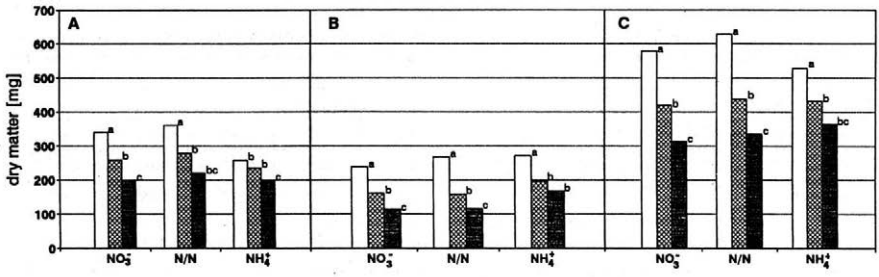
Experiments took place in a greenhouse during June, July and September of 1995. The plants were grown at three levels of solar irradiances established by shade wire net in single or double layers. At full sun light the irradiance reached 1 200 (without shading), 800 and 400 $\mu mol.m^{-2}.s^{-1}$ PPFD. Plants were harvested after 10 days growth, fresh and dry matters were established before and after drying at 70 °C in the oven with air circulation. Leaf area was established from linear measurements of leaves using correction factor 0.72 (Šesták et al., 1971). The NO_3^- content in plant tissue extracts was measured by means of selective liquid electrode (Šenkýř, Petr, 1979). Ammonium content in plant organs was determined after tissue homogenization and extraction with acidified (pH 3.0) 80% ethanol. Supernatant received after tissue extract centrifugation was evaporated at 40 °C, the rest was solved in distilled water and NH_4^+ was determined by the micro-Kjehldal method (Sugiharto, Sugiyama, 1992). Soluble N-red and protein N fractions were estimated after tissue extraction with 80% ethanol, following separation by filtration, acid digestion of both separated parts catalyzed by 30% H_2O_2 and NH_4^+ determination by means of Nessler's reagent. All analyses were at least triplicated. The experiment was repeated three times with very similar results. The results presented are from the first repetition. Experimental results were statistically processed by standard error calculation. Differences were evaluated by means of *t*-test method.

RESULTS

The values of NO_3^- content in pot eluates showed higher level of NO_3^- at the beginning of experiment and

I. Changes in NO_3^- content and pH values in the eluates of nutrient solution from the pots of sand culture with maize plants during 10 days of cultivation; all pots were irrigated every day with 0.5 l of corresponding fresh nutrient solution; the pH value of eluate in 0 day reached 7

N treatment	NO_3^- (mg.l^{-1})					pH	
Days of cultivation	0	4	6	8	10	8	10
NO_3^-	433	404	344	123	76.8	7.5	7.5
NO_3^- / NH_4^+	217	203	143	52	28.6	7.05	6.95
NH_4^+	8	10.0	7.2	1.8	0.4	6.74	6.67



1. Dry matter production of one maize plant after 10 days of growth in sand culture with 7 mM NO_3^-, 3.5 mM NO_3^- + 3.5 mM NH_4^+ (N/N) and 7mM NH_4^+ N nutrition at 1 200 µmol.m^{-2}.s^{-1} PPFD – white, 800 – cross hatched, 400 – hatched columns of solar irradiance; A – overground part, B – root, C – whole plant; values indexed by different letters are statistically different at 0.05 level

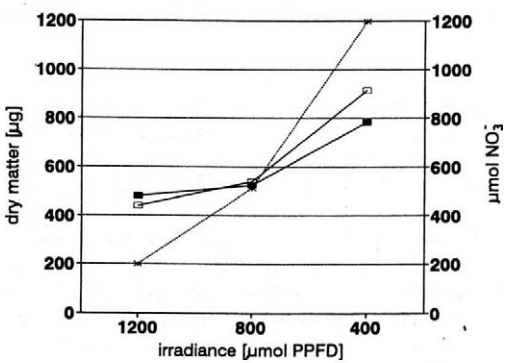
following decrease with increasing plant growth. The analyses showed that applied volume of nutrient solution was sufficient for full N nutrition during 10 days' growth of maize plants. Some low amount of NO_3^- appeared also in the eluates from the pots with NH_4^+ nutrition. It could be connected with insufficient inhibition of nitrification processes. This amount decreased rapidly with plant growth to negligible level. The pH values did not change too much and reflected moderate acidification in the case of NH_4^+ and moderate alkalinization in the case of NO_3^- nutrition which commonly occurs by the uptake of these ions (Tab. I).

Dry matter production of maize plants after 10 days' growth decreased in all three N treatments with shade. The decrease was high in NO_3^- and mixed N/N treatments and smaller in NH_4^+ treatment. It was caused by significant dry matter decrease of overground parts at full irradiance and root dry matter increase at 67% shade in comparison with NO_3^- treatment (Fig. 1).

Radiation use efficiency expressed as µg of dry matter production per µmol.m^{-2}.s^{-1} PPFD increased in both NO_3^- and NH_4^+ treatments with irradiance decrease. The values were lower for NH_4^+ treatment at full irradiance and higher at 67% shade against NO_3^- treatment (Fig. 2).

Leaf area increased in all three N treatments with shade. Ammonium nutrition diminished leaf area under full and 67% irradiance. Specific leaf area changed very similarly to leaf area which demonstrated that thinner leaves were produced as the light intensity decreased. SLA of NH_4^+ fed plants was also lowered at full irradiance (Tab. II). Root/shoot dry matter relation increased expressively with irradiance decrease in maize plants with NO_3^- nutrition. This relation was higher in NH_4^+ treated plants and decreased only moderately with shade (Tab. II).

Decreased irradiance caused remarkable increase of NO_3^- content in leaves as well as in roots – with higher level at 7 mM and lower level at 3.5 mM NO_3^- nutrition and remarkable higher content was established in roots. The content of NO_3^- in NH_4^+ fed plants was low in roots, a little higher in leaves and showed also increasing tendency with shade. The same increase of NO_3^- content in all N treatments with light decrease was also remarkable in whole plants (Tab. III). More than ten times lower content of NH_4^+ was estimated in NH_4^+ fed maize plants in comparison with NO_3^- content of NO_3^- fed plants. Moderate increase of its content was remarkable



2. Radiation use efficiency for biomass production of maize plants expressed in µg of dry matter produced per µmol.m^{-2}.s^{-1} PPFD during 10 days of growth with NO_3^- -■-, or NH_4^+ -▭- nutrition, and changes of NO_3^- content in µmol.kg^{-1} of dry matter related to irradiance unit -✳- at different irradiances

II. Leaf area (LA), specific leaf area (SLA) and root/shoot relation (R/S) of maize plants as influenced by N form and light intensity; differences in all following tables are statistically evaluated by *t*-test; values indexed with different letters are significantly different at 0.05 level

N treatment	LA ($cm^2.plant^{-1}$)			SLA ($m^2.kg^{-1}$)			R/S		
Light intensity (%)	100	67	33	100	67	33	100	67	33
NO_3^-	84.3^{ab}	86.6^{a}	96.2^{ac}	37.5^{a}	50.6^{dc}	72.8^{e}	0.70	0.62	0.58
NO_3^- / NH_4^+	87.5^{a}	91.3^{ac}	103^{c}	38.4^{a}	53.4^{d}	76.5^{e}	0.74	0.57	0.53
NH_4^+	69.0^{d}	75.3^{b}	94.5^{ac}	34.5^{b}	47.6^{c}	74.3^{e}	1.05	0.89	0.85

III. The content of NO_3^- and NH_4^+ in overground parts, roots and in whole maize plants as influenced by N form and light intensity

N treatment	Overground part			Root			Whole plant		
	NO_3^- ($\mu mol.g^{-1}$)								
Light intensity (%)	100	67	33	100	67	33	100	67	33
NO_3^-	177^{a}	253^{c}	322^{c}	333^{a}	664^{d}	748^{d}	241^{a}	410^{d}	479^{f}
NO_3^- / NH_4^+	31^{bf}	117^{d}	326^{e}	43^{b}	198^{e}	321^{a}	36^{b}	146^{e}	259^{a}
NH_4^+	28^{b}	29^{b}	37^{f}	12^{c}	14^{c}	20^{g}	19^{c}	22^{c}	29^{b}
	NH_4^+ ($\mu mol.g^{-1}$)								
NO_3^-	16.8^{a}	17.5^{a}	21^{c}	22.0^{a}	22.4^{a}	23.1^{aa}	18.9^{a}	19.4^{a}	21.8^{a}
NO_3^- / NH_4^+	20.7^{a}	27.1^{b}	29.6^{b}	28.2^{b}	40.2^{cd}	38.5^{cd}	23.9^{a}	31.8^{bc}	37.8^{c}
NH_4^+	23.9^{bc}	30.5^{b}	44.0^{d}	27.5^{b}	48.6^{de}	57.3^{e}	25.7^{b}	38.7^{c}	50.3^{d}

IV. The content of soluble N-red (S) and protein N (P) fractions and total N-red (T) in maize tissues as influenced by N form and light intensity

N treatment		Overground part			Root		
		$\mu mol.g^{-1}$					
Light intensity (%)		100	67	33	100	67	33
NO_3^-	S	682^{a}	707^{a}	776^{ab}	679^{a}	693^{a}	743^{ab}
	P	$2\,014^{e}$	$2\,116^{ef}$	$2\,255^{ef}$	$1\,007^{d}$	$1\,114^{de}$	$1\,114^{de}$
	T	$2\,696^{g}$	$2\,823^{gh}$	$3\,034^{hi}$	$1\,686^{g}$	$1\,807^{gh}$	$1\,857^{gh}$
NO_3^- / NH_4^+	S	702^{a}	806^{bc}	771^{ab}	664^{a}	729^{ab}	764^{ab}
	P	$2\,096^{ef}$	$2\,125^{ef}$	$2\,295^{f}$	$1\,086^{d}$	$1\,179^{de}$	$1\,207^{e}$
	T	$2\,798^{g}$	$2\,931^{ghi}$	$3\,065^{hi}$	$1\,750^{g}$	$1\,908^{ghi}$	$1\,971^{h}$
NH_4^+	S	743^{a}	846^{bc}	909^{cd}	750^{ab}	821^{bc}	857^{c}
	P	$2\,137^{ef}$	$2\,177^{ef}$	$2\,301^{f}$	$1\,186^{e}$	$1\,129^{def}$	$1\,253^{f}$
	T	$2\,880^{g}$	$3\,023^{ghi}$	$3\,210^{i}$	$1\,936^{hi}$	$1\,950^{hi}$	$2\,110^{i}$

with irradiance decrease in overground parts, roots as well as in whole plants. Higher content was estimated in roots than in overground parts. The same relations were noticed in N/N and NO_3^- treatments but with lower NH_4^+ contents (Tab. III).

Soluble N-red, protein N and total N contents increased in all N treatments with irradiance decrease in overground parts as well as in the roots. Ammonium nutrition increased all N-red fractions at lower irradiance in comparison with NO_3^- nutrition in both plant parts (Tab. IV).

When total N-red per plant was calculated a decreasing amounts of soluble as well as total N-red with decreasing light intensity were remarkable in all N treatments. Ammonium nutrition increased significantly total N-red amount at the lowest irradiance against NO_3^- nutrition. The highest amount of total N including NO_3^--N was found in plants with mixed and NO_3^- nutrition at full irradiance which was conditioned by the combination of higher dry matter and higher soluble N fraction. No differences were found in total N amount in plants of all N treatments at the lowest irradiance. It reflected the limitation of N uptake in the form of both ions at low irradiance (Tab. V).

DISCUSSION

Sand cultures represent relatively more comparable conditions with natural ones in comparison with hydro-

V. Soluble N-red (S) and total N-red (T) fractions, soluble and total N amounts in maize plants as influenced by N form and light intensity

N treatment		N-red			N-tot		
		µmol.plant^{-1}					
Light intensity (%)		100	67	33	100	67	33
NO_3^-	S	314^{a}	235^{b}	191^{c}	425^{a}	372^{ab}	311^{c}
	T	$1\,053^{de}$	815^{f}	649^{g}	$1\,169^{e}$	952^{f}	769^{g}
NO_3^-/NH_4^+	S	344^{a}	272^{b}	206^{c}	362^{b}	323^{bc}	275^{cd}
	T	$1\,180^{d}$	895^{ef}	722^{f}	$1\,198^{e}$	946^{f}	791^{fg}
NH_4^+	S	315^{a}	288^{ab}	258^{b}	323^{bc}	296^{c}	267^{cd}
	T	$1\,010^{e}$	875^{f}	788^{f}	$1\,018^{f}$	883^{fg}	796^{fg}

ponix. The study of numerous differences in NO_3^- and NH_4^+ nutrition is tightly connected with processes occurring in soil. As far as our experience following from preceding results concerns the reaction of some plants, especially in root-shoot relation on NH_4^+ nutrition, seems to be different in hydroponix and sand cultures (Plhák, 1994). The concentration of NO_3^- estimated in pot eluates showed that the amount and concentration of applied nutrient solution were not a limiting factor for plant growth. Biomass production of maize plants was reduced at full irradiance by NH_4^+ nutrition as compared with NO_3^- nutrition. Similar results of growth reduction were established also by Cramer, Lewis (1993b) with maize plants growing at 4 and 12 mM NH_4^+ nutrition in hydroponix at high irradiance. Growth inhibition increased with NH_4^+ concentration. This negative effects were remarkable in our experiments, namely in overground parts, they disappeared at lower (67%) irradiance and at the lowest used irradiance (33%) some benefit effects of NH_4^+ nutrition appeared. The results obtained by Magalhaes, Wilcox (1984) with tomato plants made at very similar conditions of NH_4^+ and irradiance levels are comparable with our ones with the exception of higher sensitivity of tomato plants and a positive effect at 67% shade did not appear. A positive effect of NH_4^+ against NO_3^- nutrition under lower irradiance conditions was noticed by Indica rice (Ta et al., 1981), as well as by sunflower plants (Plhák, 1994).

Negative effect of NH_4^+ nutrition at full irradiance evident as growth inhibition of overground parts was hardly connected with a direct effect of free NH_4^+ because its concentration estimated in plant tissues was rather low. Very similar low values of NH_4^+ content in maize leaves at 10 to 20 mM NH_4^+ and sufficient high level of K^+ in nutrition medium were established by Gerendas et al. (1995). Ammonium disruption of photosynthetic and especially mitochondrial electron transport processes connected with ATP formation occurred at fundamentally higher concentrations (Good et al., 1966; Lilley et al., 1975). Significant decrease in inorganic cation uptake occurs particularly at low pH values of nutrient medium (Engels, Marschner, 1993) which was not established in our experiments.

The highest biomass production appeared at mixed NO_3^-/NH_4^+ nutrition, though not significant during relatively short time of growth. It was established for numerous plants (Volk et al., 1992).

Radiation use efficiency expressed as dry matter production per irradiance unit was increasing with irradiance decrease. Plant with NH_4^+ nutrition showed lower radiation use for biomass production at full irradiance but higher radiation use at low irradiance in comparison with NO_3^- nutrition. It indicates again on some metabolic disadvantage of NH_4^+ fed plants at full irradiance and on metabolic advantage at low irradiance. After Salsac et al. (1987) energetic advantage of NH_4^+ metabolisation must be taken into account. By NO_3^- and not by NH_4^+ metabolisation 2 ATP for uptake and 15 ATP for its reduction are required.

Metabolic advantage of NH_4^+ nutrition under conditions of lower irradiance was reflected also in higher N-red contents both in soluble and protein fractions, as well as in N-red amount per plant. Higher content of soluble fractions especially amino acids and amides in connection with NH_4^+ nutrition was documented in many papers (Magalhaes, Wilcox, 1984; Cramer, Lewis, 1993a, b). The increase of NO_3^- content in maize tissues with irradiance decrease estimated also in many other plants (Magalhaes, Wilcox, 1984; Plhák, 1993, 1994) demonstrated that NO_3^- uptake did not decrease but its reduction and following metabolisation was lowered. Some decrease in nitrate reductase activity as one of possible cause was not established (data in present results not shown). It follows from the relation expressed in Fig. 2 that nitrate accumulation was highly sensitive to plant shading and a higher irradiance than approximately 900 µmol.m^{-2}.s^{-1} PPFD was necessary for sufficient NO_3^- reduction. Importance of high irradiance for NO_3^- metabolisation in sunflower and lucerne plants was demonstrated also in our preceding papers (Plhák, 1993, 1994). After these results the saturation irradiance for NO_3^- assimilation may be in some plants higher than for photosynthetic carbon assimilation.

The energetic advantage of NH_4^+ metabolisation under some level of irradiance can differ with plant species and plant localities. For plant growing in canopies only the top leaves receive full irradiance while the leaves of lower insertions as well as shade plants re-

ceive large scale of lower irradiances, much of which may be under light saturation of NO_3^- reduction.

REFERENCES

CRAMER, M. D. – LEWIS, O. A. M.: The influence of nitrate and ammonium on the growth of wheat (*Triticum aestivum*) and maize (*Zea mays*) plants. Ann. Bot., *72*, 1993a: 359–365.

CRAMER, M. D. – LEWIS, O. A. M.: The influence of NO_3^- and NH_4^+ nutrition on the carbon and nitrogen partitioning characteristics of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) plants. Pl. and Soil, *154*, 1993b: 289–301.

ENGELS, C. – MARSCHNER, H.: Influence of nitrogen supply on root uptake and translocation of cations in the xylem exudate of maize (*Zea mays* L.). J. Exp. Bot., *44*, 1993: 1695–1701.

GERENDAS, J. – RATCLIFFE, R. G. – SATTELMACHER, B.: The influence of nitrogen and potassium supply on the ammonium content of maize (*Zea mays* L.) leaves including a comparison of measurements made *in vivo* and *in vitro*. Pl. and Soil, *171*, 1995: 11–20.

GOOD, N. – IZAWA, S. – HIND, G.: Uncoupling and energy transfer inhibition in photophosphorylation. In: SANADI, D. R. (ed.): Current Topics in Bioenergetics. New York, Academic Press 1966: 75–112.

GOYAL, S. S. – LORENS, O. A. – HUFFAKER, R. C.: Inhibitory effects of ammoniacal nitrogen on growth of radish plants. I. Characterization of toxic effects of NH_4^+ on growth and its alleviation by NO_3^-. J. Amer. Soc. Hort. Sci., *107*, 1982: 125–129.

HAYNES, R. J. – GOH, K. M.: Ammonium and nitrate nutrition of plants. Biol. Rev., *53*, 1978: 465–510.

INGESTAD, T.: Nitrogen and cation nutrition of three ecologically different plant species. Physiol. Plant., *38*, 1976: 29–34.

LEWIS, O. A. M. – LEIDI, E. O. – LIPS, S. H.: Effect of nitrogen source on growth response to salinity stress in maize and wheat. New Phytol., *111*, 1989: 155–160.

LILLEY, R. M. – FITZGERALD, M. P. – RIENITS, K. G. – WALKER, D. A.: Criteria of intactness and the photosynthetic activity of spinach chloroplast preparations. New Phytol., *75*, 1975: 1–10.

MAGALHAES, J. R. – WILCOX, C. E.: Ammonium toxicity development in tomato plants relative to nitrogen form and light intensity. J. Pl. Nutr., *7*, 1984: 1477–1496.

MENGEL, K. – KIRKBY, E. A.: Ionic balance in different tissue of the tomato plant in relation to nitrate, urea, or ammonium nutrition. Pl. Physiol., *64*, 1967: 6–14.

PEUKE, A. – JESCHKE, D.: The uptake and flow of C, N and ions between roots and shoots in *Ricinus communis* L. I. Growth with ammonium or nitrate as nitrogen source. J. Exp. Bot., *44*, 1993: 1167–1176.

PLHÁK, F.: Changes of growth and nitrate content in alfalfa plants under conditions of artificial and natural irradiance. Rostl. Výr., *39*, 1993 (1): 85–91.

PLHÁK, F.: The reaction of sunflower and alfalfa on nitrate and ammonium nutrition in water and sand cultures at three levels of light intensities. Rostl. Výr., *40*, 1994: 729–739.

ROBERTS, J. K. M. – PANG, M. K. L.: Estimation of ammonium ion distribution between cytoplasm and vacuole using nuclear magnetic resonance spectroscopy. Pl. Physiol., *100*, 1992: 1571–1574.

SALSAC, L. – CHAILLOU, S. – MORROT-GAUDRY, J. F. – LESAINT, C. – JOLIVET, E.: Nitrate and ammonium nutrition in plants. Pl. Physiol. Biochem., *25*, 1987: 805–812.

SPIERS, J. M.: Effects of pH level and nitrogen source on elemental leaf content of Tifblue rabbiteye blueberry. J. Amer. Soc. Hort. Sci., *103*, 1978: 705–708.

SUGIHARTO, B. – SUGIYAMA, T.: Effects of nitrate and ammonium on gene expression of phosphoenolpyruvate carboxylase and nitrogen metabolism in maize leave tissue during recovery from nitrogen stress. Pl. Physiol., *98*, 1992: 1403–1408.

ŠENKÝŘ, J. – PETR, J.: Nitrate ion selective electrode. Chem. Listy, *73*, 1979: 1097.

ŠESTÁK, Z. – ČATSKÝ, J. – JARVIS, P. G.: Plant photosynthetic production. Manual of methods. The Hague, Dr. V. Junk, N. V. Publ. 1971: 672–701.

TA, T. C. – TSUTSUMI, N. – KURIKARA, K.: Comparative study on the response of Indica and Japonica rice plants to ammonium and nitrate nitrogen. Soil Sci. Pl. Nutr., *27*, 1981: 83–92.

VOLK, R. – CHAILLOU, S. – MARIOTTI, A. – MORROT-GAUDRY, J. F.: Beneficial effects of concurrent ammonium and nitrate nutrition on the growth of *Phaseolus vulgaris*: a ^{15}N study. Pl. Physiol. and Biochem., *30*, 1992: 487–493.

Received on January 17, 1996

Contact Address:

Doc. RNDr. František Plhák, CSc., Masarykova univerzita, Přírodovědecká fakulta, Kotlářská 2, 611 37 Brno, Česká republika, tel.: 05/41 12 95 57, fax: 05/41 21 12 14

FYZIOLOGICKÉ UKAZOVATELE RASTU A PRODUKCIE BÔBU KONSKÉHO (*VICIA FABA* L.)

PHYSIOLOGICAL INDICATORS OF GROWTH AND PRODUCTION OF FABA BEAN (*VICIA FABA* L.)

A. Kostrej

University of Agriculture, Nitra, Slovak Republic

ABSTRACT: Physiological indicators of growth and production of faba bean were quantified on the basis of pot and field trials. Pot trials were established by the method of gradual sowing rates during the growing season in partially controlled conditions of external environment. Experimental results on physiological indicators of growth and production: net output of photosynthesis *NAR*, specific rate of growth *RGR*, rate of dry matter increment per area *CGR*, leaf area index *LAI*, relative leaf area *LAR*, data on average daily temperature *T* and intensity of solar radiation I_{GR} are presented in the paper. Regression dependence of *NAR*, *T* and I_{GR} was found. This information can be used for the needs of mathematical modelling of crop models of growth and production. Field model trials were established to study the effect of density ranging from 23.0 to 139.0 plants per 1 m^2 of stand on physiological characteristics of growth and production. Regression dependence *NAR* = f(*LAI*) was set on a broad interval of stand density. It was found that *CGR* more depends on *LAI* than on *NAR*. Stand density control results in the changes of *LAI*, *NAR* and other physiological indicators of growth and production.

faba bean; physiological characteristics of growth; production; field and pot trials

ABSTRAKT: Bola urobená kvantifikácia fyziologických ukazovateľov rastu a produkcie bôbu konského z nádobových a poľných pokusov. Nádobové pokusy boli zakladané metódou postupných výsevov počas vegetácie v čiastočne regulovateľných podmienkach vonkajšieho prostredia. Sú uvedené experimentálne výsledky o fyziologických ukazovateľoch rastu a produkcie – čistý výkon fotosyntézy *NAR*, špecifická rýchlosť rastu *RGR*, rýchlosť prírastku sušiny na plochu *CGR*, index listovej pokryvnosti *LAI*, pomerná olistenosť *LAR*, údaje o priemernej dennej teplote *T* a príkon slnečného žiarenia I_{GR}. Zistila sa regresná závislosť *NAR*, *T* a I_{GR}. Tento poznatok sa môže využiť pre potreby matematického modelovania plodinových modelov rastu a produkcie. Poľné modelové pokusy sa založili za účelom štúdia vplyvu hustoty v rozsahu 23,0 až 139,0 rastlín na 1 m^2 porastu na fyziologické charakteristiky rastu a produkcie. Na širokom rozpätí zahustenia porastu sa stanovila regresná závislosť *NAR* = f(*LAI*). Zistilo sa, že *CGR* je viacej závislá od *LAI* ako od *NAR*. Reguláciou hustoty porastu sa mení *LAI*, *NAR* a ďalšie fyziologické ukazovatele rastu a produkcie.

bôb konský; fyziologické charakteristiky rastu; produkcia; nádobové a poľné pokusy

ÚVOD

Fyziologické ukazovatele rastu a produkcie bôbu konského, podobne ako u iných poľných plodín, sa realizujú v prostredí pôda – porast – atmosféra. Závisia od stavu porastu (Vrkoč, 1977), jeho fyziologických ukazovateľov (Cooper, 1975; Kostrej, Repka, 1993) a zmien podmienok a faktorov vonkajšieho prostredia (Petr a kol., 1988). Mnohé z týchto podmienok vonkajšieho prostredia sú človekom regulovateľné a ovplyvniteľné.

V predloženej práci sme si dali za cieľ experimentálne kvantifikovať niektoré fyziologické ukazovatele rastu a produkcie v závislosti od zmien človekom regulovateľných podmienok vonkajšieho prostredia.

MATERIÁL A METÓDA

Získané experimentálne výsledky sa opierajú o nádobové a poľné pokusy s odrodou bôbu konského Považsky v podmienkach juhozápadného Slovenska, experimentálne miesto bola Nitra.

Pri zakladaní nádobových pokusov metódou postupných výsevov sa počas vegetácie v krátkych intervaloch testovali rastovo rovnocenné rastliny vo fáze dvoch párov pravých listov tak, aby sa mohol vyhodnotiť účinok teploty a hustoty toku žiarenia na niektoré fyziologické ukazovatele rastu a produkcie. Počas experimentálneho obdobia sa urobilo 14 odberov v štyroch opakovaniach.

Rýchlosť celkového dýchania sa stanovila postupom, ktorý opísal McRee (1974).

I. Parametre (poradové číslo hustoty ČH, výživná plocha jednej rastliny VP.10^{-4} m^2, počet rastlín PR.m^{-2}) poľného pokusu založeného metódou rovnomerného zahustenia – Indicators (order number of density ČH, nutritive area of one plant VP.10^{-4} m^2, number of plants PR.m^{-2}) of field trial established by the method of uniform thickening

ČH	1	2	3	4	5	6	7	8	9	10
VP	444,3	341,2	294,0	218,9	179,5	146,1	118,9	107,3	92,0	71,9
PR	22,0	27,8	34,0	45,6	55,7	68,4	84,6	93,2	108,6	139,0

II. Termíny odberu vzoriek počas vegetácie – Dates of sampling during the growing season

Poradové číslo odberu[1]	Fenofáza[2]	Dátum odberu[9]	Počet dní medzi odbermi[10]
1	steblovanie[3]	5. 6.	–
2	pred kvitnutím[4]	11. 6.	6
3	plné kvitnutie[5]	18. 6.	7
4	začiatok vývoja strukov[6]	30. 6.	12
5	plný vývoj strukov[7]	14. 7.	14
6	fyziologická zrelosť[8]	11. 8.	28

[1]order number of sampling, [2]phenological stage, [3]shooting, [4]prior to anthesis, [5]full flowering, [6]start of pod development, [7]full pod development, [8]physiological ripeness, [9]date of sampling, [10]number of days between samplings

Poľné modelové pokusy (tab. I) sa založili metódou pravidelného zahusťovania podľa postupu, ktorý publikoval Kostrej (1992). Pokus prebiehal na hlinitej pôde so základnou dávkou 240 kg NPK.ha^{-1}. Na zabezpečenie presného počtu rastlín v každom stupni zahustenia sa sejba urobila ručne. Počas vegetácie sa odoberali vzorky v definovaných rastových fázach (tab. II), z každej hustoty v štyroch opakovaniach. Spôsob odberu vzoriek rastlinného materiálu v obidvoch typoch pokusov sa robil podľa zásad metódy rastovej analýzy (Kostrej, 1992). Listová plocha sa určovala metódou priameho váženia listov. Z hodnôt celkovej sušiny rastlín, sušiny listov a veľkosti listovej plochy sa vypočítali fyziologické ukazovatele rastu a produkcie: špecifická rýchlosť rastu *RGR*, čistý výkon fotosyntézy *NAR*, rýchlosť prírastku sušiny na plochu *CGR*, index listovej pokryvnosti *LAI*, pomerná olistenosť *LAR*.

Merania priemernej dennej teploty T a príkonné hustoty toku žiarenia I_{GR} sa robili na agrometeorologickej stanici VŠP v Nitre.

VÝSLEDKY A DISKUSIA

Experimentálne výsledky o fyziologických ukazovateľoch rastu a produkcie získané z pokusov v čiastočne regulovateľných podmienkach minerálnej výživy a vodného režimu ako priemerné hodnoty *NAR*, *RGR*, *LAI*, *LAR*, T a I_{GR} sú uvedené v tab. III a IV.

Porovnanie týchto údajov ukazuje, že zmeny T a I_{GR} vyvolávajú zmeny fyziologických ukazovateľov rastu a produkcie u bôbu s maximálnymi hodnotami v júni a v júli. Maximálne hodnoty *NAR* sa vždy nezhodujú s maximálnymi hodnotami T a I_{GR}, a to pravdepodobne preto, lebo *NAR* je výsledkom vzťahu medzi rýchlosťou fotosyntézy a dýchania, ktorý je teplotou rozdielne ovplyvňovaný.

III. Zmeny priemernej dennej teploty T (°C) a hustoty toku žiarenia I_{GR} (KJ.m^{-2}.d^{-1}) počas experimentálneho obdobia – Changes of average daily temperature T (°C) and radiation flux density I_{GR} (KJ.m^{-2}.d^{-1}) during experimental period

Mesiace[1]	Časový interval[2]	T	I_{GR}
5.	1	11,0	5 222
	2	15,0	5 160
	3	16,0	5 765
	$\bar{x}$	14,0	5 382
6.	1	18,0	7 329
	2	19,0	10 121
	3	25,0	18 328
	$\bar{x}$	20,6	9 892
7.	1	22,0	12 253
	2	21,5	12 873
	3	21,5	10 658
	$\bar{x}$	21,5	11 928
8.	1	19,0	7 709
	2	17,0	8 235
	3	14,0	6 666
	$\bar{x}$	16,6	7 536
9.	1	9,0	4 651
	2	8,0	3 000
	3		
	$\bar{x}$	8,5	3 825

[1]months, [2]time interval

Funkčné vzťahy s koeficientami regresnej rovnice medzi *NAR*, *R*, T a I_{GR} majú nasledovný tvar:

IV. Priemerné hodnoty čistého výkonu fotosyntézy *NAR* ($g.m^{-2}.d^{-1}$), špecifickej rýchlosti rastu *RGR* ($g.g^{-1}.d^{-1}$), rýchlosti prírastku sušiny na plochu *CGR* ($g.m^{-2}.d^{-1}$), indexu listovej pokryvnosti *LAI* ($m^2.m^{-2}$), pomernej olistenosti *LAR* ($m^2.g^{-1}$), priemernej dennej teploty *T* (°C) a príkonu žiarenia I_{GR} ($KJ.m^{-2}.d^{-1}$) – Average values of net output of photosynthesis *NAR* ($g.m^{-2}.d^{-1}$), specific rate of *RGR* growth ($g.g^{-1}.d^{-1}$), rate of dry matter increment per *CGR* area ($g.m^{-2}.d^{-1}$), leaf area index *LAI* ($m^2.m^{-2}$), relative leaf area *LAR* ($m^2.g^{-1}$), average daily temperature *T* (°C) and radiation intensity I_{GR} ($KJ.m^{-2}.d^{-1}$)

Ukazovateľ[1]	Mesiace[2]				
	5.	6.	7.	8.	9.
NAR	5,84	9,07	9,02	7,02	3,32
RGR	0,05	0,05	0,07	0,05	0,04
CGR	7,95	9,09	10,88	8,34	2,99
LAI	1,30	0,99	1,20	1,16	0,69
LAR	0,85	0,60	0,82	0,76	0,99
T	14,00	20,67	21,60	16,60	8,50
I_{GR}	5 382	9 892	11 928	7 536	3 825

[1]indicator, [2]months

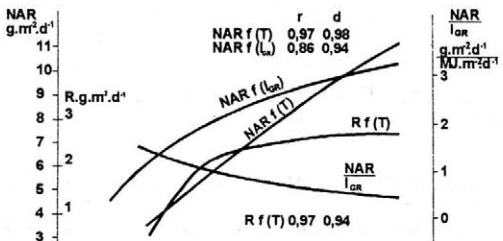
$$NAR = 0{,}635\ (T^{0{,}863}) \quad g.m^{-2}.d^{-1}$$

$$NAR = 0{,}295 + (3{,}300.\ln I_{GR}) \quad g.m^{-2}.d^{-1}$$

$$R = 0{,}179\ (T^{0{,}900}) \quad g.g^{-1}.d^{-1}$$

Kvantifikované funkčné vzťahy $NAR = f(T)$, $NAR = f(I_{GR})$, $R = f(T)$ s hodnotami korelačných koeficientov *r* a koeficientov determinácie *d* sú znázornené na obr. 1. Zo znázornených hodnôt je zrejmé, že v závislosti od hodnôt *T* a I_{GR} sa mení hodnota *NAR* v podmienkach dostatočného zásobenia rastlín vodou a živinami, so zväčšovaním I_{GR} a s prírastkom hmotnosti sušiny vyjadrenej hodnotami *NAR* sa zvyšuje nelineárne rozdielnou rýchlosťou v závislosti od hodnôt *T*. Vplyv teploty vzduchu na priebeh hodnôt *NAR* sa uskutočňuje cez vzájomné pôsobenie teploty na fotosyntézu a dýchanie.

Od vzájomných vzťahov týchto dvoch procesov v nelimitujúcich podmienkach zásobenia rastlín vodou a minerálnymi živinami závisí potom konečný výsledok fotosyntetickej činnosti. Tento poznatok sa môže využiť nielen pre modelovanie fotosyntézy a dýchania, ale aj pre určenie rastu biomasy ako kvantitatívneho výsledku ich vzájomných vzťahov. Využívajúc tento princíp, môžeme zmeny rastu suchej biomasy určiť pomocou matematického modelovania v rôznych ekologických situáciách a kombináciách základných faktorov vonkajšieho prostredia.

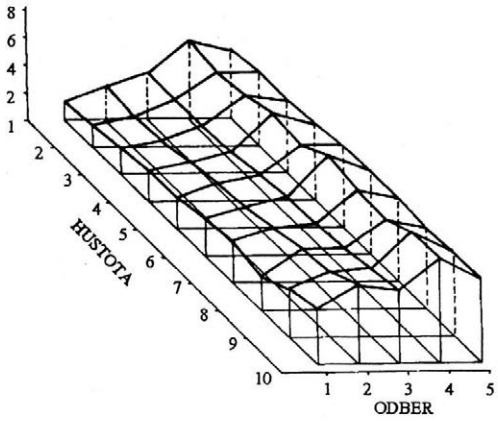


1. Funkčné vzťahy medzi čistým výkonom fotosyntézy *NAR* ($g.m^{-2}.d^{-1}$), celkovým dýchaním *R* ($g.g^{-1}.d^{-1}$), hustotou toku žiarenia I_{GR} ($KJ.m^{-2}.d^{-1}$) a priemernou dennou teplotou *T* (°C) – Functional relationships between net output of photosynthesis *NAR* ($g.m^{-2}.d^{-1}$), total respiration *R* ($g.g^{-1}.d^{-1}$), radiation flux density I_{GR} ($KJ.m^{-2}.d^{-1}$) and average daily temperature *T* (°C)

Priebeh fyziologických ukazovateľov rastu a produkcie bôbu konského, zisťovaný v nelimitujúcich podmienkach pestovania, ukázal, že ich realizácia je determinovaná špecifickými reakciami rastlín na konkrétne podmienky prostredia. Súbežne s nádobovými pokusmi boli preto založené aj poľné pokusy za účelom sledovania vplyvu rozdielnej hustoty porastu na fyziologické ukazovatele rastu a produkcie.

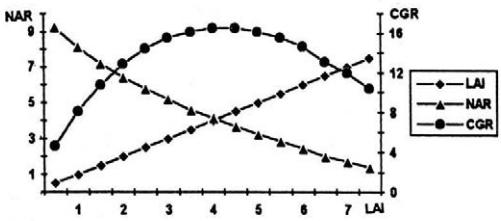
S procesom zahustenia porastu sa zvyšuje celková produkcia biomasy za súčasného znižovania individuálnej hmotnosti rastlín. Celková produkcia porastu je daná integráciou počtu rastlín a individuálnej hmotnosti. Zvolená štruktúra zahustenia porastu v rozsahu od 23 do 139 rastlín na 1 m^2 už v začiatočných fázach rastu určuje rozdielnu individuálnu hmotnosť, a tým aj celkovú produkciu biomasy. S postupom rastu sa tieto rozdiely zvyšujú natoľko, že na konci vegetačného obdobia pri zbere úrody u hustejších porastov nastáva také veľké zníženie individuálnej hmotnosti rastlín, že počet rastlín nestačí kompenzovať túto veličinu a dochádza k zníženiu celkovej produkcie.

Z hľadiska fyziológie produkčného procesu ovplyvňuje zahustenie veľmi výrazne veľkosť listovej plochy a celkovú listovú pokryvnosť porastu (Petr a kol., 1974; Vrkoč, 1977). Priebeh rastu listovej plochy uvádza obr. 2. V závislosti od zmien indexu listovej plochy sa mení aj výkonové zložky produkčného procesu, ako je *NAR* a *CGR*. So zvyšovaním *LAI* sa hodnoty *NAR* znižujú. *NAR* ako funkciu *LAI* môžeme vyjadriť rovnicou $NAR = 17{,}15 - (7{,}88\ LAI)^{1/3}$ s hodnotou korelačného koeficientu $r = 0{,}95$ a koeficientu determinácie $d = 0{,}91$. Hodnoty *CGR* sa so zvyšovaním *LAI*



2. Zmeny indexu listovej pokryvnosti *LAI* ($m^2.m^{-2}$) počas vegetácie v závislosti od zahustenia porastu – Changes of leaf area index *LAI* ($m^2.m^{-2}$) during the growing season as depended on stand thickening

hustota – density
odber – sampling



3. Zmeny čistého výkonu fotosyntézy *NAR* ($g.m^{-2}.d^{-1}$) a rýchlosti rastu *CGR* ($g.m^{-2}.d^{-1}$) v závislosti od zmien veľkosti listovej plochy *LAI* ($m^2.m^{-2}$) – Changes in net output of photosynthesis *NAR* ($g.m^{-2}.d^{-1}$) and rate of growth *CGR* ($g.m^{-2}.d^{-1}$) as depended on changes of leaf area index *LAI* ($m^2.m^{-2}$)

zvyšujú. Vzájomné vzťahy medzi *NAR* a *CGR* v závislosti od veľkosti *LAI* sú znázornené na obr. 3.

Z urobenej analýzy môžeme usudzovať, že fyziologické ukazovatele rastu produkcie a tvorby úrody (Walter, 1971; Cooper, 1973) podliehajú konkurenčným vzťahom o faktory prostredia. Týka sa to nielen koreňovej výživy, ale predovšetkým faktorov určujúcich tvorbu a výkonnosť nadzemných častí, najmä veľkosti *LAI*. Veľkosť listovej plochy reguluje svetelné pomery v poraste, ktoré v procese morfogenézy ovplyvňujú tvorbu reprodukčných orgánov. Toto spolu so znižovaním výkonových jednotiek produkcie, zvlášť *NAR* a *CGR*, znižuje po prekročení istého stupňa zahustenia produkčnú výkonnosť z porastu.

LITERATÚRA

COOPER, J. P.: Controls of photosynthesis production in terestrial systems. In: Photosynthesis and productivity indifferent environments. Int. Biol. Prog. Cambridge, London, New York, Melbourne, 1973: 593–621.

COOPER, J. P.: Actual and potential production in photosystems. In: Photosynthesis and productivity indifferent environments. Cambridge, London, Cambridge Univ. Press 1975: 198–210.

KOSTREJ, A.: Kvantitatívne charakteristiky a modelovanie produkčného procesu poľných plodín. Nitra, 1992.

KOSTREJ, A. – REPKA, J.: Závislosť fotosyntézy na zmenách príkonu slnečného žiarenia a teploty. Rostl. Výr., *39*, 1993 (7): 595–601.

McREE, K. J.: Equations for the rate of dark respiration of white clover and grain sorghum as function of dry weight, photosynthetic rate and temperature. Crop Sci., *14*, 1974 (4): 509–514.

PETR, J. a kol.: Hrach a bôb. Praha, SZN 1974.

PETR, J. a kol.: Yield formation in the main field crops. Praha, 1988: 186–190.

VRKOČ, F.: K dynamice růstu a produktivitě hlavních polních plodin. In: Produkce biomasy a tvorba výnosu polních plodin, Praha, ČSVTS SZ 1977: 13–23.

WALTER, R.: Vzťah agrotechniky a výživy k tvorbe reprodukčných orgánov u kolbu. Praha, ÚVTI 1971.

Došlo 17. 1. 1996

Kontaktná adresa:

Prof. Ing. Anton Kostrej, DrSc., Vysoká škola poľnohospodárska, A. Hlinku 2, 949 76 Nitra, Slovenská republika, tel.: 087/51 17 51, fax: 087/41 14 51

ENDOGÉNNY ETYLÉN V PROCESE KLÍČENIA JARNÉHO JAČMEŇA (*HORDEUM VULGARE* L.)

ENDOGENOUS ETHYLENE IN THE PROCESS OF SPRING BARLEY (*HORDEUM VULGARE* L.) GERMINATION

Z. Jureková, J. Mika

University of Agriculture, Nitra, Slovak Republic

ABSTRACT: In spring barley plants (*Hordeum vulgare* L.) cultivated in natural conditions of southern Slovakia aging and and dying of leaves of highest layers by the desiccant Reglone was induced. Subsequently, their relationship to biological properties of new grain generation was studied. Our results did not confirm the predicted relationship. Grain germination after dormancy was not affected significantly by premature dying of leaves of parent plant. Endogenous ethylene, whose amount increased in dependence on length of germination, was detected in germinating grains. There were no differences between germinative plants of the control treatment and treatments with induced aging of leaves in amount of ethylene produced. Dry matter weight of germinating grain fell in the process of germination what was in negative correlation with amount of releasing ethylene. Their regression relationship is expressed by correlation coefficient $r = -0.725163$.

aging of leaves; grain germination; endogenous ethylene; dry matter weight; content of assimilation pigments

ABSTRAKT: U rastlín jarného jačmeňa (*Hordeum vulgare* L.) pestovaných v prirodzených podmienkach južného Slovenska sme indukovali starnutie a odumieranie listov najvyšších etáží desikantom Reglone. Následne bol sledovaný ich vzťah k biologickým vlastnostiam novej generácie zŕn. Naše výsledky predpokladaný vzťah nepotvrdili. Klíčenie zŕn po dormancii nebolo preukazne ovplyvnené predčasným odumieraním listov materskej rastliny. V klíčiacich zrnách sme detekovali endogénny etylén, ktorého množstvo stúpalo v závislosti od dĺžky klíčenia. V množstve produkovaného etylénu sme nezaznamenali rozdiely medzi klíčnymi rastlinami kontrolného variantu a variantov s indukovaným starnutím listov. V procese klíčenia klesala hmotnosť sušiny klíčiaceho zrna, čo je v negatívnej korelácii s množstvom uvoľňovaného etylénu. Ich regresný vzťah je vyjadrený korelačným koeficientom $r = -0,725163$.

starnutie listov; klíčenie zrna; endogénny etylén; hmotnosť sušiny; obsah asimilačných farbív

ÚVOD

U jednoročných rastlín sú korelatívne vzťahy vegetatívnych a generatívnych orgánov v posledných fázach ontogenézy charakteristické kompetíciou o asimiláty a endogénne hormóny. Ako uvádzajú Prioul, Dugué (1992), starnutie vegetatívnych častí je urýchľované prednostnou translokáciou asimilátov do mladých rastových centier, u obilnín predstavovaných zrnami.

Proces starnutia asimilačných orgánov negatívne ovplyňuje dĺžku a rýchlosť akumulácie organickej hmoty zrnami, pričom dĺžka napĺňania zrna je v pozitívnej korelácii s produktivitou (Crafts-Brandner, Poneleit, 1992). Je otázne, či sa pozitívna korelácia vzťahuje i na kvalitatívne, resp. biologické vlastnosti novej generácie zŕn.

Životné prejavy semien po dormancii sú obyčajne charakterizované procesom klíčenia. Z hormonálneho hľadiska je najmä u obilnín dobre preštudovaná úloha giberelínov (Yamada, 1982; Gaskin et al., 1984) a kyseliny abscisovej (King, 1982; Jacobsen, Chandler, 1985). Takmer absentujú údaje o regulačnej funkcii a dynamike endogénneho etylénu v procese klíčenia obilnín.

V našej experimentálnej práci sme v prirodzených podmienkach pestovania urýchľovali starnutie a odumieranie listov najvyšších etáží jarného jačmeňa aplikáciou desikantu Reglone v definovaných rastovo-fenologických fázach. Po dozretí a dormancii zrna sme testovali klíčenie a dynamiku endogénneho etylénu uvoľňovaného klíčnymi rastlinami.

MATERIÁL A METÓDA

Zrno jarného jačmeňa odrody Bonus bolo získané z rastlín dopestovaných v prirodzených podmienkach poľného pokusu. Pokus bol založený na hnedozemi so základným hnojením 80 kg $P.ha^{-1}$, 120 kg $K.ha^{-1}$ a jarným prihnojením LAV v dávke 30 kg $N.ha^{-1}$. Výsevok v prepočte na 1 ha bol 4 mil. klíčivých zŕn. V zapoje-

nom poraste boli vytvorené štyri pokusné parcelky (20 m^2), na ktorých boli pokusné rastliny ošetrené 0,2% vodným roztokom Reglone v nasledovných rastovo-fenologických fázach (Zadoks et al., 1974):

1. fáza objavenie posledného listu (7. fáza podľa Zadoksa);
2. fáza zdurenie pošvy posledného listu (10. fáza podľa Zadoksa);
3. fáza koniec kvitnutia, začiatok tvorby zrna (fáza 10.5.4. podľa Zadoksa);
4. súbežne s každou aplikáciou boli rastliny kontrolného variantu ošetrené vodou.

Množstvo roztoku aplikovaného na rastliny v rámci pokusnej parcely bolo 1 800 ml. U pokusných rastlín bola sledovaná rastovo-fenologická fáza, etapa organogenézy podľa Kupermanovej a vizuálne príznaky starnutia a odumierania listov. Na siedmy deň po ošetrení rastlín v rámci variantu bol v troch najvyššie postavených listoch stanovený celkový obsah asimilačných farbív (Šesták, Čatský, 1966).

Po zbere bolo získané zrno uložené do sklenených prachovníc, ktoré boli umiestnené v stabilných teplotných podmienkach, v tme. Dormancia trvala 210, 224 a 238 dní. V uvedených termínoch bolo zo vzorky náhodným výberom vybraných 4 x 100 zŕn, v ktorých bolo testované klíčenie.

Klíčenie prebiehalo v Petriho miskách na dvoch vrstvách filtračného papiera pravidelne zvlhčovaného destilovanou vodou. Kultivácia sa uskutočnila v klimatizovanom boxe pri teplote 17 °C ± 0,2 °C, 80% vlhkosti, intenzite osvetlenia 78,4 $KJ.m^{-2}.s^{-1}$ a 14h dennom režime. Klíčenie bolo vyhodnocované po 48, 72, 96 a 120 h od založenia pokusu, posudzované bolo vizuálne. Za klíčenie sme považovali stav, keď bolo prerazené osemenie a viditeľný vrchol radikuly. V každom intervale hodnotenia počtu vyklíčených zŕn bola časť materiálu odobratá na stanovenie hmotnosti sušiny. Sušina bola stanovená vysušením vzoriek do konštantnej hmotnosti pri 105 °C.

Súbežne s testami klíčenia boli pripravené v rovnakých podmienkach aj vzorky klíčiacich zŕn, v ktorých bol detekovaný endogénny etylén. Časové intervaly detekcie sú v súhlase s intervalmi, v ktorých bolo testované klíčenie.

Zrná klíčili v kyvete z plexiskla vystlanej filtračným papierom s pravidelným zvlhčovaním. Kyvetu sme použili na minimalizovanie (odstránenie) poranení spôsobených poškodením klíčnych rastlín (pretrhnutím koreňov pri manipulácii). V každom intervale boli kyvety pred začiatkom inkubačnej doby na stanovenie endogénneho etylénu hermeticky uzatvorené. Dĺžka inkubácie bola 5 h pri teplote 23 °C a osvetlení 78,4 $KJ.m^{-2}.s^{-1}$.

Vzorky plynu boli odobraté injekčnými striekačkami cez silikónové septum a na niekoľko minút (do začiatku analýzy) fixované zapichnutím do gumovej zátky. Z každej kyvety (opakovania) boli odobraté dve až tri vzorky pre analýzu.

Pre kvantifikáciu etylénu sme použili plynový chromatograf CHROM 5 so sklenenou náplňovou kolónou s vnútorným priemerom 3 mm a dĺžkou 2,5 m. Použitá stacionárna fáza bola Porapak Q (100/120 mesh), nosný plyn bol dusík. Na detekciu bol použitý plamenný ionizačný detektor (FID). Po analýze bol zistený presný objem plynu v kyvete a neskôr stanovená suchá hmotnosť klíčiacich zŕn. Produkcia etylénu je vyjadrená v $nl.g^{-1}$ suchej hmoty za 1 h. Na štatistickú analýzu sme použili počítačový štatisticko-grafický systém Statgraphics.

VÝSLEDKY

Rastovo-fenologické pozorovania pokusných rastlín ošetrených prípravkom urýchľujúcim starnutie a odumieranie listov jarného jačmeňa potvrdili skutočnosť, že príznakmi starnutia, ktoré sa prejavovali žltnutím a postupným usýchaním listu, bol postihnutý vždy najstarší list. Teda orgán, u ktorého sa aj v rámci jeho individuálnej ontogenézy začali degradačné procesy súvisiace so starnutím. Z hľadiska ontogenézy rastliny to bol po ošetrení v 7. rastovo-fenologickej fáze 8. list, v 10. rastovo-fenologickej fáze 9. list, vo fáze 10.5.4. 10. list.

Stanovenia celkového obsahu asimilačných farbív v jednotlivých listoch pri odbere 7 dní po ošetrení posledného variantu (tab. I) poukazujú na výrazný pokles celkového obsahu farbív v najstarších listoch každého variantu a korešpondujú s uvedenými pozorovaniami.

I. Celkový obsah asimilačných farbív ($mg.m^{-2}$) v listoch najvyšších etáží jarného jačmeňa po desikácii – Total content of assimilation pigments ($mg.m^{-2}$) in leaves of highest layers of spring barley after desiccation

Postavenie listu na stonke[1]	Variant[2]			
	1	2	3	kontrola[3]
8.	51,89	–	–	139,64
9.	76,24	63,71	–	147,94
10.	98,42	104,15	49,92	159,74
11.	118,22	127,5	129,15	167,12

[1]position of leaf on stem, [2]treatment, [3]control

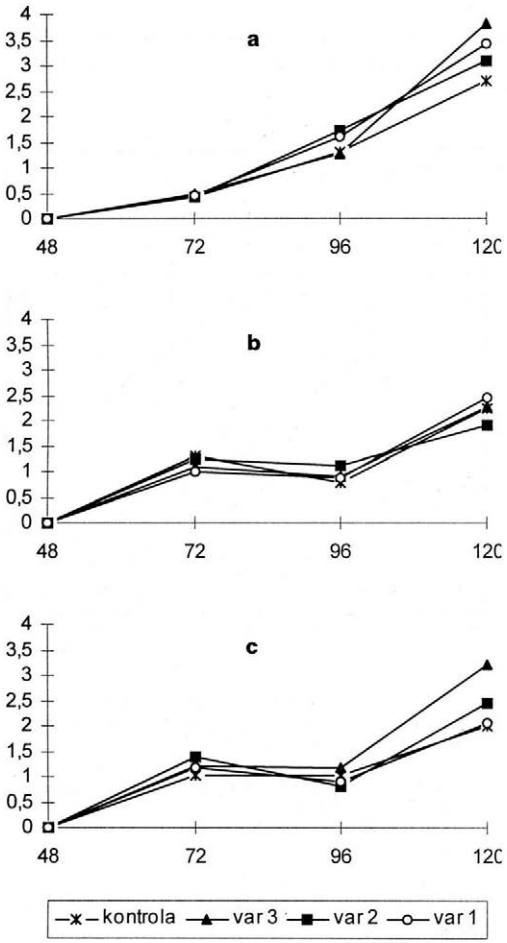
Zrná z jednotlivých pokusných variantov boli po diferencovanej dormancii testované na rýchlosť klíčenia. Vzhľadom na to, že dĺžka dormancie neovplyvnila proces klíčenia, pre ilustráciu vyberáme len priemerné výsledky testov zhrnuté v tab. II. Klíčenie zŕn jednotlivých variantov môžeme charakterizovať ako rovnomerný proces. V žiadnom z hodnotených intervalov sme nezaznamenali rozdielne hodnoty v porovnaní s kontrolou. Naše výsledky nepotvrdzujú negatívny vplyv urýchleného odumierania listov na životné prejavy novej generácie zŕn.

Dynamické merania produkcie endogénneho etylénu v klíčiacich zrnách po 210 dňoch dormancie (obr. 1a) ukázali, že množstvo endogénneho etylénu v závislosti

II. Množstvo vyklíčených zŕn v jednotlivých intervaloch – Number of germinated grains at different intervals

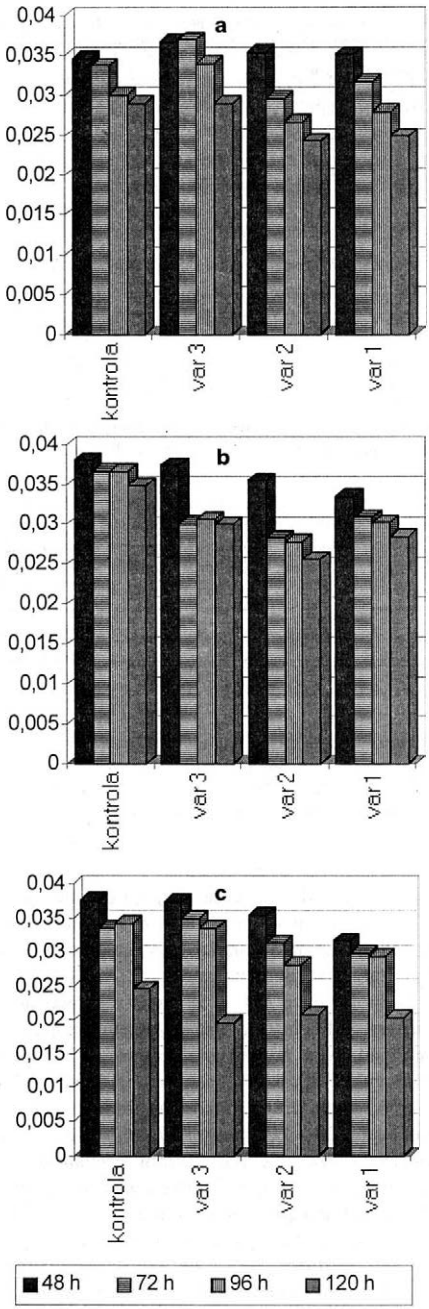
Interval[1] (h)	Variant[2]			
	1	2	3	kontrola[3]
48	87,56	91,47	91,41	88,53
72	95,43	96,6	96,53	94,17
96	96,7	98	97,1	96,9
120	96,8	98	97,3	97

[1]interval, [2]treatment, [3]control



1. Dynamika endogénneho etylénu klíčnych rastlín jarného jačmeňa po diferencovanej dormancii: a) 210 dní po dormancii; b) 224 dní po dormancii; c) 238 dní po dormancii – Dynamics of endogenous ethylene of germinative plants of spring barley after differentiated dormancy: a) 210 days after dormancy; b) 224 days after dormancy; c) 238 days after dormancy

os x: čas (h) – x axis: time (h)
os y: etylén (nl/g sušiny/h) – y axis: ethylene (nl/g of dry matter/h)
kontrola – control



2. Zmeny hmotnosti sušiny klíčnych rastlín jarného jačmeňa po diferencovanej dormancii: a) 210 dní po dormancii; b) 224 dní po dormancii; c) 238 dní po dormancii – Changes in dry matter weight of germinative plants of spring barley after differentiated dormancy: a) 210 days after dormancy; b) 224 days after dormancy; c) 238 days after dormancy

os x: variant – x axis: treatment
os y: hmotnosť sušiny (g/rastlina) – y axis: dry matter weight (g/plant)
kontrola – control

III. Viacfaktorová analýza rozptylu pre etylén – Multifactor analysis of variance for ethylene

Zdroj rozptylu[1]	Suma štvorcov[2] (stupeň voľnosti[3])	Stupeň volnosti[4]	Priemerný štvorec[5]	*F*-koeficient[6]	Hladina významnosti[7]
Hlavný efekt[8]	43,360989	8	5,420124	125,781	0,000
Hodiny[12]	42,674578	3	14,224859	330,106	0,000
Variant[13]	0,294347	3	0,098116	2,277	0,1144
Pokus[14]	0,392064	2	0,196032	4,549	0,0252
Dvojfaktorové interakcie[9]	5,2489920	21	0,2499520	5,800	0,002
Hodiny x variant	0,8203929	9	0,0911548	2,115	0,0842
Hodiny x pokus	4,1449826	6	0,6908304	16,032	0,000
Variant x pokus	0,2836165	6	0,0472694	1,097	0,4016
Rezíduá[10]	0,7756524	18	0,0430918		
Celkovo[11]	49,385634	47			

[1]source of variance, [2]sum of squares, [3]degree of performance, [4]degree of freedom, [5]mean square, [6]*F*-coefficient, [7]significance level, [8]main effect, [9]two-factor interactions, [10]residua, [11]total, [12]hours, [13]treatment, [14]trial

IV. Mnohonásobné porovnávanie stredných hodnôt pre etylén podľa času Scheffeho metódou s 99% koeficientom spoľahlivosti – Multiplicity comparison of mean values for ethylene according to the time by Scheffe method with 99% coefficient of reliability

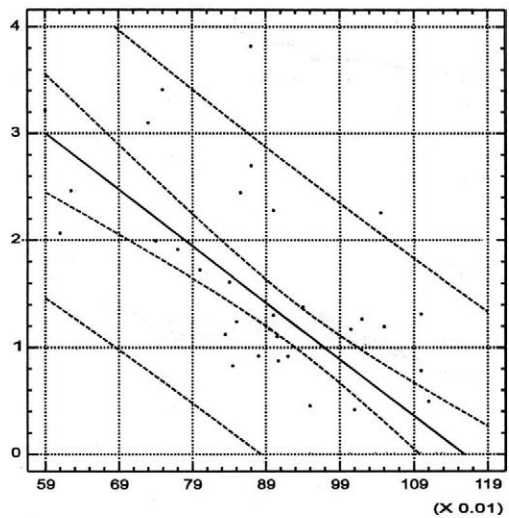
Interval[1]	Počet[2]	Priemer[3]	Homogénne skupiny[4]
48	12	0,010800	*
72	12	0,9355500	*
96	12	1,1284500	*
120	12	2,6383500	*

[1]interval, [2]number, [3]mean, [4]homogeneous groups

od času (počte hodín klíčenia) stúpa a dosahuje maxima u 120 h starých klíčnych rastlín. Rozdiely v produkcii etylénu v rámci pokusných variantov sú bezvýznamné, štatisticky nepreukazné.

Zrná odpočívajúce 224 a 238 dní si zachovávajú vysoké percento klíčivosti a výraznú dynamiku endogénneho etylénu. Jeho množstvo stúpa po 72 h klíčenia, keď bolo vyklíčených 95 % zŕn s priemernou dĺžkou koleoptyly 8 mm. Druhé maximum produkcie sme zaznamenali po 120 h klíčenia pri 97 % vyklíčených zŕn a priemernej dĺžke koleotyly 20 mm. Preukaznosť výsledkov potvrdzuje viacfaktorová analýza rozptylu (tab. III), keď pri porovnávaní rozdielov stredných hodnôt pri koeficiente spoľahlivosti 99 % sme získali homogénne skupiny (tab. IV), ktoré korešpondujú s obr. 1a, b, c. Uvedené výsledky zaujímavo dokresľujú údaje o zmenách obsahu suchej hmotnosti v priebehu 48 až 120 h klíčenia.

Hmotnosť sušiny klíčiaceho zrna v závislosti od času klesá. Negatívna korelácia sa zachováva vo všetkých variantoch pokusu, ako aj u zŕn s rôznou dĺžkou dormancie (obr. 2a, b, c). Porovnanie výsledkov o zmenách hmotnosti sušiny zrna so zmenami v produkcii endogénneho etylénu potvrdzuje ich negatívny korelačný vzťah. Regresná analýza s korelačným koeficientom



3. Regresná analýza medzi produkciou etylénu a hmotnosťou sušiny semien ($r = -0{,}725163$) – Regression analysis between ethylene production and seed dry matter ($r = -0.725163$)

os x: hmotnosť sušiny semien – x axis: seed dry matter weight
os y: etylén – y axis: ethylene

$r = -0{,}725163$, vyjadrená na obr. 3, s uvedeným v plnej miere korešponduje.

DISKUSIA

V literatúre je nemálo dôkazov o tom, že vek a fyziologická aktivita listov, ako aj ich hormonálna bilancia, ktorá sa utvára pod vplyvom vonkajších faktorov, má rozhodujúci význam pri ovplyvňovaní kvantity a kvality novej generácie semien.

Funkčný vzťah medzi starnúcimi a mladými aktívnymi orgánmi je interpretovaný najmä dôkazmi z oblasti tzv. nutričných vzťahov orgánov (Salette, Lemaire, 1981). Práce z oblasti stresovej fyziológie však potvrdzujú, že stres pôsobiaci počas fyziologického dozrievania redukuje počet klíčiacich zŕn a ich životnosť (Vieira et al., 1982; Krony et al., 1984). Dornbos et al. (1989) uvádzajú, že sucho pôsobiace počas napĺňania semien sóje signifikantne redukuje počet klíčivých zŕn.

V našich pokusoch sme nepotvrdili negatívny vplyv indukovaného starnutia listov najvyšších etáží na životné prejavy zrna. V tomto zmysle sú naše poznatky porovnateľné s údajmi z literatúry (Vieira et al., 1991). Citovaní autori po poškodení listov rastlín suchom zistili zníženie produkcie o 35 až 41 %, ale nenašli negatívny vplyv faktora na klíčenie a životnosť semien.

V našom experimentálnom prístupe sme sledovali aj dynamiku endogénneho etylénu, ktorý má v procese klíčenia významnú úlohu. V dynamike jeho produkcie sme u kontrolných a pokusných klíčnych rastlín nezistili preukazné rozdiely. Výsledky naznačujú dve maximá v produkcii etylénu a stanovili sme ich v neskorších fázach klíčenia. Z tohto pohľadu sú naše výsledky porovnateľné s výsledkami z literatúry (Takayanagi, Harrington, 1971; Ketring, Morgan, 1972; Greef, Proft, 1978; Sinska, 1989; Gorecki et al., 1991). Obe nami detekované maximá spadajú do obdobia intenzívneho predlžovacieho rastu etiolovaných orgánov klíčnych rastlín (obr. 1b, c). Zvýšená produkcia etylénu môže súvisieť so zvýšenou akumuláciou stimulačných hormónov v intenzívne sa predlžujúcich orgánoch klíčnych rastlín.

Takýto pomer stimulačných a inhibičných hormónov je popisovaný v prípadoch, kde vonkajšie podmienky su priaznivé pre nadmerný rast orgánov. Predpokladá sa, že obsah etylénu brzdí prednostný rast jedného z orgánov na úkor ostatných.

LITERATÚRA

CRAFTS-BRANDNER, S. J. – PONELEIT, G. G.: Effect on leaf senescence in maize. Crop Sci,. *32*, 1992: 127–131.

GREEF, J. A. DE – PROFT, M. DE: Kinetic measurement of small ethylene changes in open system designed for physiological studies. Pl. Physiol., *42*, 1978: 79–84.

DORNBOS, D. L. – MULLEN, R. E. – SHIBLES, R. M.: Drought stress effect during seed fill on soybean seed germination and vigor. Crop Sci., *29*, 1989: 476–480.

GORECKI, R. J. – ASHINO, H. – SATO, S. – ESASHI, Y.: Ethylene production in pea and cocklebur seeds of differing vigour. J. Exp. Bot., *42*, 1991 (236): 407–414.

GASKIN, P. – GILMOUR, S. J. – LENTON, J. R. – MacMILLAN, J. – SPONSEL, V. M.: Endogenous gibberellins and karothenoids identified from developing and germinating barley grains. J. Pl. Growth Regul., *2*, 1984: 229–242.

JACOBSEN, J. V. – CHANDLER, P. M.: Gibberelline and abscisic acid in germinating cereals. In: Regulation of plant growth and development. Martinus Nijhoff, Dr. W. Junk Publ. 1985: 163–193.

KETRING, D. L. – MORGAN, P. W.: Physiology of oil seeds. IV. Role of endogenous ethylene and inhibitory regulators during natural and induced afterripening of dormant Virginia-type peanut seeds. Pl. Physiol., *50*, 1972: 382–387.

KING, R. W.: Abscisic acid in seed development. In: KHAN, A. A. (ed.): The physiology and biochemistry of seed development, dormancy and germination. Amsterdam, Elsevier Biomedical Press 1982: 157–181.

KRONY, D. M. TE – EGLI, D. B. – BALLES, J. – TOMES, L. – STUCKEY, R. E.: Effect of date of harvest maturity on soybean seed quality and *Phomopsis* sp. seed infection. Crop Sci., *24*, 1984: 189–193.

PRIOUL, J. L. – DUGUÉ, N.: Source-sink manipulation and carbohydrate metabolism in maize. Crop Sci., *32*, 1992: 754–756.

SALETTE, J. – LEMAIRE, G.: Sur la variation de la teneur en azote des graminées fourragères pendant leur croissance; formulation d'un loi de dilution. Paris, C. R. Acad. Set. (ser. III), *292*, 1981: 875–878.

SINSKA, I.: Interaction of ethephon with cytokinine and gibberelline during the removal of apple seed dormancy and germination of embryos. Pl. Sci., *64*, 1989: 39–44.

ŠESTÁK, Z. – ČATSKÝ, J.: Metody studia fotosyntetické produkce rostlin. Praha, Academia 1966.

TAKAYANAGI, K. – HARRINGTON, J. F.: Enhancement of germination rate of aged seeds by ethylene. Pl. Physiol., *47*, 1971: 521–524.

VIEIRA, R. D. – KRONY, D. M. TE – EGLI, D. B.: Effect of drought stress on soybean seed germination and vigour. J. Seed Technol., *16*, 1991: 12–21.

VIEIRA, R. D. – SEDIYAMA, T. – SIILVA, R. F. – SEDIYAMA, C. S. – THIEBAUT, J. T. L.: Effect of delayed harvest on soybeans seed quality. Rev. Bras. Sem., *4*, 1982: 9–22.

YAMADA, K.: Determination of endogenous gibberellins in germinating barley by combined gas chromatografy – mass spectrometry. J. Amer. Soc. Brew. Chem., *40*, 1982: 18–25.

ZADOKS, J. C. – CHANG, T. T. – KONZAK, C. F.: A decimal code for the growth stages of cereals. Weed Res., *14*, 1974: 415–421.

Došlo 17. 1. 1996

Kontaktná adresa:

Doc. RNDr. Zuzana Jureková, CSc., Vysoká škola poľnohospodárska, A. Hlinku 2, 949 76 Nitra, Slovenská republika, tel.: 087/51 17 51, fax: 087/41 14 51

Z VĚDECKÉHO ŽIVOTA

Za doc. Ing. Ludvíkem Kunclem, CSc.

Doc. Ing. Ludvík Kuncl, CSc., se narodil 5. srpna 1941 v Dobronicích u Bechyně v rodině zemědělce. Malebná jihočeská krajina v okolí Lužnice a výchova v rodině mu vtiskly hluboký vztah k přírodě a k zemědělství, jimž zůstal věrný po celý svůj život.

Po středoškolských studiích v Táboře pracoval nejprve jako agronom na Státním statku ve Vyšším Brodě. Po vystudování fytotechnického oboru na Provozně ekonomické fakultě VŠZ v Českých Budějovicích v roce 1968 zůstal na této, nyní Zemědělské fakultě JU, kde pracoval nejprve jako technik, samostatný odborný pracovník pro vědu a výzkum, odborný asistent a konečně jako docent v oboru hodnocení jakosti a zpracování produktů rostlinné výroby a pícninářství. V letech 1993 až 1994 působil ve funkci vedoucího Katedry pícninářství a od roku 1994 vykonával funkci proděkana pro vědeckovýzkumnou činnost.

Vědecká i pedagogická práce doc. Kuncla byla příkladná. Věnoval se především studiu agroekologických podmínek uplatnění a genotypové podmíněnosti kvalitativních vlastností víceletých pícnin, přičemž zvláštní pozornost věnoval těmto otázkám u mezidruhových a mezirodových hybridů trav včetně aspektů jejich konzervace. Zabýval se též problematikou uplatnění víceletých pícních cenóz v podmínkách zvýšených ekologických požadavků a dále kvalitou brambor, pěstovaných při omezených vstupech.

Na Zemědělské fakultě JU se mimořádnou měrou zasloužil o vybudování společné mezikatedrové laboratoře pro hodnocení jakosti zemědělských produktů. Své bohaté vědomosti a zkušenosti dokázal s pedagogickým citem předávat studentům jak ve vlastní výuce, tak i při vedení diplomových a doktorských prací.

Bohaté životní zkušenosti doc. Kuncla a jeho schopnosti široké syntézy našly své uplatnění v členství a v aktivním zapojení do práce Vědecké rady Zemědělské fakulty JU, Vědecké rady JU a Oborové rady doktorandského studia pro obor speciální produkce rostlinná. Byl též členem ČAZV, a to Odboru jakosti zemědělských produktů a Odboru výživy obyvatelstva a jakosti potravin.

Náhlým odchodem doc. Ing. Ludvíka Kuncla, CSc., ztrácíme vynikajícího učitele a odborníka.

Doc. Ing. František Klimeš, CSc.

VLIV ZASTOUPENÍ VÁPNÍKU A HOŘČÍKU V ŽIVNÉM ROZTOKU NA TOXICITU HLINÍKU U OZIMÉ PŠENICE

EFFECT OF CALCIUM AND MAGNESIUM ABUNDANCE IN NUTRIENT SOLUTION ON ALUMINIUM TOXICITY IN WINTER WHEAT

J. Černohorská, Z. Kadlecová, M. Dvořák

Charles University, Faculty of Science, Praha, Czech Republic

ABSTRACT: It is suggested that Al affects Ca homeostasis in plants. In our work we have studied the effect of Ca : Mg molar ratio in nutrient solution on the growth parameters of wheat (*Triticum aestivum* L., cv. Sparta) in the presence of Al. If Al causes reduction of Ca^{2+} uptake and so Ca homeostasis is disturbed, it would be possible to alleviate Al-toxicity by increasing Ca^{2+} concentration in nutrient solution. This will change the Ca : Mg ratio what can lead to changes in the plasmalemma permeability. As the control variant we used Ca : Mg ratio 2 : 1, which is very near to Ca : Mg ratio in Knop nutrient solution. The ratio Ca : Mg 5 : 1 was used to increase Ca^{2+} concentration and Ca : Mg 1 : 4 ratio was used to increase Mg concentration. Total concentration of Ca + Mg ions in the nutrient solution was $5{,}7.10^{-3}$ M. Al was used in the form of $Al_2(SO_4)_3$, $7.4.10^{-4}$ M, pH 4 of nutrient solutions was adjusted with HCl. Supplement of Ca^{2+} in the variant with Ca : Mg 5 : 1 ratio in the presence of Al caused decrease in longitudinal growth of roots when compared with the control variant (Fig. 2). Ca content in this variant increased in the roots, but Ca supplement had no effect on the Al content in whole plants (Figs 5, 6). In the case of increased Mg concentration (Mg : Ca 4 : 1) in the presence of Al longitudinal growth of roots decreased even more (Figs 3, 4). It had no effect on the Ca content in roots, while in shoots Ca content slightly decreased (Figs 5, 6). This would indicate that Al-toxicity was not induced by Ca and Mg deficiency. Our results did not confirm alleviating effect of Ca supplement in nutrient solution on Al-toxicity if there is constant low pH. These are more in agreement with the results of Grant, Racz (1990), who found increased efflux of K^+ from the roots of barley in case of changed Ca : Mg ratio in both directions. According to these findings we suppose that disturbance of plasmalemma semipermeability of wheat root tissue would be the primary effect of Al-toxicity.

Al-toxicity; Ca; Mg; roots; *Triticum aestivum* L.

ABSTRAKT: Vliv Al na růst rostlin byl podrobně studován, není však dosud jasné, co je primární odpovědí na působení Al, a není tudíž znám ani princip tolerance některých rostlin k Al. Předpokládá se, že primární efekty Al jsou spojeny s poklesem akumulace Ca – obecně s porušením symplazmické homeostáze Ca^{2+} iontů. Sledovali jsme vliv vzájemných poměrů Ca a Mg v kultivačním médiu na růstové parametry pšenice (*Triticum aestivum* L., odrůda Sparta) v přítomnosti Al. Je-li působením Al snížen příjem Ca, a tak porušena jeho homeostáze v buňkách, mohlo by nadstandardní zvýšení koncentrace Ca v médiu tento syndrom zmírňovat. Jako kontrolní variantu jsme použili poměr Ca : Mg 2 : 1, který odpovídá poměru obou iontů v Knopově živném roztoku. Nadstandardní přídavek Ca byl použit v poměru k Mg 5 : 1 a nadstandardní přídavek Mg byl použit v poměru k Ca 4 : 1. Nadbytek Ca ve variantě Ca : Mg 5 : 1 v přítomnosti Al měl za následek snížení délkového růstu kořenů oproti kontrole s Al. Obsah Ca v kořenech byl nadstandardním přídavkem Ca zvýšen, ale obsah Al v rostlině tím nebyl ovlivněn. V případě nadstandardního přídavku Mg v poměru k Ca 4 : 1 v přítomnosti Al byl délkový růst kořenů výrazně snížen, více než v případě obráceného poměru. Obsah Ca v kořenech nebyl ovlivněn, obsah Ca v nadzemní části byl nepatrně snížen proti kontrole. Naše výsledky tedy nepotvrdily meliorační efekt nadstandardní koncentrace Ca v médiu na toxicitu Al, pokud je nízké pH konstantní.

Al-toxicita; Ca; Mg; kořeny; *Triticum aestivum* L.

ÚVOD

Kyselé půdy jsou pro pšenici fytotoxické, což celosvětově vede k varujícímu snížení její produkce. Tato fytotoxicita je výsledkem celého komplexu disbalancí živin v půdním roztoku v důsledku nízkého pH: deficience základních živin Ca a Mg, ale především toxicita Al a s ní spojená snížená dostupnost P. Přes rozsáhlé studium vlivu Al na růst rostlin není dosud jasné, co je primární odpovědí na působení Al, a není tudíž znám ani princip tolerance některých rostlin k Al. Poslední práce v tomto směru předpokládají, že Al může být

vylučován kořenovými špičkami Al-tolerantních rostlin pšenice mechanismy, které exkretují ligandy pro chelatizaci Al^{3+}, imobilizují Al v buněčných stěnách, zvyšují pH okolí kořenových špiček, a tím precipitují Al^{3+} nebo aktivně transportují Al z cytoplazmy (Delhaize, Ryan, 1995).

Nejvýraznějším symptomem a zároveň uznávanou mírou Al-toxicity je inhibice růstu kořenů. V literatuře jsou uváděny dvě základní hypotézy o tom, jak k této inhibici, ztotožňované s vlastním principem Al-toxicity, dochází. První předpokládá porušení symplazmické homeostáze Ca^{2+}, a tím i porušení buněčného metabolismu závislého na Ca (Rengel, Elliot, 1992). V tom případě by mohl nadstandardní přídavek Ca (případně Mg) zmírňovat toxický efekt Al. Druhá hypotéza uvažuje o interakci Al s cytoplazmatickými membránami kořenových buněk (Kinraide et al., 1994).

MATERIÁL A METODA

Experimentální rostlinou byla ozimá pšenice (*Triticum aestivum* L.), odrůda Sparta. Rostliny byly pěstovány ve vodních kulturách v Knopově živném roztoku v konstantních podmínkách: teplota 20 ± 2 °C, relativní vlhkost vzduchu 65 až 70 %, světelná perioda 16 h světla, 8 h tmy, osvětlení sodíkovými výbojkami s průměrnou hodnotou ozáření 300 $\mu M.m^{-2}.s^{-1}$.

Zvýšená koncentrace H^+ iontů (pH 4) je podmínkou pro sledování Al-toxicity, ale zároveň je sama toxická pro kořeny rostlin. Proto jsme vždy užívali dvou kontrolních variant, a to jednak při pH 5, jednak při pH 4.

V prvé části práce jsme sledovali vliv vzájemných poměrů Ca : Mg v živném médiu na Al-toxicitu u pšenice. Je-li působením Al snížen příjem Ca, a tím porušena jeho homeostáze v kořenových buňkách, mohlo by nadstandardní zvýšení koncentrace Ca v živném médiu tento syndrom zmírňovat. Tím se však změní vzájemný poměr Ca : Mg, který může ovlivnit permeabilitní funkci plazmalemy.

Jako kontrolní variantu jsme použili poměr Ca : Mg 2 : 1, který odpovídá poměru obou iontů v Knopově živném roztoku. Nadstandardní přídavek Ca byl použit v poměru k Mg 5 : 1 a nadstandardní přídavek Mg byl použit v poměru k Ca 4 : 1. Celková koncentrace iontů Ca + Mg v kultivačních médiích byla vždy $5{,}7.10^{-3}$ M. Al byl použit ve formě $Al_2(SO_4)_3$ v koncentraci $7{,}4.10^{-4}$ M, acidita kultivačních médií byla upravena na pH 4 pomocí HCl, kontrolní varianta byla tedy dvojí, jednak s pH 4 a jednak s pH 5.

Byly měřeny parametry: délka nejdelšího kořene, délka nadzemní části, sušina obou částí. Naměřené hodnoty růstových parametrů byly statisticky vyhodnoceny nelineární regresní analýzou pomocí programového systému NONLIN. V grafech je uvedena pouze dynamika délkového růstu kořene, kde jsou rozdíly mezi jednotlivými variantami nejvýraznější. Chemické analýzy rostlin byly provedeny atomovou absorpční spektroskopií. Pro tento účel byl použit rozklad na mokré cestě ($HNO_3 + H_2O_2$) pomocí mikrovlnné technologie CEM. Složení kultivačních roztoků pro jednotlivé varianty uvádí tab. I.

I. Složení kultivačních roztoků pro jednotlivé varianty – Composition of cultivation solutions for different variants

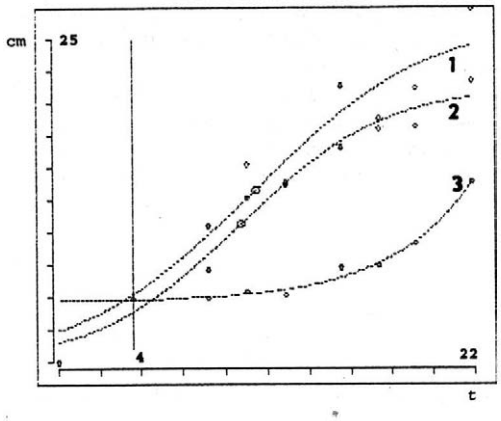
Varianta[1]	Označení[2]	Ca : Mg	Al [10^{-4} M]	pH
1	ŽR	2 : 1	0	5
2	pH 4	2 : 1	0	4
3	+Al	2 : 1	7,4	4
4	+Ca +Al	5 : 1	7,4	4
5	+Mg +Al	1 : 4	7,4	4

[1]variant, [2]denotation

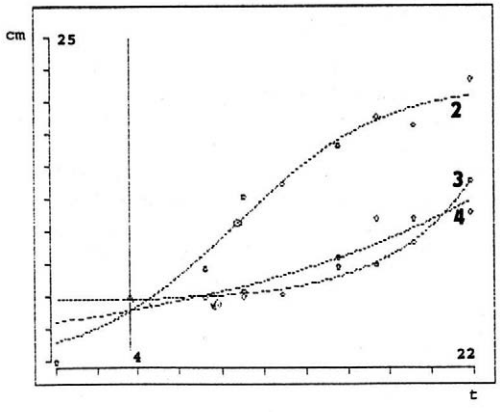
VÝSLEDKY A DISKUSE

Dynamiku délkového růstu kořenů u výchozích variant pokusu [kontrola pH 5 (1), kontrola pH 4 (2) a přítomnost Al (3)] popisuje obr. 1. Je dobře patrné, že stres nízkého pH sám o sobě snižuje délkový růst kořenů, v součinnosti s Al se však mění i charakter této dynamiky. Pro přehlednost je dále v grafech uváděna pouze kontrola v pH 4. Nadbytek Ca ve variantě (4) Ca : Mg 5 : 1 v přítomnosti Al snižoval po 18 dnech kultivace délkový růst kořenů i oproti kontrole s Al (obr. 2). V případě nadstandardního přídavku Mg v poměru k Ca 4 : 1 v přítomnosti Al byl délkový růst kořenů výrazně snížen, více než v případě obráceného poměru (obr. 3, 4). Obsah Ca v kořenech byl nadstandardním přídavkem Ca zvýšen, ale obsah Al v rostlině tím nebyl ovlivněn. Nadstandardním přídavkem Mg nebyl obsah Ca v kořenech ovlivněn, obsah Ca v nadzemní části byl nepatrně snížen proti kontrole (obr. 5, 6).

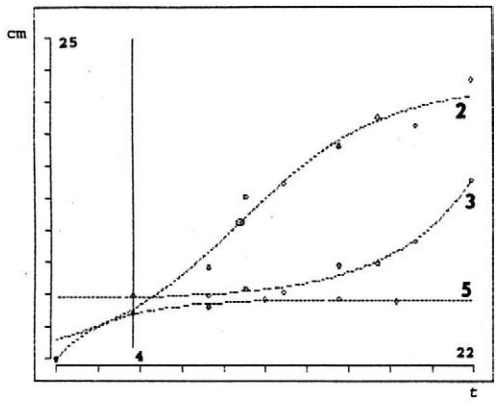
Naše výsledky tedy nepotvrdily meliorační efekt nadstandardní koncentrace Ca nebo Mg v médiu na toxicitu Al. Ca dosud užívaný k melioraci toxického působení Al zvyšuje pH prostředí, a tím vlastně eliminuje fytotoxickou formu Al. Efekt Ca a Mg na dynamiku růstových parametrů pšenice není v žádném vztahu k obsahu obou prvků v rostlině, což naznačuje, že příčinou Al-toxicity není indukovaná deficience Ca nebo Mg. Shodu nacházíme spíše s výsledky, které uveřejnili Grant, Racz (1990), kteří potvrdili zvýšený výtok iontů K z kořenů ovsa v případě porušení poměru Ca : Mg v médiu, a to ať už ve prospěch Ca nebo Mg. Také Wheeler, Edmeades (1995) uvádějí, že zvýšená koncentrace Ca v roztoku prohloubila Al-toxicitu u pšenice v případě, že koncentrace Mg v médiu byla velmi nízká. Protože přítomnost Al ještě prohlubuje efekt porušeného poměru Ca : Mg, domníváme se, že primárním efektem Al-toxicity bude změna v regulaci funkce plazmalemy kořenových buněk pšenice.



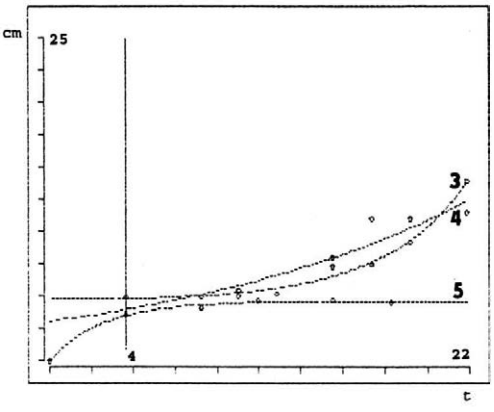
1. Dynamika délkového růstu kořenů pro varianty 1, 2, 3 – The dynamics of longitudinal roots growth for variants 1, 2, 3



2. Dynamika délkového růstu kořenů pro varianty 2, 3, 4 – The dynamics of longitudinal roots growth for variants 2, 3, 4

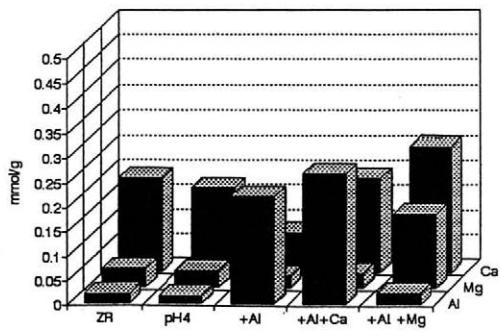


3. Dynamika délkového růstu kořenů pro varianty 2, 3, 5 – The dynamics of longitudinal roots growth for variants 2, 3, 5

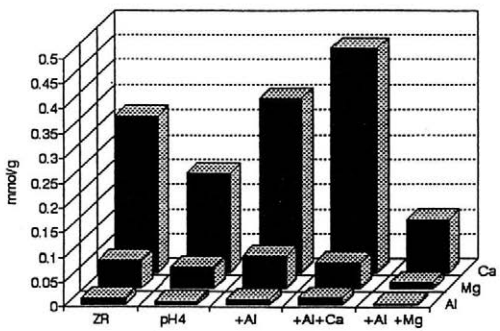


4. Dynamika délkového růstu kořenů pro varianty 3, 4, 5 – The dynamics of longitudinal roots growth for variants 3, 4, 5

Vysvětlivky k obr. 1 až 4 – Explanation to Figs 1 to 4:
osa x: čas, dny – x axis: time, days
osa y: délka kořenů, cm – y axis: length of roots, cm
čísla variant u křivek – variants numbers at the curves



5. Obsah Ca, Mg, Al v sušině kořenů rostlin jednotlivých variant – Content of Ca, Mg, Al in the dry matter of plant roots of single variants



6. Obsah Ca, Mg, Al v sušině nadzemní části rostlin jednotlivých variant – Content of Ca, Mg, Al in the dry matter of plant upper parts of single variants

LITERATURA

DELHAIZE, E. – RYAN, P. R.: Aluminium toxicity and tolerance in plants. Pl. Physiol., *107*, 1995: 315–321.

GRANT, C. A. – RACZ, G. J.: Effect of varying concentrations and ratios of calcium and magnesium on solute leakage from barley roots. J. Pl. Nutr., *13*, 1990 (6): 651–665.

KINRAIDE, T. B. – RYAN, P. R. – KOCHIAN, L. V.: Al^{3+}–Ca^{2+} interactions in aluminium rhizotoxicity. II. Evaluating the Ca^{2+} displacement hypothesis. Planta, *192*, 1994: 104–109.

RENGEL, Z. – ELLIOT, D. C.: Aluminium inhibits net $^{45}Ca^{2+}$ uptake by *Amaranthus* protoplasts. Biochem. Physiol. Pfl., *188*, 1992: 177–186.

WHEELER, D. M. – EDMEADES, D. C.: Effect of varying solution calcium or magnesium concentrations in the presence or absence of aluminium on yield and plant calcium or magnesium concentrations in wheat. J. Pl. Nutr., *18*, 1995 (10): 2229–2245.

Došlo 17. 1. 1996

Kontaktní adresa:

RNDr. Jana Černohorská, CSc., Univerzita Karlova, Přírodovědecká fakulta, Viničná 5, 128 44 Praha 2-Nové Město, Česká republika, tel.: 02/24 91 55 20, fax: 02/29 36 43

POKYNY PRO AUTORY

Časopis uveřejňuje původní vědecké práce, krátká sdělení a výběrově i přehledné referáty, tzn. práce, jejichž podkladem je studium literatury a které shrnují nejnovější poznatky v dané oblasti. Práce jsou uveřejňovány v češtině, slovenštině nebo angličtině. Rukopisy musí být doplněny krátkým a rozšířeným souhrnem (včetně klíčových slov).

Autor je plně odpovědný za původnost práce a za její věcnou i formální správnost. K práci musí být přiloženo prohlášení autora o tom, že práce nebyla publikována jinde.

O uveřejnění práce rozhoduje redakční rada časopisu, a to se zřetelem k lektorským posudkům, vědeckému významu a přínosu a kvalitě práce.

Rozsah vědeckých prací nemá přesáhnout 15 stran psaných na stroji včetně tabulek, obrázků a grafů. V práci je nutné používat jednotky odpovídající soustavě měrových jednotek SI (ČSN 01 1300).

Vlastní úprava rukopisu má odpovídat státní normě ČSN 88 0220 (formát A4, 30 řádek na stránku, 60 úhozů na řádku, mezi řádky dvojité mezery), k rukopisu je vhodné přiložit disketu s prací pořízenou na PC v některém textovém editoru, nejlépe v T602, a s grafickou dokumentací. Tabulky, grafy a fotografie se dodávají zvlášť, nepodlepují se. Na všechny přílohy musí být odkazy v textu.

Pokud autor používá v práci zkratek jakéhokoliv druhu, je nutné, aby byly alespoň jednou vysvětleny (vypsány), aby se předešlo omylům. V názvu práce a v souhrnu je vhodné zkratek nepoužívat.

Název práce (titul) nemá přesáhnout 85 úhozů. Jsou vyloučeny podtitulky článků.

Krátký souhrn (Abstrakt) je informačním výběrem obsahu a závěru článku, nikoliv však jeho pouhým popisem. Musí vyjádřit všechno podstatné, co je obsaženo ve vědecké práci, a má obsahovat základní číselné údaje včetně statistických hodnot. Musí obsahovat klíčová slova. Nemá překročit rozsah 170 slov. Je třeba, aby byl napsán celými větami, nikoliv heslovitě. Je uveřejňován a měl by být dodán ve stejném jazyce jako vědecká práce.

Rozšířený souhrn (Abstract) je uveřejňován v angličtině, měly by v něm být v rozsahu cca 1–2 strojopisných stran komentovány výsledky práce a uvedeny odkazy na tabulky a obrázky, popř. na nejdůležitější literární citace. Je vhodné jej (včetně názvu práce a klíčových slov) dodat v angličtině, popř. v češtině či slovenštině jako podklad pro překlad do angličtiny.

Úvod má obsahovat hlavní důvody, proč byla práce realizována a velmi stručnou formou má být popsán stav studované otázky.

Literární přehled má být krátký, je třeba uvádět pouze citace mající úzký vztah k problému.

Metoda se popisuje pouze tehdy, je-li původní, jinak postačuje citovat autora metody a uvádět jen případné odchylky. Ve stejné kapitole se popisuje také pokusný materiál.

Výsledky – při jejich popisu se k vyjádření kvantitativních hodnot dává přednost grafům před tabulkami. V tabulkách je třeba shrnout statistické hodnocení naměřených hodnot. Tato část by neměla obsahovat teoretické závěry ani dedukce, ale pouze faktické nálezy.

Diskuse obsahuje zhodnocení práce, diskutuje se o možných nedostatcích a práce se konfrontuje s výsledky dříve publikovanými (požaduje se citovat jen ty autory, jejichž práce mají k publikované práci bližší vztah). Je přípustné spojení v jednu kapitolu spolu s výsledky.

Literatura musí odpovídat státní normě ČSN 01 0197. Citace se řadí abecedně podle jména prvních autorů. Odkazy na literaturu v textu uvádějí jméno autora a rok vydání. Do seznamu se zařadí jen práce citované v textu. Na práce v seznamu literatury musí být odkaz v textu.

Na zvláštním listě uvádí autor plné jméno (i spoluautorů), akademické, vědecké a pedagogické tituly a podrobnou adresu pracoviště s PSČ, číslo telefonu a faxu, popř. e-mail.

INSTRUCTIONS FOR AUTHORS

Original scientific papers, short communications, and selectively reviews, that means papers based on the study of technical literature and reviewing recent knowledge in the given field, are published in this journal. Published papers are in Czech, Slovak or English. Each manuscript must contain a short and a longer summary (including the key words).

The author is fully responsible for the originality of his paper, for its subject and formal correctness. The author shall make a written declaration that his paper has not been published in any other information source.

The board of editors of this journal will decide on paper publication, with respect to expert opinions, scientific importance, contribution and quality of the paper.

The paper extent shall not exceed 15 typescript pages, including tables, figures and graphs.

Manuscript layout shall correspond to the State Standard ČSN 88 0220 (quarto, 30 lines per page, 60 strokes per line, double-spaced typescript). A PC diskette should be provided with the paper, written in an editor program, preferably T602, and with graphical documentation. Tables, figures and photos shall be enclosed separately. The text must contain references to all these annexes.

The **title** of the paper shall not exceed 85 strokes. Subtitles of the papers are not allowed either.

Abstract is an information selection of the contents and conclusions of the paper, it is not a mere description of the paper. It must present all substantial information contained in the paper. It shall not exceed 170 words. It shall be written in full sentences, not in form of keynotes, and comprise base numerical data including statistical data. It must contain key words. It should be submitted in English and if possible also in Czech or Slovak.

Introduction has to present the main reasons why the study was conducted, and the circumstances of the studied problems should be described in a very brief form.

Review of literature should be a short section, containing only literary citations with close relation to the treated problem.

Only original method shall be described, in other cases it is sufficient enough to cite the author of the used method and to mention modifications of this method. This section shall also contain a description of experimental material.

In the section **Results** figures and graphs should be used rather than tables for presentation of quantitative values. A statistical analysis of recorded values should be summarized in tables. This section should not contain either theoretical conclusions or deductions, but only factual data should be presented here.

Discussion contains an evaluation of the study, potential shortcomings are discussed, and the results of the study are confronted with previously published results (only those authors whose studies are in closer relation with the published paper should be cited). The sections Results and Discussion may be presented as one section only.

The citations are arranged alphabetically according to the surname of the first author. References in the text to these citations comprise the author's name and year of publication. Only the papers cited in the text of the study shall be included in the list of references. All citations shall be referred to in the text of the paper.

If any abbreviation is used in the paper, it is necessary to mention its full form at least once to avoid misunderstanding. The abbreviations should not be used in the title of the paper nor in the summary.

The author shall give his full name (and the names of other collaborators), academic, scientific and pedagogic titles, full address of his workplace and postal code, telefon and fax number or e-mail.

ROSTLINNÁ VÝROBA PLANT PRODUCTION

Volume 42, No. 6 June 1996

OBSAH – CONTENTS

Z VĚDECKÉHO ŽIVOTA – FROM THE SPHERE OF SCIENCE

Vědecký časopis ROSTLINNÁ VÝROBA ● Vydává Česká akademie zemědělských věd – Ústav zemědělských a potravinářských informací ● Redakce: Slezská 7, 120 56 Praha 2, tel.: 02/25 25 41, fax: 02/25 70 90 ● Sazba: Studio DOMINO – ing. Jakub Černý, Bří Nejedlých 245, 266 01 Beroun, tel.: 0311/22 959 ● Tisk: ÚZPI Praha ●

Rozšiřuje Ústav zemědělských a potravinářských informací, referát odbytu, Slezská 7, 120 56 Praha 2