Phyton (Horn, Austria)	**Vol. 37**	Fasc. 2	253–261	2. 3. 1998

Effect of Benzyladenine on Peroxidase Activity During Senescence of Sunflower (*Helianthus annuus* L.) Cotyledons

By

Nuran DURMUŞ*) and Asim KADIOĞLU*)

With 3 Figures

Received June 6, 1997

Accepted August 14, 1997

Key words: Benzyladenine, *Helianthus annuus*, peroxidase, senescence

Summary

DURMUŞ N. & KADIOĞLU A. 1998. Effect of benzyladenine on peroxidase activity during senescence of sunflower (*Helianthus annuus* L.) cotyledons. – Phyton (Horn, Austria) 37 (2): 253–261, with 3 figures. – English with German summary.

The influence of benzyladenine (BA, a cytokinin) on peroxidase activity during the senescence of sunflower cotyledons was investigated. For this purpose, changes in the activity of peroxidase were determined during senescence of the cotyledons by spectrophotometric method and the effect of BA on peroxidase isoenzymes was determined by polyacrylamide gel electrophoresis. However, the level of total chlorophyll was measured as the sign of senescence. While the senescence stage of cotyledons progressed, peroxidase activity was found increasing, whereas there was a decline in the level of total chlorophyll. In the BA- treated cotyledons, the activity of peroxidase and the number of isoperoxidases decreased compared with the control whereas the level of total chlorophyll was higher than the control.

From these results, it was concluded that reduced peroxidase activity in the BA-treated cotyledons was a contributing mechanism to the overall antisenescence action of cytokinins, if not at least a close relationship.

Zusammenfassung

DURMUŞ N. & KADIOĞLU A. 1998. Einfluß von Benzyladenin auf die Peroxidaseaktivität während der Seneszenz von Keimblättern der Sonnenblume (*Helianthus*

*) N. DURMUS and A. KADIOGLU, Karadeniz Technical University, Department of Biology, Faculty of Arts and Sciences, 61080, Trabzon, TURKEY.

annuus L.). – Phyton (Horn, Austria) 37 (2): 253–261, 3 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Es wurde der Einfluß von Benzyladenin (BA, ein Cytokinin) auf die Peroxidaseaktivität während der Seneszenz von Keimblättern der Sonnenblume untersucht. Dazu wurden die Änderungen in der Peroxidaseaktivität während der Alterung in den Keimblättern spektrophotometrisch und die Wirkung von BA auf die Peroxidaseisoenzyme mittels Polyacrylamidelelektrophorese untersucht. Der Gesamtchlorophyllgehalt diente als Kennzeichen für die Seneszenz. Mit dem Fortschreiten des Alterungsprozesses der Kotyledonen stieg die Aktivität der Peroxidase an, gleichzeitig war eine Abnahme im Gesamtchlorophyllgehalt zu verzeichnen. In den BA-behandelten Keimblättern sank jedoch die Peroxidaseaktivität und die Zahl an Isoperoxidasen nahm im Vergleich zu den Kontrollen ab; der Gehalt an Gesamtchlorophyll war hingegen höher als in der Kontrolle.

Aus diesen Ergebnissen kann geschlossen werden, daß die verminderte Peroxidaseaktivität in den BA-behandelten Keimblättern ein aktiver Bestandteil der allgemeinen Wirkung des Cytokinins gegenüber Seneszenzprozessen ist; zumindest aber steht sie damit in engem Zusammenhang.

Introduction

Senescence is a process of the dying of some cell, tissue and organs to complete normally growth of plants. It is characterised by a decline in photosynthesis rate, catabolism of pigments and changes in the levels of protein and nucleic acid (Ford & Shiples 1988, Kasemir & al. 1988, Martin & Sabater 1989). One of the most obvious symptoms of senescence in photosynthetic tissues of higher plants is the gradual loss of chloroplast pigments. This usually results in the yellowing of such tissues, particularly during the later stages of senescence (Young & al. 1991, Gut & al. 1987).

During senescence, plant organs exhibit a sharp fall in the amount of chlorophylls, proteins and nucleic acids accompanied by a general increase in some enzymes activities (Thimann 1980, Stoddart & Thomas 1982). The activities of protease and peroxidase (EC 1.11.1.7) have been reported to show an increase with the advancement in senescence (Grover & Sinha 1985). There are several reports that peroxidase activity increases during senescence of detached leaves or leaf discs (Kisban & Mishra 1975, Parish 1968, Mukherjee & Rao 1993). Increase in the activity of this enzyme with the physiological age of the leaves has also been reported (Parish 1968, Ford & Simon 1972, Maraite 1973). Parish 1968 also suggested that the increase in the activity of peroxidase is one of the most reliable indicators of maturity and senescence . However, Srivastava & al. 1983 found no difference in peroxidase activity between young and mature leaves of barley.

A lot of studies have been done on the subjects of the senescence of cotyledons (Kasemir & al. 1988, Kraus & al. 1993), leaves (Martin & Sabater 1989, Young & al. 1991) and ripening of fruits (Mukherjee & Rao 1993). Cotyledon senescence is not fundamentally different from leaf senescence (Gilbert & al. 1980). The process of leaf senescence appears to be

regulated by complex interactions among various plant hormones (THIMANN 1980, STODDART & THOMAS 1982, NOODEN & LEOPOLD 1978). However, cytokinins have been regarded as the most potent senescence- retarding hormones in plants and play a significant role in the regulation of leaf senescence (THIMANN 1980, RICHMOND & LANG 1957). Retardation of senescence by cytokinin, including BA, in excised leaves and cotyledons has been reported in several plant species and this synthetic growth regulator has little or no effect as a retardant of senescence in attached organs (KRAUS & al. 1993, GILBERT & al. 1980, NOODEN & LEOPOLD 1978).

It is probable that antisenescent action mechanism of cytokinins might be in relation to the enzymes. For example, GROSSMAN & LESHEM 1978 demonstrated that cytokinins lowered endogenous lipoxygenase levels in intact pea leaves and decelerated the increase in lipoxygenase activity following excision. So, it might be thought that BA will contribute to the mechanism of the overall antisenescent action of cytokinins by reducing the activity of peroxidase.

For this reason, in this study, the effect of BA on the activity of peroxidase during senescence of sunflower cotyledons was investigated. However, the level of total chlorophyll was determined and the relation between this parameter and peroxidase activity was discussed in the tissue.

Materials and Methods

Plant material

Sunflower (*Helianthus annuus* L.) seeds which were obtained from Agriculture Research Center in Trabzon were sown in pots after they were incubated in the distilled water overnight. The cotyledons of 18 day-old seedlings were used for the experiments. The discs in 1 cm diameter were punched from cotyledons and rinsed in distilled water for 10 min to remove broken cells and then 9 discs were floated on 10 ml of sterilized distilled water or 1, 10 and 100 ppm aqueous solutions of BA in a petri dish. All samples were incubated at 25 °C under continuous dark in a growth chamber. All analyses were done 1-d intervals after incubation (KRAUS & al. 1993).

Chlorophyll content

For chlorophyll determination, the discs were weighed and homogenised in 5 ml of 80% acetone. The samples were centrifuged at 3000 rpm for 5 min and the optical density of the supernatant was read at 663 nm and 645 nm with a spectrophotometer. Total chlorophyll was estimated according to ARNON 1949.

Peroxidase activity

For peroxidase activity, the cotyledon discs were gently homogenised in 10 ml of cold 0.2 M sodium phosphate buffer (pH 7.0). The homogenate was filtered through two layers of cheesecloth and then centrifuged at 20 000 g for 20 min at 4 °C (CANAL

& al. 1988). Protein content of the supernatant was determined according to BRADFORD 1976. Then the extract was dialysed overnight against 0.02 M sodium phosphate buffer (pH 7.0). The dialyzed extract was assayed for peroxidase activity spectrophotometrically by a modification of the method described by RODRIGUEZ & SANCHEZ 1982. The assay mixture contained 1.4 ml of 0.05 M phosphate citrate buffer (pH 4.6), 1 ml of 40 mM guaiacol and 0.5 ml of 26 mM H_2O_2. The mixture was incubated for 15 min at 25 °C and finally 0.1 ml of the enzyme extract was added to the cuvvette. Changes in the absorbance at 420 nm were measured for 3 min using a Schimadzu UV-120-01 spectrophotometer. Peroxidase activity was expressed as ΔA_{420}/min/g fresh weight.

Electrophoresis for isoperoxidases

Polyacrylamide gel electrophoresis was conducted as described by LIU 1973. A separating gel of 3% acrylamide was used. The enzyme extract in 50% glycerol with 1% bromophenol blue was applied to gel. Electrophoresis was conducted in a cold room at +4 °C using reservoir buffer (14.4 g glycine and 3 g tris per litre, pH 8.3) at 10 mA. For analysis of peroxidase isoenzymes, the gel was incubated for 1 h in the solution containing 2.5 ml 3% of H_2O_2/100 ml benzidine solution (0.1 g benzidine/ 100 ml 0.2 M sodium acetate buffer, pH 5.0) at 35 °C . Then it was stored in 30% ethyl alcohol.

Results and Discussion

In the present study, it was determined that the amount of total chlorophyll decreased gradually during senescence of sunflower cotyledons and the loss of total chlorophyll in the controls was greater than that of BA-treated cotyledons (Fig. 1). It was found that the most effective concentration of BA which delays the loss of photosynthetic pigment levels is 100 ppm. At the end of the experiments, the loss of total chlorophyll in the controls was 52.05% whereas in the 100 ppm concentration of BA-treated cotyledons was 26.02%. That is, in the cotyledons treated with 100 ppm BA retained 26.03% more total chlorophyll after 5-d of detachment than in the controls. Similarly, many workers (STODDART & THOMAS 1982, SATLER & THIMANN 1983, CHEN & KAO 1991, JORDI & al. 1993) have reported that exogenously applied cytokinins retarded the loss of photosynthetic pigments during the senescence of leaves and cotyledons.

Fig. 2 shows the time courses of changes of peroxidase activity in sunflower cotyledons treated with water or BA. Peroxidase activity gradually increased in the cotyledon discs treated with water during the 5-d incubation period. MUKHERJEE & RAO 1993 have also obtained similar results in *Cajanus cajan* leaves. Their studies showed that peroxidase activity during maturation of the leaves continuously increased and the leaves in the stage of senescence, have 23-fold higher peroxidase activity than the youngest leaves. In another study, BARTOLI & al. 1995 reported that there was 5-fold enhancement in the peroxidase activity during the senescence of *Chrysanthemum morifolium* petals. Similar results were also obtained

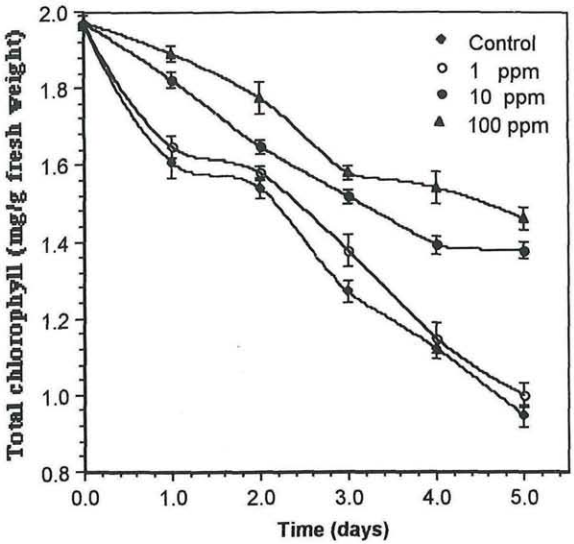

Fig. 1. Effects of BA on total chlorophyll content of detached sunflower cotyledons in the dark. Vertical bars represent standard errors (n = 3).

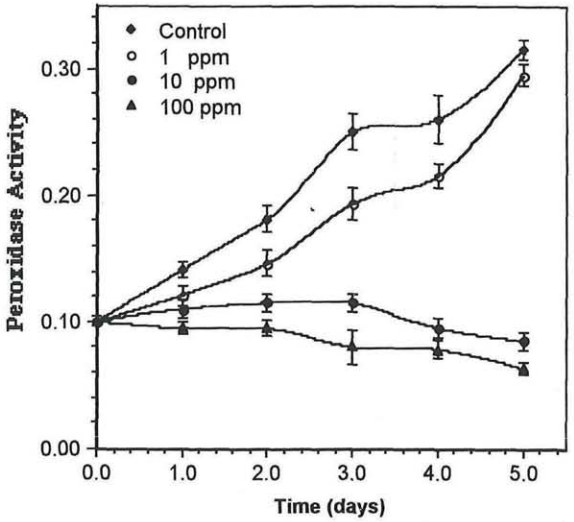

Fig. 2. Effects of BA on peroxidase activity of detached sunflower cotyledons in the dark. Vertical bars represent standard errors (n = 3).

from other plants (KISBAN & MISHRA 1975, PARISH 1968, MUKHERJEE & RAO 1993). The results showed that there was a correlation between chlorophyll content and peroxidase activity during the senescence of the discs. The decrease of peroxidase activity and retardation of chlorophyll loss in 10

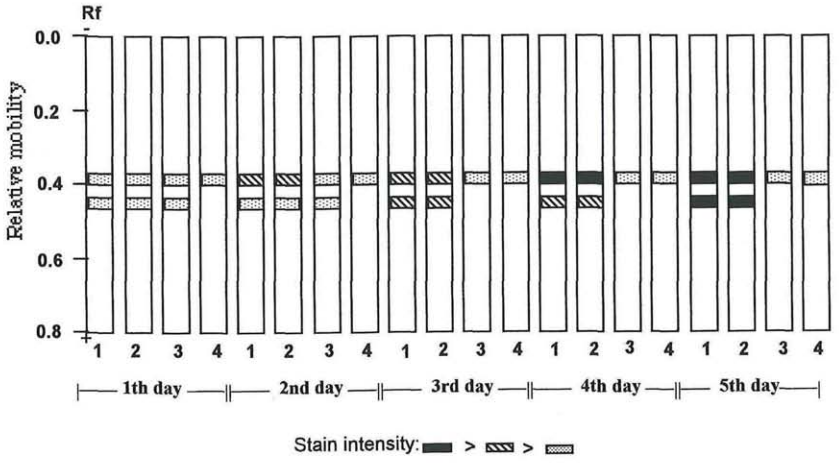

Fig. 3. Effects of BA on the number of peroxidase isoenzymes of detached sunflower cotyledons in the dark. Following discontinuous native polyacrylamide gel electrophoresis, isoenzymes were stained with 2.5 ml 3% of H_2O_2/100 ml benzidine solution for 1 h, the mobilities of the isoenzymes recorded, and the relative intensities of the bands were rated as indicated. The direction of electrophoretic migration was from top (–) to bottom (+). Lane 1: control; Lane 2: 1 ppm; Lane 3: 10 ppm; Lane 4: 100 ppm.

and 100 ppm BA treatments support the idea of peroxidase has a role in chlorophyll degradation (EVERSE & al. 1991).

No significant changes of peroxidase activity were observed in water and 1 ppm of BA-treated cotyledon discs during the senescence but peroxidase activity gradually decreased in 10 ppm and 100 ppm of BA-treated cotyledon discs in the present study. The decrease of peroxidase activity in 10 ppm of BA-treated cotyledon discs was found after 3-d incubation, whereas that of 100 ppm of BA-treated cotyledon discs was observed at 1-d after incubation. We determined that especially 100 ppm of BA significantly decreased the activity of peroxidase as compared with the control. The study done by VENKATARAYAPPA & al. 1984 supports our results. These workers reported that 50 ppm of BA decreased the activity of peroxidase in bean leaf discs. In contrast to above-mentioned findings, GASPAR & XHAUFFLAIRE 1967, 1968 reported that kinetin and isopentenyl adenine increase peroxidase activity in the root sections. This result supports the idea that cytokinins have opposite effect in different organs and ages. Furthermore, BA decreased lipoxygenase (GROSSMAN & LESHEM 1978) and arginine decarboxylase activities (CHEN & KAO 1992) respectively during the senescence of pea and rice leaves.

In addition, we determined the changes in the contents of peroxidase isoenzymes in cotyledon discs which were treated water or BA during 5-d

incubation period (Fig. 3). There was no significant difference in the number of peroxidase isoenzymes and their activities between 1 ppm of BA and the control treatments. However, the effects of 10 ppm and 100 ppm of BA on the number of peroxidase isoenzymes and their activities were found lower than the controls.

It has been shown that the mechanism of action of cytokinins is in relation to nucleic acid metabolism, because adenine is in the structure of cytokinins and isopentenyl adenine which is a hormone in group of cytokinins is in the structure of some tRNAs (LESHEM 1973). On the other hand, it has been suggested that cytokinins retard the senescence by their inhibiting effect on the synthesis of mRNAs coding for some catabolic enzymes such as protease, RNAase and chlorophyllase (HEINZ & BOPP 1981). In the present study, it is likely that the decrease in the number of peroxidase isoenzymes and peroxidase activity in the cotyledons treated with BA in comparison with the control might occur by these ways and consequently retards the senescence. On the other hand, to determine which one was effective on peroxidase, it is necessary to do more experiments about effecting mechanism of BA in the senescent tissues.

Acknowledgement

This work was supported by the Research Fund of Karadeniz Technical University.

References

ARNON D. I. 1949. Copper enzymes in chloroplasts, polyphenoloxidase in *Beta vulgaris*. – Plant Physiol. 24: 1–15.

BARTOLI C. G., SIMONTACCHI M., GUIAMET J. J., MONTALDI E. & PUNTARULO S. 1995. Antioxidant enzymes and lipid peroxidation during aging of *Chrysanthemum morifolium* RAM petals. – Plant Science. 104: 161–168.

BRADFORD M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein- dye binding. – Anal. Biochem. 72: 248–254.

CANAL M. J., TAMES R. S. & FERNANDEZ B. 1988. Peroxidase and polyphenol oxidase activities in *Cyperus esculentus* leaves following glyphosate applications. – Physiol. Plant. 74: 125–130.

CHEN C. T. & KAO C. H. 1991. Senescence of rice leaves XXX, levels of endogenous polyamines and dark- induced senescence of rice leaves. – Plant Cell Physiol. 32: 935–941.

— 1992. Senescence of rice leaves XXXII, effects of abscisic acid and benzyladenine on polyamines and ethylene production during senescence. – J. Plant Physiol. 139: 617–620.

EVERSE J., EVERSE K. E. & GRISHAM M. B. 1991. Peroxidases in chemistry and biology, RATON B. & ARBOR A., Vol II, CRC Press, Boston.

FORD D. M. & SHIPLES R. 1988. Photosynthesis and other traits in relation to chloroplast number during soybean leaf senescence. – Plant Physiol. 86: 108–111.

FORD T. W. & SIMON E. W. 1972. Peroxidase and glucose-6-phosphate dehydrogenase levels in cotyledons of *Cucumis sativus* L. – J. Exp. Bot. 23: 423–431.

GASPAR T. & XHAUFFLAIRE A. 1967. Effect of kinetin on growth, auxin catabolism, peroxidase and catalase activities. – Planta 72: 252–257.

— & — 1968. Action comparée de la 6-furfurylaminopurine et de la 6-(γ, γ-diméthylallylamino) purine sur la croissance, l'activité peroxydasique, la teneur en chlorophylles et en caroténoides. – Physiol. Plant. 21: 792–799.

GILBERT M. L., THOMPSON J. E. & DUMBROFF E. B. 1980. Delayed cotyledon senescence following treatment with a cytokinin, an effect at the level of membranes. – Can. J. Bot. 58: 1797–1803.

GROSSMAN S. & LESHEM Y. Y. 1978. Lowering of endogenous lipoxygenase activity in *Pisum sativum* foliage by cytokinin as related to senescence. – Physiol. Plant. 43: 359–362.

GROVER A. & SINHA S. K. 1985. Senescence of detached leaves in pigeon pea and chick pea: Regulation by developing pods. – Physiol. Plant. 65: 503–507.

GUT H., RUTZ C., MATILE R. & THOMAS H. 1987. Leaf senescence in a non-yellowing mutant of *Festuca pratensis* : Degradation of carotenoids. – Physiol. Plant. 70: 659–663.

HEINZ H. & BOPP M. 1981. Phytohormone: Die Cytokinine. – Biol. Uns. Zeit. 11: 113–120.

JORDI W., DEKHUIJZEN H. M., STOOPEN G. M. & OVERBEEK J. H. M. 1993. Role of other plant organs in gibberellic acid-induced delay of leaf senescence in *Alstroemeria* cut flowers. – Physiol. Plant. 87: 426–432.

KASEMIR H., ROSEMANN D. & OELMÜLLER R. 1988. Changes in ribulose-1,5-bisphosphate carboxylase small subunit during senescence of mustard (*Sinapis alba*) cotyledons. – Physiol. Plant. 73: 257–264.

KISBAN M. & MISHRA D. 1975. Inorganic pyrophosphatase activity during rice leaf senescence. – Can. J. Bot. 53: 503–510.

KRAUS T. E., HOFSTRA G. & FLETCHER R. A. 1993. Regulation of senescence by benzylaminopurine and uniconazole in intact and excised soybean cotyledons. – Plant Physiol. Biochem. 31: 827–834.

LESHEM Y. 1973. The molecular and hormonal basis of plant growth regulation. – Pergamon Press, New York.

LIU E. H. 1973. A simple method for determining the relative activities of individual peroxidase isoenzymes in a tissue extract. – Anal. Biochem. 56: 149–154.

MARAITE H. 1973. Changes in polyphenol oxidases and peroxidases in muskmelon (*Cucumis melo* L.) infected by *Fusarium oxysporum* f.sp. melonis. – Physiol. Plant. Pathol. 3: 29–49.

MARTIN M. & SABATER B. 1989. Translational control of chloroplast protein synthesis during senescence of barley leaves. – Physiol. Plant. 75: 374–381.

MUKHERJEE D. & RAO K. V. M. 1993. Alteration patterns of hill activity, peroxidase activity and sugars of pigeon pea during maturation and senescence. – Indian J. Plant Physiol. 36: 13–16.

NOODEN L. D. & LEOPOLD A. C. 1978. Phytohormones and related compounds. LETHAM D. S., GOODWIN T. W. & HIGGINS T. J., Biomedical Press, Amsterdam.

PARISH R. W. 1968. Studies on senescing tobacco leaf disks with special reference to peroxidase, I. The effects of cutting and of inhibition of nucleic acid on protein synthesis. – Planta 82: 1–13.

RICHMOND A. E. & LANG A. 1957. Effect of kinetin on protein content and survival of detached *Xanthium* leaves. – Science 125: 650–651.

RODRIGUEZ R. & SANCHEZ T. R. 1982. Peroxidase and IAA oxidase in germinating seeds of *Cicer arientium* L. – Rev. Esp. Fisiol. 38: 183–188.

SATLER S. O. & THIMANN K. V. 1983. Relation between respiration and senescence in oat leaves. – Plant Physiol. 72: 540–546.

SRIVASTAVA S. K., VASHI D. J. & NAIK B. L. 1983. Control of senescence by polyamines and guanidines in young and mature barley leaves. – Phytochemistry 22: 2151–2154.

STODDART J. L. & THOMAS H. 1982. Encyclopedia of plant physiology, BOULTER D. & THIER P. P., New series, Springer-Verlag, Berlin.

THIMANN K. V. 1980. Senescence in plants. – CRC Press, Florida.

VENKATARAYAPPA T., FLETCHER R. A. & THOMPSON J. E. 1984. Retardation and reversal of senescence in bean leaves by benzyladenine and decapitation. – Plant Cell Physiol. 25: 407–418.

YOUNG A. J., WELLINGS R. & BRITTON G. 1991. The fate of chloroplast pigments during senescence of primary leaves of *Hordeum vulgare* and *Avena sativum*. – J. Plant Physiol. 137: 701–705.

Phyton (Horn, Austria) 37 (2): 261–262 (1998)

Recensiones

ESCHRICH Walter 1995. Funktionelle Pflanzenanatomie. – Gr. 8°, XI + 393 Seiten, 425 Abbildungen; geb. – Springer-Verlag Berlin, Heidelberg, New York. – DM 78,–. – ISBN 3-540-59131-1.

Dem Konzept, bei der Besprechung des Baues der Pflanzen die Funktion voranzustellen, entsprechend, lauten die Titel der Hauptabschnitte des Buches Wasserversorgung, Wassertransport, Gaswechsel, Lichtwirkung, Transport von Nährstoffen, Blattdifferenzierung, Sekretion, Reizreaktionen, cambiales Wachstum, Statik sowie Reproduktion. Diese sind in je sieben bis 26 Kapitel untergliedert, in denen die der jeweiligen Funktion entsprechenden anatomischen Strukturen behandelt werden. Besonders bezeichnend ist für den vorliegenden Band die überaus reiche Ausstattung mit Abbildungen, die teils Originale darstellen, teils von anderen Autoren übernommen sind. Die Abbildungen (Strichzeichnungen und Schwarzweißphotos) sind meist von sehr guter Qualität, sehr übersichtlich beschriftet und mit ausführlichen Legenden versehen. Sowohl beim knappen, präzisen Text als auch bei der Auswahl der Abbildungen erkennt man einen Autor mit großer eigener Erfahrung, der viele Dinge durch Bilder darstellt, die man in gängigen Lehrbüchern nicht findet, sodaß dem Buch durchaus eine originelle Note zukommt. Auch scheint dem Rezensenten hier das harmonische Zusammenfügen von physiologischer und anatomischer Information besser geglückt als bei einem früher besprochenen Werk mit ähnlichem Titel [Phyton 31(1): 171–178]. Der letzte Abschnitt Reproduktionsbiologie ist mit 36 Seiten im Vergleich zu den vegetativen Organen allerdings äußerst dürftig ausgefallen; es ist eigentlich nur der Bau der Samenschale ausreichend dargestellt. Nektarien sind ganz unbefriedigend behandelt. Die im Zuge der späten Pollenentwicklung und des Pollenschlauchwachstums so wichtige „male germ unit" fehlt. Öffnungsmechanismen