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Effect of Radiation Quality on Phenylalanine Ammonia-Lyase and Pigment Content in the Shoots of Broad Bean (*Vicia faba*) Seedlings

By

Nabil SABER*), Hanan ABOU-ZEID*) and Salah BARAKAT*)

With 5 Figures

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Summary

SABER N., ABOU-ZEID H. & BARAKAT S. 1998. Effect of radiation quality on phenylalanine ammonia-lyase and pigment content in the shoots of broad bean (*Vicia faba*) seedlings. – Phyton (Horn, Austria) 38 (2): 269–279, 5 figures. – English with German summary.

The synthesis and accumulation of photosynthetic and non-photosynthetic pigments as well as the activity of phenylalanine ammonia-lyase (PAL) in the shoot of broad bean (*Vicia faba*) seedlings grown under different irradiations were investigated. The enzyme reached its maximum activity at 25° C and pH 8.5; it was inhibited in the presence of trans-cinnamic acid. Continuous "white light" and light/dark treatments significantly promoted the synthesis of photosynthetic and non-photosynthetic pigments and induced PAL activity. Blue and red irradiation treatments inhibited the synthesis of photosynthetic pigments but induced PAL activity and synthesis of non-photosynthetic pigments. Flavonoid compounds in the shoots and light-harvesting pigment-proteins in the chloroplasts varied under the different irradiations.

Zusammenfassung

SABER N., ABAI-ZEID H. & BARAKAT S. 1998. Einfluß der Strahlungsqualität auf die Phenylalanine-Ammonium-Lyase und den Pigmentgehalt in den Sproßen von *Vicia faba* Sämlingen. – Phyton (Horn, Austria) 38 (2): 269–279, 5 figures. – Englisch mit deutscher Zusammenfassung.

*) N. SABER, H. ABOU-ZEID and S. BARAKAT, Botany Department, Faculty of Science, Alexandria University Alexandria, Egypt.

Sowohl die Synthese und die Akkumulation von Photosynthese und Nicht-Photosynthesepigmenten als auch die Aktivität der Phenylalaine-Ammonium-Lyase (PAL) wurden in den Sproßen von *Vicia faba* Sämlingen untersucht, welche unter verschiedenen Bestrahlungen wuchsen. Das Enzym erreichte seine maximale Aktivität bei 25° C und pH 8,5. Es wurde in Gegenwart der Transzimtsäure inhibiert. Ständiges „Weißlicht" und Licht/Dunkelbehandlungen förderten die Synthese von photosynthetischen und nichtphotosynthetischen Pigmenten und induzierten die PAL-Aktivität. Behandlungen mit rotem und blauem Licht inhibierten die Synthese von photosynthetischen Pigmenten, induzierten jedoch die PAL-Aktivität und die Synthese von Nicht-Photosynthesepigmenten. Flavonoid-Komponenten in den Sproßen und light-harvesting Proteine in den Chloroplasten variierten bei den verschiedenen Behandlungen.

Introduction

The plant pigments include photosynthetic pigments and secondary metabolic products (flavonoids and anthocyanins). In general, low irradiance promotes a large size photosynthetic unit, whereas high irradiance elicits a smaller chlorophyll antena size (Mawson & al. 1994, Webb & Melis 1995).

It has been reported that flavonoids and anthocyanins biosynthesis are controlled internally by multiple regulatory genes (Taylor & Briggs 1990, Kubaseka & al. 1992) and by phytohormones (Weiss & al. 1995) and externally by several factors such as light (Weiss & Halevy 1991).

Phenylalanine ammonia-lyase (PAL, E.C. 4.3.1.5) catalyzes the conversion of phenylalanine (which is synthesized via shikimic acid pathway) to cinnamic acid, the key intermediate in flavonoids biosynthesis. Zucker 1972 reported that the induction of PAL involves at least two of three overlapping aspects: (a) synthesis of PAL, (b) repression of PAL synthesis and (c) inactivation of PAL. The induction of PAL activity is highly controlled by light (Castaneda & Quintero 1990, Weiss & al. 1990).

The aim of this study was to elucidate the effect of irradiation on the induction of photosynthetic pigments and pigment proteins as well as on PAL activity and, consequently, its relation with synthesis of flavonoids and anthocyanins.

Materials and Methods

Broad bean seeds (*Vicia faba* L.) were surface sterilized by soaking in 4% sodium hypochlorite for 5 min, washed thoroughly with tap water and then with distilled water. The sterilized seeds were transferred to plastic pots filled with pure quartz sand. The pots were divided into four sets and placed in growth chambers under the following irradiation treatments: light/dark treatment (16L/8D), photon flux density (PFD) of 870 μmol m^{-2} s^{-1}. WL continuous "white light", PFD of 430 μmol m^{-2} s^{-1}. BR continuous blue irradiation, PFD of 370 μmol m^{-2} s^{-1}. RR continuous red irradiation, PFD of 560 μmol m^{-2} s^{-1}. WL was furnished by a bank of 40 W fluorescent tubes. BR was done with blue fluorescent lamps (TL18/36; Philips) and projector equipped with

a 300 W projector lamp and blue Plexiglas filter (Rroehm and Hass, Hayward, CA). RR was obtained by red fluorescent lamps (TL 15/40 W; Philips) and passing light from a 300 W projector lamp through red filter (Schott RG-610). Photon fluence rates were measured with Li-Cor quantum sensor. During the experimental period enough water was added every other day to each pot to keep the soil moisture content at approximately 80% of the field capacity. The temperature was $25 \pm 2°$ C.

Extraction and enzyme assay of PAL were done according to the method described by ZUCKER 1965. Purification of PAL enzyme of the shoots of 11-day old seedlings was carried out as follows: to the crude enzyme extract 2.5 volumes of acetone (–10° C) were added and held for 20 min to allow for precipitation of protein, centrifuged at 5,000 g for 10 min, and the pellets were collected. After rinsing the pellets with distilled water, the precipitated protein was dissolved in borate buffer, and centrifuged at 20,000 g. The supernatant was dialyzed overnight against two changes of borate buffer containing 4 ml of mercaptoethanol. With crystalline $(NH_4)_2SO_4$, the materials insoluble between 32 and 70% saturation were collected by centrifugation at 5,000 g and dissolved in a minimal volume of borate buffer. The $(NH_4)_2SO_4$-dissolved fraction was carefully layered on top the agarose gel column (40×3.5 cm) equilibrated with 1.5 liters of 0.1 M borate buffer at a flow rate of 1 ml/min. Aliquots of 5 ml were collected with the same borate buffer as eluent, and these were analyzed for protein and enzyme activity. The most active fractions were combined, mixed with crystalline $(NH_4)_2SO_4$, 70% saturation, and the precipitate was collected by centrifugation for 10 min at 5,000 g. The purified enzyme was dissolved in 5 ml of 0.1 M borate buffer, pH 8.5.

The photosynthetic pigments were extracted and determined using the spectrophotometric method recommended by METZNER & al. 1965. Flavonoids were extracted from the shoots of 11-day old seedlings and estimated according to the method described by THIEME & KHOGALI 1975. Flavonoid compounds were analyzed, in comparison with authentic samples, by HPLC on HC-ODS column with 0 to 55% acetonitrile in water as eluent at a flow rate of 0.5 ml min^{-1} for 30 min (GRAHAM 1991). Protein was estimated after LOWRY & al. 1951.

Isolation of chloroplasts of the leaves from 11-day old seedlings was carried out according to MACHOWICZ & WIECKOWSKI 1978. For extraction of chloroplast proteins, 2 ml of chloroplast suspension were diluted with tris-HCl buffer, pH 8.9, and the suspension was sonicated for 2 min on ice bath, then centrifuged at 20,000 rpm (4° C) for 20 min. Electrophoresis (SDS-PAGE) was carried out according to LAEMMLI 1970. The calculations of F-test (ANOVA) were made according to SNEDECOR & COCHRAN 1973.

Results and Discussion

There was an increase in PAL activity and in flavonoid and anthocyanin content of the shoots of broad bean seedlings under all irradiation treatments (Fig. 1), particularly under continuous "white light". Compared with L/D treatment (control) blue and red irradiations had a similar but slightly less promotive effect on anthocyanin synthesis, whereas PAL activity and flavonoid content were slightly enhanced. In contrast, BR and RR were markedly inhibitory to the synthesis of photosynthetic pigments

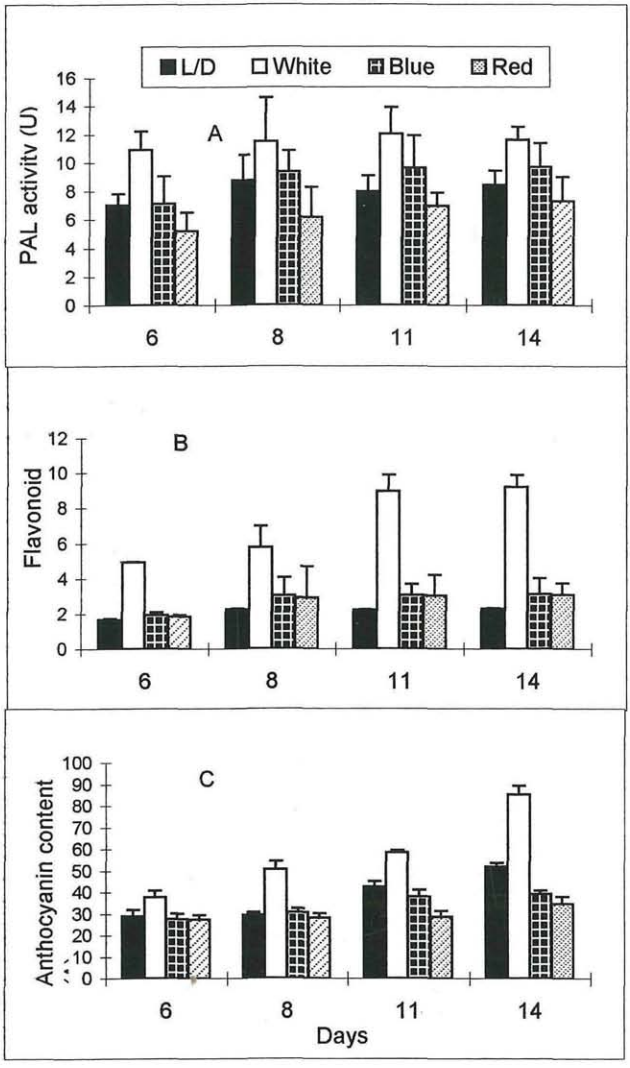

Fig. 1. Effect of different irradiations on: A, PAL activity (U, increase in A_{290} g^{-1} fr. wt); B, flavonoid content (mg g^{-1} d.wt); C, anthocyanin content (A, difference A_{530}-A_{675} g^{-1} d.wt). Each value is the mean of three indpendent samples readings ± SE. L/D, light/dark cycle; WL, white light; BR, blue radiation; RR, red radiation.

content (Fig. 2). The analysis of variance (F-test) indicated that with the different irradiation treatments the overall variations were highly significant (P = 0.001) for the PAL activity, flavonoids, anthocyanins and photosynthetic pigments (Table 1). These results indicate that the synthesis

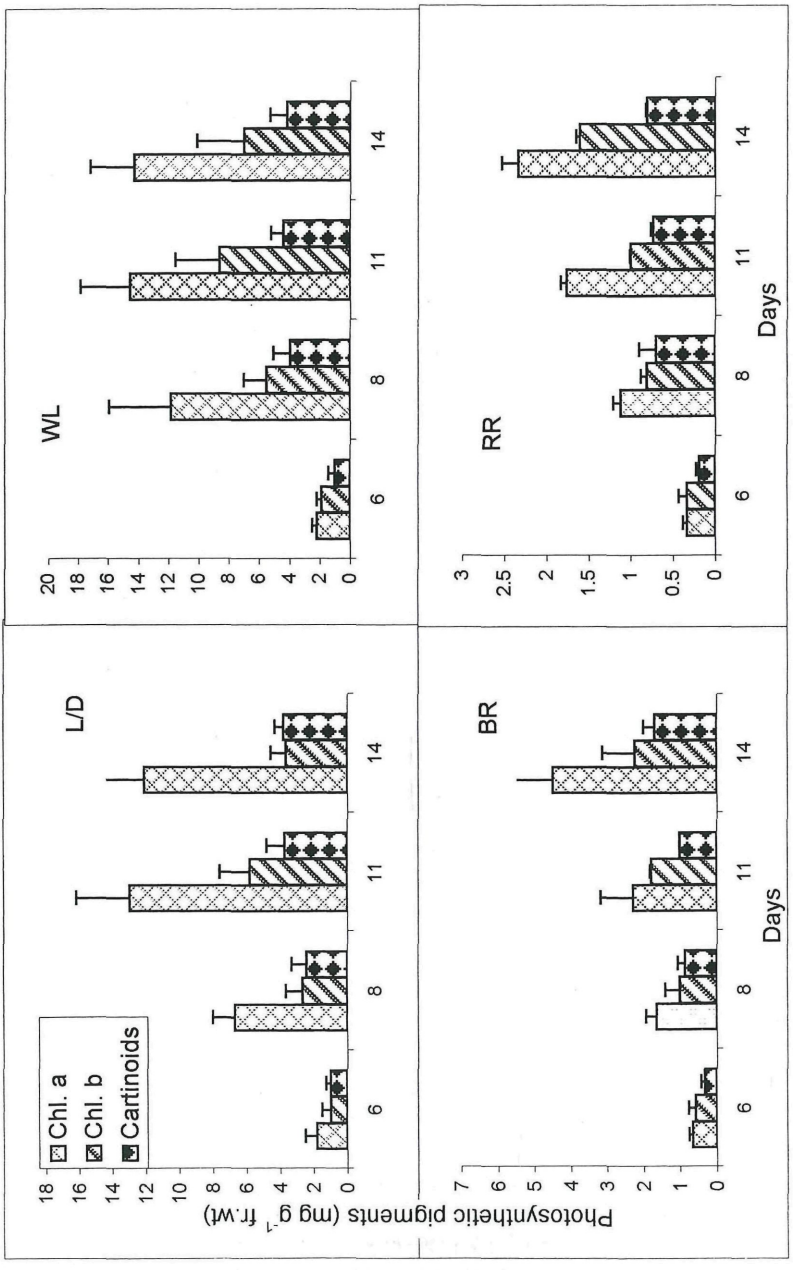

Fig. 2. Effect of different irradiations on the photosynthetic pigments in the shoots of broad bean seedlings. L/D, light/dark cycle; WL, white light; BR, blue radiation; RR, red radiation.

Table 1

F-values (analysis of variance) for variations in the PAL activity, flavonoids, anthocyanins and total photosynthetic measurements in the shoot of broad bean plants, with various light treatments and days, and their interaction at the probability level of 0.001. All figures are highly significant except flavanoids with days under red irradiation (*) and anthocyanin with days under blue irradiation (**).

Source of variations	PAL activity	Flavonoids	Anthocyanins	Total photosynthetic pigments
L/D	829	958	1591	283
treatment	1225	4309	2100	586
Days	1704	1920	1826	900
Interaction				
White light	494	449	153	555
Days	593	1280	622	1081
Interaction	655	561	795	898
Blue light	370	118	137	426
Days	738	642	193**	911
Interaction	264	683	247	1150
Red light	416	167	152	339
Days	1826	252*	450	810
Interaction	454	728	260	1020

of photosynthetic and non-photosynthetic pigments and the induction of PAL activity in broad bean shoots are light quality-dependent rather than irradiance-dependent. Under continuous RR (PFD of 560 μmol m^{-2} s^{-1}) the pigment contents and PAL activity in the shoots were lower than under WL (PFD of 430 μmol m^{-2} s^{-1}) or BR (PFD of 370 μmol m^{-2} s^{-1}). Furthermore L/D treatment enhanced the synthesis of photosynthetic pigments more than the BR or RR but less than the continuous "white light" treatment. WEISS & al. 1990 found that blue light was more effective than red light in promoting anthocyanin synthesis and PAL activity in *Petunia* petals. In contrast, KOES & al. 1989 reported that pigmentation of *Petunia hybrida* (V30) was induced by red light, but not by blue light. CASTANEDA & QUINTERO 1990 showed that PAL activity in *Gompherna globosa* under blue light was higher than under white light, whereas MOSCOVICI & al. 1996 have recently reported that blue and red light, of the same PFD, had similar effects on anthocyanin synthesis in *Petunia* flower. Therefore, the results of this study suggest the possible involvement of photosynthetic system and photoreceptors (such as phytochrome-mediated high irradiance responses, HIR) in the light-induced PAL activity and synthesis of flavonoids and anthocyanins in the shoots of broad bean seedlings.

Table 2

Purification steps of PAL enzyme extracted from the shoots of 11-day old broad bean seedlings grown under L/D treatment as described in Materials and Methods. The enzyme activity was expressed as U (increase in A_{290})

Purification Steps	Volume ml	PAL activity U/ml	Total activity	Total protein mg	Specific activity	Recovery	Purification fold
Crude extract	94.6	18.4	1740.6	272.0	6.4	100	1.0
ppt. by acetone	56.7	21.4	1213.4	102.6	11.8	44	1.8
Dialysis	25.9	24.3	629.4	48.2	13.1	23	2.0
32–70% $(NH_4)_2SO_4$	18.4	27.2	500.5	3.3	151.7	18	23.7
Agarose	14.2	29.9	424.6	1.3	326.6	15	51.0
70% $(NH_4)_2SO_4$	3.8	33.2	126.2	0.2	631.0	5	98.6

The purification stages of PAL enzyme (Table 2) show that the second $(MH_4)_2SO_4$ precipitate had the highest specific activity and the purification of enzyme reached about 99% of crude extract. The rate of reaction increased gradually up to 60 μM of phenylalanine and K_m for PAL was 0.0312 μM. The purified enzyme exhibited its maximum activity at 30° C and pH 8.5. Trans-cinnamic acid was a potent inhibitor of the purified PAL enzyme, but coumaric acid had no effect. Chlorogenic and caffeic acids at 0.3mM were strongly inhibitory to enzyme activity. This indicates that cinnamic, caffeic and chlorogenic acids are feedback inhibitors. Tyrosine had no effect and, therefore, did not serve as substrate (Table 3).

Table 3

Effect of some aromatic compounds on the activity of purified PAL of the shoots of 11-day old broad bean seedlings grown under L/D cycle. The control value was 0.262 U. The activity was expressed as U (increase in A_{290} ml^{-1} hr^{-1}). A, activity; RA, relative activity to control.

Aromatic comps.	Cinnamic		Coumaric		Chlorogenic		Caffeic		Tyrosine	
Coc. (mM)	A	RA	A	RA	A	RA	A	RA	A	RA
0.05	0.259	99	0.262	100	0.262	100	0.262	100	0.262	100
0.1	0.225	86	0.262	100	0.259	99	0.262	100	0.262	100
0.2	0.222	85	0.262	100	0.246	94	0.225	86	0.262	100
0.3	0.157	60	0.262	100	0.196	75	0.209	80	0.262	100
0.5	0.112	43	0.262	100	0.186	71	0.196	75	0.262	100
1.0	0.091	35	0.262	100	0.131	50	0.125	48	0.262	100

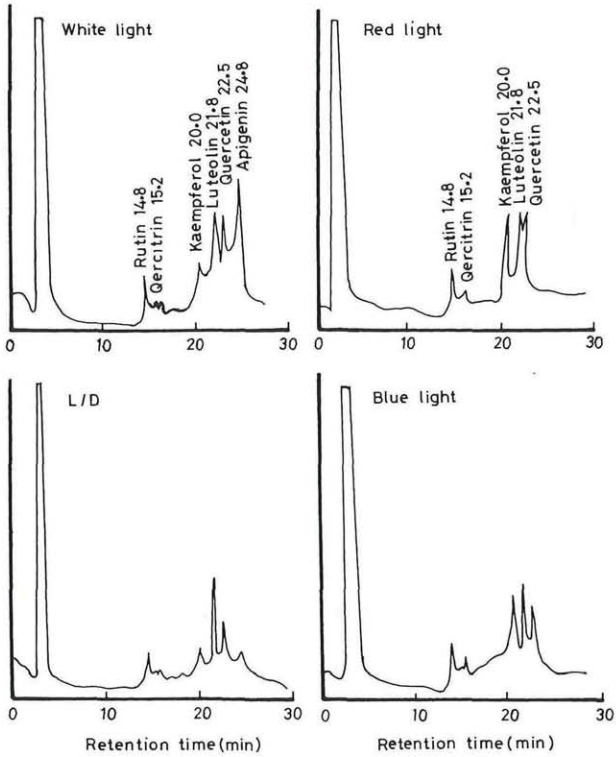

Fig. 3. HPLC analysis of flavonoid compounds of the shoots of 11-day old broad bean seedlings grown under different irradiations.

Six flavonoid compounds, viz quercetin, kaempferol (flavonol); apigenin, luteolin (flavone); quercitrin and rutin (flavonol glycoside) were isolated from the shoots of 11-day old broad bean seedlings grown under continuous "white light" and L/D treatments. Apigenin was not detected under blue or red irradiations (Fig. 3). Although we did not estimate these compounds quantitatively the results may still indicate that light quality had different effects on specific regulatory genes for the synthesis of flavonoid compounds (BEVAN & al. 1989, TAYLOR & BRIGGS 1990, KUBASEKA & al. 1992).

Scans of electrophoretic separation of chloroplast-protein complex of isolated chloroplasts of the leaves of 11-day old broad bean seedlings grown under L/D treatment showed that there are six polypeptides with molecular mass ranging from 24 to 100 kD associated with photosystems I and II (Fig. 4). The polypeptide with 86 kD completely disappeared in plants grown under continuous white radiation. Under continuous blue

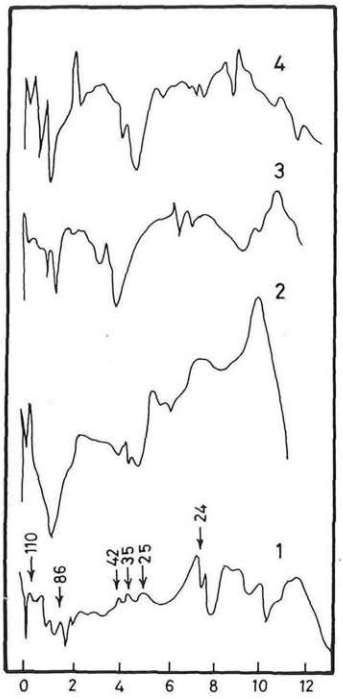

Fig. 4. Scans of SDS-PAGE separation pigment proteins of isolated chloroplasts of the shoots of 11–day old broad bean seedlings grown under different light treatments. 1, L/D; 2, continuous "white light"; 3, continuous blue radiation and 4, continuous red radiation.

radiation the polypeptides with 32 and 25 kD completely disappeared, whereas under continuous red radiation the polypeptide with 32 kD could not be detected. These results in accordance with MELIS 1991 & MAWSON & al. 1994 who reported that the level of irradiance during plant growth modulates the size and composition of the light-harvesting pigment-proteins of the photosystems. Thus, the variation of the light-harvesting proteins associated with the photosystems of broad bean seedlings grown under the varying irradiations may affect photosynthesis and hence the photosynthetic dependent-synthesis of flavonoids and anthocyanins.

The results of this study suggest that there are two mechanisms, photosynthetic and non-photosynthetic, involved in the induction of PAL activity and promotion of flavonoids and anthocyanins synthesis in the shoots of broad bean seedlings; both mechanisms are affected by light quality rather than by light irradiance.

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Recensio

VAUGHAN J. G. & GEISSLER C. 1997. The New Oxford Book of Food Plants. Illustrated by B. E. NICHOLSON. With additional illustrations by Elisabeth DOWLE and Elizabeth RICE. – Lex. 8°, XX + 239 Seiten, 100 Farbabbildungen; geb. – Oxford University Press, Oxford, New York, Tokyo. – £ 25,–. – ISBN 0-19-854825-7.

Das Oxford Book of Food Plants von S. G. HARRISON, G. B. MANSFIELD & M. WALLIS aus 1969 ist fast schon als ein Klassiker zu bezeichnen. Es war sehr beliebt und weit verbreitet, die Gründe dafür dürften im wesentlichen wohl in der ausgewogenen Auswahl, in den schönen Abbildungen nach den Aquarellen von B. E. NICHOLSON und dem präzisen, recht zuverlässigen Text mit Beschreibungen und Hinweisen auf Herkunft und Nutzung gelegen sein.

Es ist daher höchst erfreulich, daß dieses Standardwerk nun in einer völligen Neubearbeitung vorliegt. Der gesamte Text wurde durchgesehen und gegebenenfalls aktualisiert und einige zusätzlichen Aren sind aufgenommen worden. Eines der alten Schlußkapitel ist – stark verändert – zu einem etwas über sechs Seiten langen Einleitungskapitel geworden (mit Hinweisen über Domestikation und Ausbreitung von Kulturpflanzen über die Erde, sowie allgemeinen Hinweisen über die Nutzungsgruppen, denen die Arten in diesem Buch zugeordnet sind). Das am Ende stark erweiterte Kapitel „Nutrition and health" umfaßt 14 Seiten, u.a. mit Ausführungen über Nahrungsbestandteile wie Kohlenhydrate, Fette, Eiweiß, Vitamine, Mineralstoffe, sekundäre Pflanzenstoffe, Gift- und Hemmstoffe, über Wirkung von Verarbeitung und Lagerung, über den täglichen Bedarf des Körpers etc. „Nutrition tables" enthält 14 Tabellen (und eine Abbildung), vor allem über Zusammensetzung pflanzlicher Nahrungsmittel aber auch über Vitamin-Quellen, Vitaminverluste beim Kochen u.a.

Die Farbbilder der alten Ausgabe sind alle unverändert übernommen worden, es sind jedoch auch neue hinzugekommen, einerseits durch Einfügen zusätzlicher Abbildungsseiten, andererseits durch Abbildungen am Fuße von nicht ganz für Text genutzten Seiten. So wird diese neue Version sicher auch rasch weite Verbreitung bei allen an Kulturpflanzen Interessierten finden.

Einige Fehler haben sich (z.T. schon in der Erstausgabe) leider eingeschlichen, die zwar von der Zahl her nicht ins Gewicht fallen, aber besonders unangenehm sind, wenn sie Abbildungen betreffen. Die Blüte von *Canavalia* (p. 45, Fig. 2A) ist falsch orientiert, die Platte der Fahne gehört nach unten bzw. horizontal gerichtet. Der angebliche *Lablab niger* auf p. 49 ist eine *Phaseolus vulgaris*-Sorte mit Anthozyan-ge-