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Uniconazole-induced changes of stress responses of *Vicia faba*: polyphenole oxidase activation pattern serves as an indicator for membrane stability

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Summary

Influence of uniconazole and different salt concentrations on growth and development of broad bean (*Vicia faba* L.) were investigated. Addition of salt to the irrigation water caused significant reduction of plant growth but uniconazole treated plants revealed better biomass. The number of phenolic compounds in *V. faba* leaf extracts changed with the age of the leaves, and the antioxidative potentials of uniconazole treated plants were slightly lower. The activity of polyphenoloxidase and its activation after plastid membrane desintegration by SDS were lower in uniconazole treated plants. Further studies with varying SDS-concentrations revealed a remarkable difference in membrane disintegration by SDS in uniconazole pretreated plants. This finding supports the hypothesis that uniconazole enhanced stress tolerance after uniconazole treatment is based on physiological change in thylakoid membrane stability.

Introduction

Uniconazole ((E)(p-chlorophenyl(-4.4-dimethyl-2-(1,2,4-triazol-1-yl)-1penten-3yl is a fungitoxic triazole which reveals plant growth regulator properties (MACKAY et al., 1990; FLETCHER and HOFSTRA, 1988; CAVINS et al., 2003). Furthermore, uniconazole treated plants develop a broad tolerance against a number of environmental stresses (FLETCHER and HOFSTRA, 1988) including salt stress (BEKHETA, 2000) and cold stress (ZHOU and LEUL, 1998). The influence of uniconazole on plant metabolism is obviously due to interactions of this triazole compound with various endogenous growth regulators. Triazoles in general, but uniconazole in particular, are known to block the biosynthetic way to gibberellin in several plant species at the step from ent-kaurene to ent-kaurenoic acid. This action interferes with the activity of endogenous giberellins. In fact, application of triazoles to *Rhododendron catawbiense* and *Kalmia latifolia* markedly reduced stem elongation (GERT, 1995, 1997). The complex nature of plant responses to uniconazole comprise growth responses and morphological modifications as well as biochemical characteristics of the plants, i.e. enhanced chlorophyll content, accumulation of stress related amino acids, and variations in phospholipid composition of biomembranes (ZHOU and YE, 1996).

The general biochemical stress responses of uniconazole treated plants against environmental factors has been compared to the reaction pattern of plants treated with abscisic acid (ABA). Exogenous treatment of plants with ABA rises plant resistance against salinity, ozone, heat, chilling and freezing (e.g. MACKAY et al., 1990). ABA is known to induce a fast stomatal closure in response to water deficit (ZEEVAAT and CREELMANN, 1988). Concomittant with water deficit induced stress the formation of reactive oxygen species (SMIRNOFF, 1993, 1998) may occur, which cause membrane damages and lipid peroxidation.

After treatment of plants with 2-aminoethanol or with uniconazole, membranes are transferred into a state which provides better resistance to stress factors (AZIZ et al., 1998; MASCHER et al., 2005; ZHOU and LEUL, 1998), and even the structural stability of organelles may be enhanced as a consequence of stress related biochemical changes. MASCHER et al. (2005) described the protective action of choline and other amines against oxidative membrane deterioration in pretreated plants.

From the complex protective pattern of uniconazole arises the assumption that triazoles may cause changes of membrane properties. In order to develop a test system for membrane stability of salt stressed plants under uniconazole influence we chose the phenolase activation test with *Vicia faba*. The broad bean is known to contain a tightly membrane bound, latent phenolase in its plastids (SWAIN et al., 1966; ANGELTON and FLURKEY, 1984). The enzyme is integrated in the thylakoids in a latent state and becomes active after following liberation from the membrane. This liberation from the membrane is slow in physiological buffers but can be enhanced by detergent-driven membrane disintegration (HUTCHESON et al., 1980). In some plants, especially in mosses, PPO latency is strongly expressed and has been correlated to strong membrane association by lipophilic domains (RICHTER et al., 2005). This publication describes the PPO test system used in *V. faba* and presents some arguments for the assumption that enhancement of stress tolerance of *V. faba* caused by uniconazole is due to changes in membrane properties.

Material and methods

Seeds

Broad bean (*Vicia faba* L.) seeds (variety: Dicke Bohne (Goldblume O'Lacys' GmbH, Düsseldorf Z 020021/05) were soaked in water or uniconazole solution, respectively, at 20 ppm uniconazole for 8 hours at environmental temperature (ca. 18°C). After soaking, the seeds were air-dried on filter paper and were placed in Floraton-3® Garden soil: sand mixture of 3:1. The seeds were planted in 200 ml pots (2 seeds/pot) from which they were repotted after 3 weeks into 1l pots. The seeds were covered by 1 cm of soil. Irrigation started directly after seedling emergence with sodium chloride containing water with 2000, 4000 ppm of salt and salt free control solution.
Cultivation was carried out under greenhouse conditions with L:D of 14:10 at an overall range of about 400 μmol photons / m^2 x s. The average temperature was 20 to 25°C at day time and 18°C at night period.

Root, shoot and leaves were harvested and quantified after given growth times, root length was estimated according to TENNANT (1975), root to shoot relation was used as relative growth parameter. The plants from soaking experiments were cultivated under the same greenhouse conditions as the plants cultivated for spraying treatments.
After 28 days of cultivation uniconazole treated plants (seed soaking in 20 ppm aqueous solution) revealed better shoot and root biomass than those of the respective control plants.

Plant treatment

Plants in four developmental leaf stages were sprayed with a 20 ppm uniconazole-solution in water. After regrowing under greenhouse conditions, the plants were taken for chloroplast isolation, for estimation of total phenolics and for the analysis of the composition of phenolic compounds by HPLC-standard separation procedure.
The morphological features of sprayed plants showed the well-known and described morphological changes after uniconazole treatment: internodial stunting, even in the inflorescence region, and formation of smaller leaves in size.

Chloroplast isolation

The plants can be divided into two groups:
a) 1st group: plants produced from soaked seeds in 20 ppm uniconazol and irrigated with different concentrations of salt (NaCl) (0, 2000 and 4000 ppm)
b) 2nd group: plants sprayed with uniconazol at 20 ppm and irrigated with tap water.

Chloroplasts were isolated from freshly harvested young leaves of *V. faba*, ground in icecold posphate buffer 10 mmol/l, pH 6.4, which contained 0.6 mol/l sucrose. X g leaves were ground in 2 X ml of buffer volume. The homogenate was filtered through 4 layers of cheesecloth, and centrifuged at 900 x g for 8 min. The resulting sediment was the fraction called „unwashed chloroplasts". The sediment was taken up in the original buffer volume, thoroughly resuspended and centrifuged again at 900 x g. The sediment was again taken up in the original buffer volume. The fraction was called „washed chloroplasts" or C1.

Polyphenol analyses

The quantitative analyses of polyphenolic substances were carried out in a RP-HPLC method with detection by a Photodiode Array Detector (PAD), 225 to 540 nm, according to TIKKANEN and JULKUNEN-TIITTO (2003).
Preparation of samples, modified: 0.200 g fresh leaf material was weighed into a 10 ml-centrifugation tube. Each sample was extracted three times for 30 s with 5 ml ice-cold methanol using an ultra-thurrax. After each extraction, the mixture was centrifuged for 10 min at 5,000 rpm. After centrifugation the supernatant was collected in a 50 ml round flask. The methanol was completely evaporated in a rotation evaporator at 40°C and 100 mbar.
The residues were solubilized in 1.5 ml methanol (Lichrosolv) for analysis.

An acetonitrile gradient was used, which was composed of elution solutions:
A: acetic acid solution, w = 2 % and
B: acetonitile/H_2O lichrosolv/glacial acetic acid, 400+90+10 (v/v/v)

Gradient parameter:

Time	Flow rate	Percentage of elution solution	
Min	ml/min	% A	% B
0	1.2	90	10
8	1.2	90	10
38	1.1	77	23
50	1.0	60	40
70	1.0	10	90
73	1.0	10	90
78	1.2	90	10
93	1.2	90	10

HPLC-conditions:

Separating column:	Merck RP 18 (250 mm, Ø 4 mm, 5 µm, endcapped)
Injection volume:	20 µl
Temperature:	column temperature: 26°C
Detection:	PDA-detector (Waters Millipore 996; Spectrum 225 nm -540 nm, polyphenols quantified at 280 nm)
Pump:	2 x Knauer Model 64
Gradient-former	Knauer Programmer Model 50
Autosampler:	Merck-Hitachi AS-2000
Quantification:	Area integration sample

Estimation of antioxidative potential

All samples were tested for antioxidative activity by means of TEAC method (=Trolox® Equivalent Antioxidative Capacity). It refers to a „in vitro"-test based on the ABTS substance (2.2-azino-di-[3-ethyl-benzothiazolin-6-sulfonate]), which was developed by MILLER et al. (1993). By adding hydrogen peroxide, under catalysis of metmyoglobin, a colour develops by the formation of ABTS radicals in a time-limited increase at an extinction maximum at 734 nm. Antioxidative substances, such as polyphenols, delay the beginning of the staining reaction, i.e. the onset of radical formation. The standard substance Trolox®, a water soluble tocopherol derivative, causes also delays in radical formation. The delay produced by the polyphenolic substances from leaf discs of *Vicia faba* material was correlated to the concentration of Trolox® which causes the same delay during equivalent test-conditions. The antioxidative capacity of the samples is expressed as mmol/l Trolox ® equivalents/cm².

For preparation of samples 3 leaf discs (1 disc = 0.785 cm²) were put into a 2 ml flask containing a mixture of acetone/methanol/phosphate buffer (c = 5 mmol, pH 7.4) 3+1+1 (v/v/v), suspended and shaken for 15 min. at 1,400 rpm. The mixture was finally centrifuged at 13,000 rpm for 10 min. 20 µl of the supernatant was used for the test and buffer was added to 1 ml. 600 µl 0.5 mm/l ABTS was pipetted in a macro cuvette, 70 µl metmyoglobin-solution was added, and 20 µl sample solution followed. After adding 300 µl of a 2 % solution of H_2O_2 in distilled water, the reaction started and was measured at 734 nm.

Total phenolic content

The determination of the total phenolic content was carried out using Folin-Ciocalteu-phenolic reagent. For this purpose, 1 ml raw polyphenolic extract was resolved in 2 ml 2.5 % acetic acid. 0.2 ml of this diluted sample was given into a 10 ml graduated flask, 0.8 ml 2.5 % acetic acid, 2.0 ml 20 % Na_2CO_3 solution and 0.5 ml Folin-Ciocalteu-phenolic reagent were added. The solution was shaken after the adding of each reagent. The flask was filled with distilled water. After mixing, the samples were heated in a water bath of 70°C for 10 min. After cooling down to room temperature, the blue colour of the polyphenols is stable for some hours. The polyphenolic content was measured at 730 nm; epicatechin was used as a reference.

Chlorophyll estimation

Chlorophyll a and chlorophyll b content were determined in an 80% acetone extract of the leaves according to LICHTENTHALER (1987).

PPO estimation

PPO activity was determined polarographically at 25°C with a YSI oxygen electrode 5331. The air saturated reaction mixture (3 ml)

contained 7.5 mmol/l 4-methylcatechol in 67 mmol/l phosphate buffer (LIEBEREI et al.,1981).
The SDS-activation of broad bean PPO was carried out according to SWAIN et al. (1966).

Results

Plants grown from seeds soaked in 20 ppm uniconazole solution revealed a higher biomass production in roots, shoots and leaves than controls (Tab. 1). Under 2000 ppm salt, control plants were slightly higher in their fresh weight, under higher salt conditions (4000 ppm) growth was impaired. Plants grown from uniconazole treated seeds were similar in qualitative stress response, but were higher in root growth compared with the corresponding salt treated plants without uniconazole. The root to shoot ratios under control conditions and in plants under low salt were higher than the in non-treated plants. Thus, there is long lasting interference of uniconazole with growth pattern, which lasts at least up to 28 days after soaking. Obviously the short uniconazole treatment of seeds causes changes in the plants which are leading to the long lasting differences in their reaction to environmental stress.

The spectrum of phenolic compounds in *V. faba* methanolic leaf extracts changes with the age of the leaves. Young leaves contain five groups of peaks, which form a typical qualitative pattern (Fig. 1a). During leaf maturation, the number of groups is reduced and the number of peaks in the groups is also lower. Uniconazole spraying of leaves leads to less extractable phenolics per leaf weight and to a peak composition in the young leaves (1b) which resembles the peak pattern of mature leaves in untreated plants (1c).

A considerable part of the antioxidative potential of leaves is based on the extractable phenolics. In all cases studied, the uniconazole treated plants were lower in their antioxidative compounds, but the differences were not significant. The long lasting unionazole effect seems not to be due to an interference of the triazole treatment with the phenolics metabolism (Fig. 2).

Chloroplast bound (or thylakoid bound) phenolase of *V. faba* is latent in vivo and thus cannot be detected by oxygen consumption in intact leaves. Under standard conditions (7.5 mmol/l 4-Methylcathechol) for phenolase quantification, a low activity of about 36 µm O_2 / h x g FW was detectable in control plants and in uniconazole treated plants. Addition of SDS in a final concentration of 0.1 % w/v gave rise to an immediate strong time dependent acceleration of phenolase activity and after 10 min of incubation, already 95 µmol O_2 / h x g FW were consumed. The activity in the sample stored at 4 °C rose over the
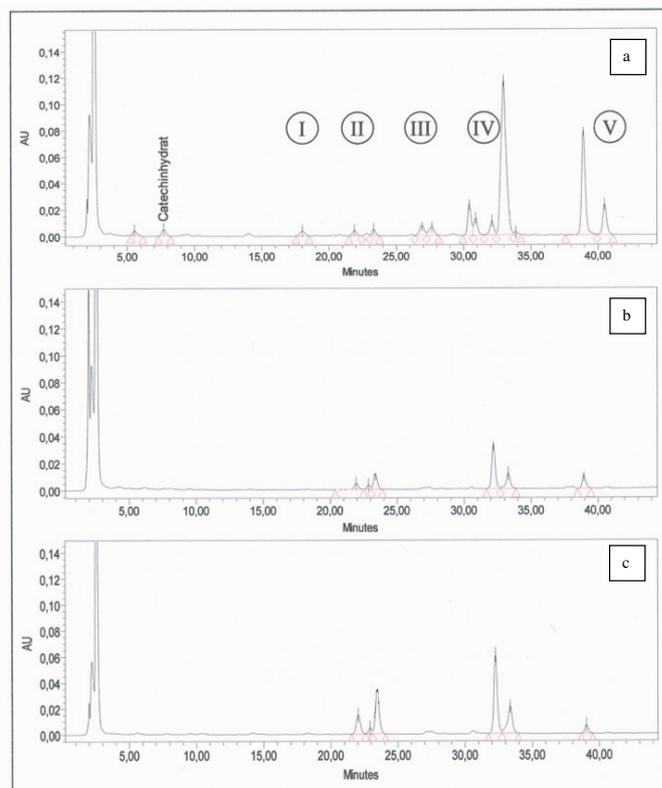

Fig. 1: Phenolic compounds in leaves of *Vicia faba*
Effect of maturation and Uniconazole treatment.
a = young leaves, b = Uniconazole-treated leaves, c = nature leaves.

whole storage time of more than 24 hrs, after which the activity of phenolase was about 20 times higher than the initial value (Fig. 3). Higher concentration of SDS normally degrade the active phenolases of many plants, and the treatment results in irreversible loss of phenolase enzyme (LIEBEREI et al., 1981; MOOR and FLURKEY 1990; KANADE et al., 2006).
The phenolase of *V. faba,* in contrast, is remarkably stable in the presence of SDS. Concentrations of 1.5 % SDS lead to a very high activity, which is reached after about 2 hrs of incubation. No losses of activity, which might be the result of enzyme deterioration, are detectable within this time span (Fig. 4).

Tab. 1: Influence of salt stress on uniconazole treated *Vicia faba* plants. Morphological characteristics of non flowering plants after 4 weeks of culture.

| Samples | Number of leaves | Leaf-FW | Stem-FW | Root-FW | Nodulation | Stem-length | Length of primary root | R |
								S + L
		[g]	[g]	[g]		[cm]	[cm]	
0-0	7	3,9	4,24	6,12	-	28	16	0,75
0-2000	8	5,19	5,03	7,6	-	22	27	0,74
0-4000	7	3,7	3,41	4,84	+	19	18	0,68
20-0	8	5,4	5,26	9,1	+	27	35	0,85
20-2000	8	5,97	6,21	11,33	+	20	27	0,93
20-4000	7	4,57	4,64	6,18	-	17	20	0,67

R = roots, S = shoots, L = leaves.

This result underlines that *V. faba* phenolase is set free from the thylakiods by detergents in an active state, which is far more stable than in many other plants. The molecular basis for this enzyme stability remains to be explained.

With respect to membrane stability, it is remarkable that after uniconazole treatment the activation process proceeds slower than in control plants (Fig. 5), and the enzyme activity per µg of chlorophyll does not reach values of untreated plants.

The activation of phenolase in leaf extracts was different in uniconazole treated samples compared to untreated control plants. The treated plants were slower in activation. In order to verify if this behaviour is due to properties of the plastids or the plastid membranes themselves, isolated plastids from a hypertonic extraction procedure were treated with 0.1 % SDS directly after isolation. The phenolase activity, expressed in activity per µg chlorophyll is rising in both groups of plastids, but it is easy to be seen that plastids derived
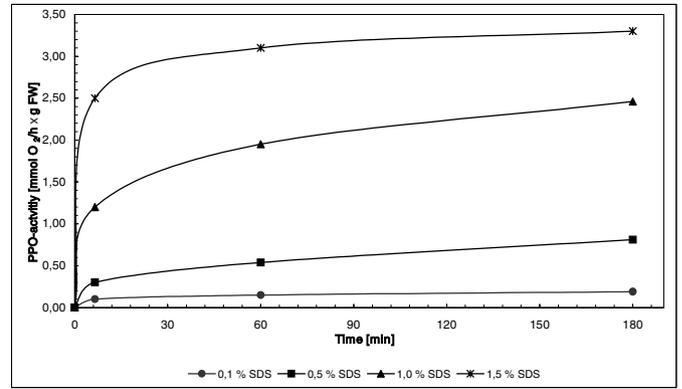

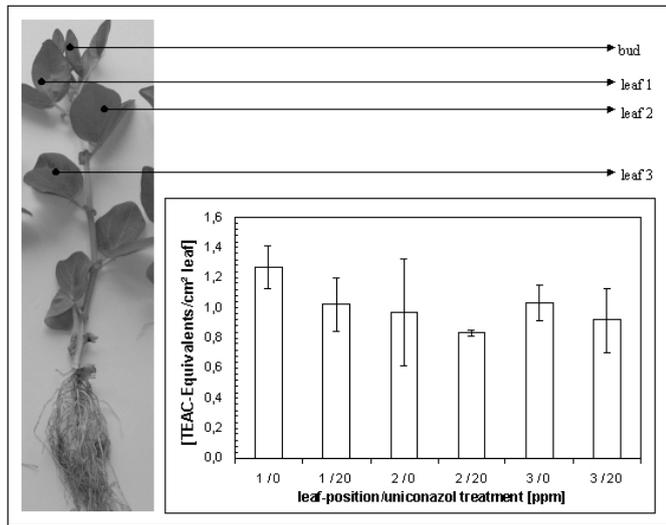**Fig. 4:** Time-dependence of PPO-activation of *Vicia faba* by different SDS-concentrations.

from uniconazole treated plants were slower in their activation pattern and remained at a lower activity level than the control plants (Fig. 5). Obviously the plastid membranes do not undergo such a fast desintegration as the plastids isolated from non-treated plants.

When samples of both groups were treated by 1.5 % SDS for 12 hrs, their activities finally reached the same values (data not shown).

Discussion

Uniconazole treatment leads to polyfactoral changes in plant responses to environmental stress factors. Detailed descriptions on reduced electrolyte leakage or lower liberation of malondialdehyde after heat stress have been reported in rape plants (ZHOU and LEUL, 1999), the amelioration of NaCl stress in peanut seedlings by a host of metabolic changes are known (MUTHUKUMARASAMY and PANNEERSELVAM, 1997). Leaves of *V. faba*, artificially dwarfed by uniconazole-P (FUKUDA et al., 2001) revealed many microstructural changes, e.g. the size and density of mesophyll cells. Typical for all descriptions are a) the polyfactorial changes and b) the indirect action of uniconazole.
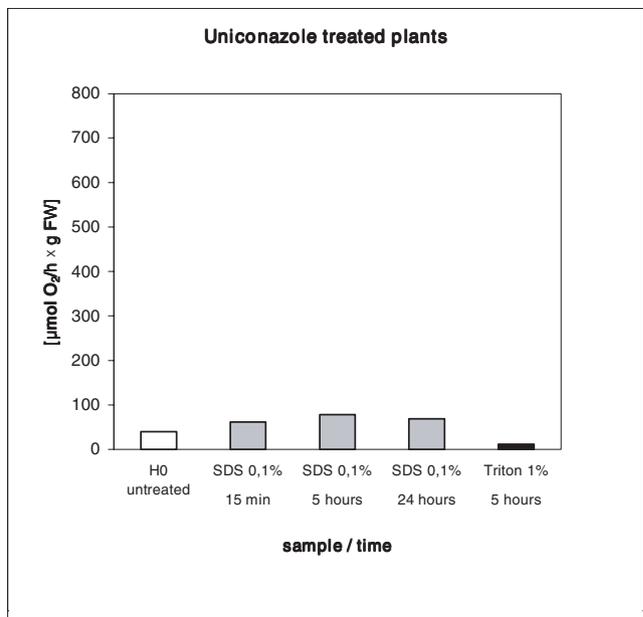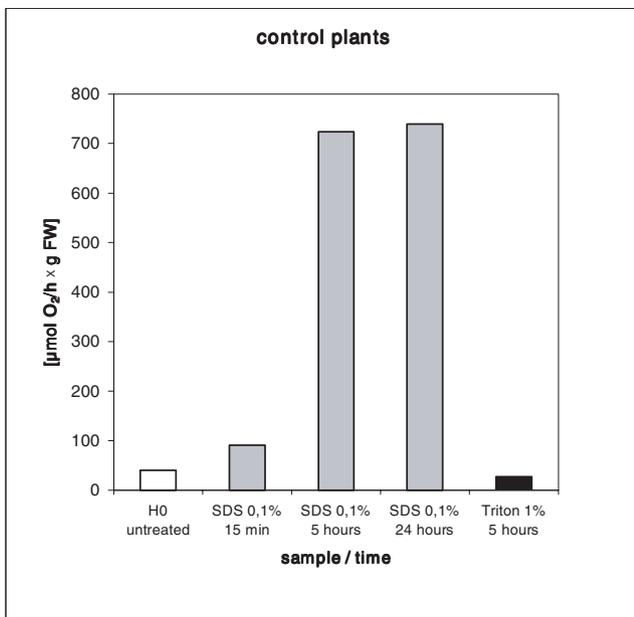

Fig. 2: Trolox-equivalents after spraying with Uniconazole (20 ppm).

Fig. 3: PPO-activity under influence of Uniconazole (UNI) (20ppm) and detergents.
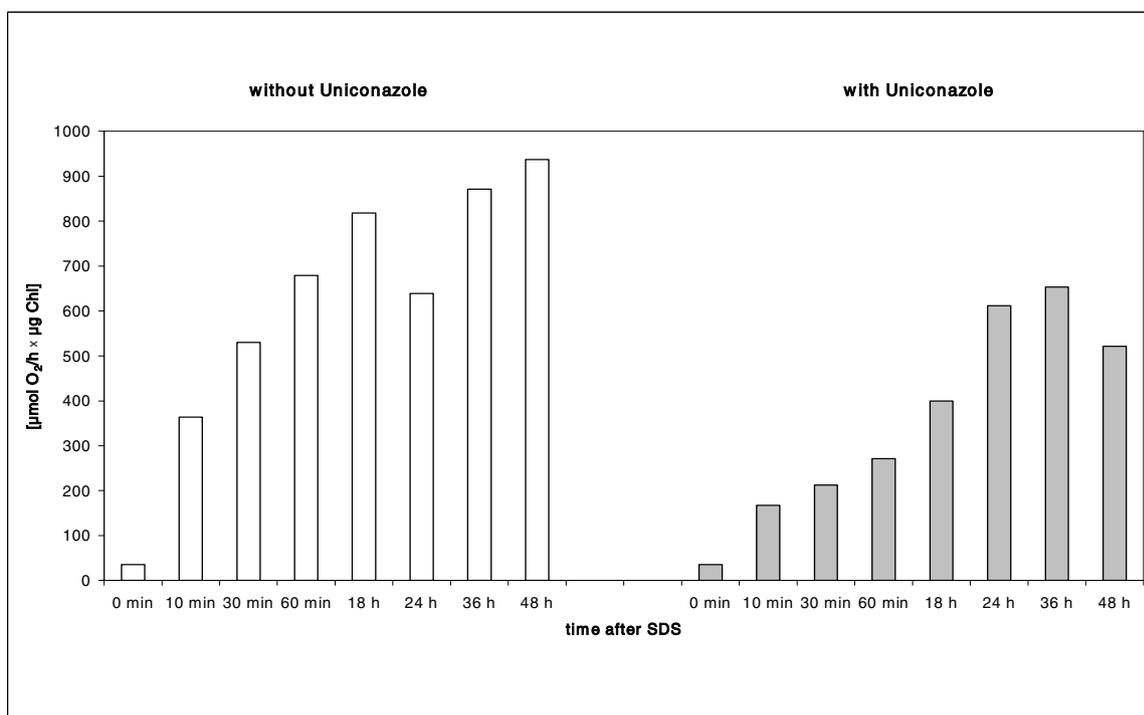
Fig. 5: PPO-activity and activitation after treatment with 0.1 % SDS.

The direct interaction of uniconazole with cytokinins and ABA are often regarded as the explanation for the widespread action on metabolic factors (FLECHER and ARNOLD 1986; NISHIJIMA et al. 1997; ZHOU and LEUL, 1999; HENDERSON et al., 2004). It seems to be more and more evident that the long-lasting effect of short uniconazole treatments is the induced change of metabolism, which obviously causes changes in membrane function and stability. So far, it is not clear, in which way the postulated membrane stabilization may occur.

In order to test the impact of uniconazole on the thylakoids, we choose the *Vicia faba* phenolase system. For a long time it is known that *V. faba* contains a strongly membrane integrated phenolase (SWAIN et al., 1966), which turns into an active enzyme after thylakoid disintegration by SDS. The enzyme activation has been used as a test parameter for the overall stability of the thylakoid. It could easily be shown that uniconazole treatment changed the membrane properties in that way that desintegration and concommittant phenolase activation were retarded compared to control plants. There are both a concentration dependent and a time dependent effect on the activation pattern.

The phenolase test allows us to start a series of experiments to get deeper in the analyses of the molecular interaction of uniconazole with membrane physiology.

Recently, MASCHER et al. (2005) reported on the important role and stress-physiological significance of 2-aminoethanole in drought stressed barley. He used sublethal doses of the stressor paraquat as a causal agent of oxidative stress and combined this treatment with the application of 2-aminoethanol. The pretreatment with the 2-aminoethanol protected the sensitive chloroplast thylakoids. The electron microscopic study revealed an expressed membrane structure protection in plants pretreated with 2-aminoethanol.

According to this finding, general studies on the interaction of triazoles like uniconazole with biogenic amines and their stress-physiological significance should be intensified. They might be helpful to understand a long series of experiments which have been published in the working group of Bergmann (BERGMANN et al., 1994 a-b,; LEINHOS and BERGMANN, 1995), who studies enhanced productivity of cereals after application of biological amines and triazoles.

References

ANGELTON, E.A., FLURKEY, W.H., 1984: Activation and alteration of plant and fungal polyphenoloxidases iso-enzymes in sodium dodecyl sulfate electrophoresis. Phytochemistry 23, 2723-2725.

AZIZ, A., MARTIN-TANGUY, J., LARHER. F., 1998: Stress-induced changes in polyamine and tyramine levels can regulate proline accumulation in tomato leaf discs treated with sodium chloride. Physiol. Plant. 104, 195-202.

BEKHETA, M.A.E.A., 2000: Physiological studies on the effect of uniconazole on the growth and productivity of *Vicia faba* plants grown under different levels of salinity, Ph.D. thesis, Faculty of Science, Cairo University.

BERGMANN, H., LEINHOS, V., MACHELETT, B., 1994a: Increase of stress resistance in crop plants by using phenolic compounds. Acta Horticult. 381, 390-397.

BERGMANN, H., MACHELETT, B., LEINHOS, V., 1994b: Effect of natural amino alcohols on the yield of essentiell amino acids and the amino acid pattern in stressed barley. Amino Acids 7, 327-331.

CAVINS, T.J., GREER, L., GIBSON, J.L., WHIPKER, B.E., DOLE, J.M., 2003: Response of marguerite daisy (*Argyranthemum frutescens*) „Comet Pink" to plant growth regulators. Quarterly Reports on Plant Growth Regulation and Activities of the PGRSA 31, 2-7.

FLETCHER, R.A., ARNOLD, V., 1986: Stimulation of cytokinins and chlorophyll synthesis in cucumber cotyledons by triadimefon. Physiol. Plant. 66, 197-201.

FLETCHER, R.A., HOFSTRA, G., 1988: Triazoles as potential plant protectans. In: Berg, D., Plenpel, M. (eds.), Sterol Biosynthesis inhibitors: pharmaceutical and agrochemical aspects, 321-331. Ellis Harwood Ltd., Cambridge UK.

FLETCHER, R.A., HOFSTRA, G., GAO, J.-G., 1996: Comparative fungitoxic and plant growth regulating properties of triazole derivatives. Plant Cell Physiol. 27, 367-371.

FUKUTA, N., ARAI, M., YUKAWA, T., MATSUMARA, O., 2001: Effect of dwarfing induced by Uniconazole-P on snow tolerance of the Faba bean (*Vica faba* L.). Plant Prod. Sci. 4, 189-195.

GERT, M.P.N., 1995: Paclobutrazole or uniconazole applied in the previous season promotes a lowering of field grown *Rhododendron* and *Kalmia*. J. Plant Growth. Regul. 14, 205-210.

GERT, M.P.N., 1997: Persistence of triazole growth retardants on stem elongation of *Rhododendron* and *Kalmia*. J. Plant Growth Regul. 16, 197-203.

HIRON, R.W.P., WRIGHT, S.T.C., 1973: The role of endogenous abscisic acid in the response of plants to stress. J. Exp. Bot. 81, 769-774.

HENDERSON, J. H., LI, H.C., RIDER, S.D., MORDHORST, A.P., SEVERSON, R.J., CHENG, J.C., ROBEY, J., SUNG, Z.R., DE VRIES, S.C, OGAS, J., 2004: PICKLE acts throughout the plant to repress expression of embryonic traits and may play a role in gibberellin-dependent responses. Plant Physiol. 134, 995-1005.

HUTCHESON, S.W., BUCHANAN, B.B., MONTALBINI, P., 1980: Polyphenol oxidation by *Vicia faba* chloroplast membranes. Plant Physiol. 66, 1150-1154.

KANADE, R.S., PAUL, B., APPU RAO, A.G., GOWDA, L.R., 2006: The conformational state of polyphenol oxidase from field bean (*Dolichos lablab*) upon sodium dodecyl sulphate and acid-pH activation. Biochem. J. 395, 551-562.

LEINHOS, V., BERGMANN, H., 1995: Changes in yield, lignin content and protein pattern of barley (*Hordeum vulgare* cv. Alexis) induced by drought stress. J. Appl. Bot. 69, 206-210.

LICHTENTHALER, H.K., 1987: Chlorophylls and carotenoids – pigments of photosynthetic membranes. Methods Enzymol. 148, 350-382.

LIEBEREI, R., BIEHL, B., VOIGT, J., 1981: Serological studies on phenolase from spinach chloroplasts. Phytochemistry 20, 2109-2116.

MACKAY, C.E., HALL, J.C., HOFSTRA, G., FLETCHER, R.A., 1990: Uniconazole-induced changes in abscisic acid, total amino acids, and proline in *Phaseolus vulgaris*. Pesticide Biochem. Physiol. 37, 74-82.

MASCHER, R., NAGY, E., LIPPMANN, B., HÖRNLEIN, S., FISCHER, S., SCHEIDING, W., NEAGOE, A., BERGMANN, H., 2005: Improvement of tolerance to paraquat and drought in barley (*Hordeum vulgare* L.) by exogenous 2-aminoethanol: effects on superoxide dismutase activity and chloroplast ultrastructure. Plant Sci. 168, 691-698.

MILLER, N.J., RICE-EVANS, C., DAVIES, M.J., GOPINATHAN, V., MILNER, A., 1993: A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. Clin. Sol. 84, 407-412.

MOOR, B.M., FLURKEY, W.H., 1990: Sodium dodecyl activation of a plant polyphenol oxidase. J.Biol.Chem. 265, 4982-4988.

MUTHUKUMARASAMY, M., PANNEERSELVAM, R., 1997: Amelioration of NaCl stress by triadimefon in peanut seedlings. Plant Growth Regulation 22, 157-162.

NISHIJIMA, T., KATSURA1, N., KOSHIOKA, M., YAMAZAKI, H., MANDER L.N., 1997: Effects of uniconazole and GA3 on cold-induced stem elongation and flowering of *Raphanus sativus* L. Plant Growth Regulation 21, 207-214.

RICHTER, H., LIEBEREI, R., VON SCHWARTZENBERG, K., 2005: Identification and characterisation of a bryophyte polyphenol oxidase encoding gene from *Physcomitrella patens*. Plant Biol. 7, 283-291.

SMIRNOFF, N., 1993: The role of active oxygen response to water deficit and desiccation. New Phytol. 125, 27-58.

SMIRNOFF, N., 1998: Plant resistance to environmental stress. Curr. Opin. Biotechnol. 9, 214-219.

SWAIN, T., MAPSON, L.W., ROBB, D.A., 1966: Activation of *Vicia faba* (L.) tyrosinase as effected by denaturing agents. Phytochemistry 5, 469-482.

TENNANT, D., 1975: A test of a modified line intersect method of estimating root length. J. Ecol. 63, 995-1001.

TIKKANEN, O.P., JULKUNEN-TIITTO, R., 2003: Phenological variation as protection against defoliating insects: The case of *Quercus robur* and *Operophthera brumata*. Oecologia 138, 244-251.

ZEEVAAT, J.A.D., CREELMANN, R.A., 1988: Metabolism and physiology of abscicic acid. Ann. Rev. Plant Physiol. 39, 439-473.

ZHOU, W.J., YE, Q.F., 1996: Physiological and yield effects of uniconazole on winter rape (*Brassica napus* L.). J. Plant Growth Regul. 15, 69-73.

ZHOU, W.J., LEUL, M., 1998: Uniconazole-induced alleviation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. Plant Growth Regulation 26, 41-47.

ZHOU, W. J., LEUL, M., 1999: Uniconazole induced tolerance of rape plants to heat stress in relation to changes in hormonal levels, enzyme activities and lipid peroxidation. Plant Growth Regulation. 27, 99-104.

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