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Influence of Sulphur and Nitrogen Supply on the Pigment Content of Oilseed Rape, Marigold, and Beetroot

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With 2 Figures

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Summary

BLOEM E., SCHROETTER S., EL-KHAYAT A. S. M. & SCHNUG E. 2011. Influence of sulphur and nitrogen supply on the pigment content of oilseed rape, marigold, and beetroot. – Phyton (Horn, Austria) 50 (2): 301–317, with 2 figures.

In the past, it was shown that severe symptoms of sulphur (S) deficiency and nitrogen (N) deficiency are related to the pigment content in leaves and flowers of oilseed rape. N and S deficiency both cause chlorosis of leaves by interfering with chlorophyll biosynthesis. S-deficient flowers of oilseed rape change in colour from bright yellow to pale yellow or nearly white flowers, a phenomenon closely linked to the formation of pigments. In the present study, the impact of S and N nutrition on the synthesis of different plant pigment classes was investigated in different plants and plant organs in order to assess a possible influence of fertilisation practices on this food quality parameter.

In a pot experiment with oilseed rape (*Brassica napus*), marigold (*Calendula officinalis*) and red beetroot (*Beta vulgaris* subsp. *vulgaris*) the influence of a graded N and S supply on the content of photosynthetic pigments in leaves (chlorophyll,

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carotenoids) and colour intensity of flowers (flavonoids and carotenoids) of oilseed rape and marigold and in tubers (betacyanin and betaxanthin) of beetroot was determined. The chlorophyll content in leaves of oilseed rape and beetroot increased with increasing N nutrition, while the N and S supply did not affect the chlorophyll content of marigold leaves. Changes in the S supply had no effect on the pigment content of beetroot. The N supply had the strongest influence on the betalain content in beetroot tubers and the colour intensity of flowers of oilseed rape and marigold. In case of oilseed rape, interactions between the S and N supply were found. The colour intensity of oilseed rape flowers increased with increasing S level, while a higher N supply reduced the pigment content.

Zusammenfassung

Bloem E., Schroetter S., El-Khayat A. S. M. & Schnug E. 2011. Influence of sulphur and nitrogen supply on the pigment content of oilseed rape, marigold, and beetroot. [Einfluss der Schwefel- und Stickstoffversorgung auf den Pigmentgehalt von Sommerraps, Ringelblumen und Roter Beete]. – Phyton (Horn, Austria) 50 (2): 301–317, mit 2 Abbildungen.

In der Vergangenheit wurde bereits der enge Zusammenhang zwischen der Schwefel (S)- und Stickstoff (N)-versorgung und dem Pigmentgehalt in Blättern und Blüten von Raps aufgezeigt. N- und S-Mangel haben beide Blattchlorosen zur Folge, was auf eine Störung der Chlorophyllbiosynthese hindeutet. Auch die Blüten von Raps weisen bei S-Mangel eine gestörte Pigmentbiosynthese auf: S-Mangelpflanzen entwickeln im Gegensatz zu den typischen, leuchtend gelben Blüten gut mit S versorgter Pflanzen nur schwach gelbliche bis hin zu weißen Blüten. In der vorliegenden Untersuchung wurde der Einfluss der N- und S-Versorgung auf die Synthese verschiedener Klassen von Pflanzenpigmenten in unterschiedlichen Pflanzen und Pflanzenorganen untersucht, um den Einfluss der Pflanzenernährung auf diesen Qualitätsparameter von Pflanzen zu ermitteln.

In einem Gewächshausversuch mit Raps (*Brassica napus*), Ringelblume (*Calendula officinalis*) und Rote Beete (*Beta vulgaris* subsp. *vulgaris*) wurde der Einfluss der N- und S-Versorgung auf den Gehalt an photosynthetischen Pigmenten (Chlorophyll, Carotinoide) in Blättern untersucht sowie die Farbintensität der Blüten von Raps und Ringelblume (Flavonoide, Carotinoide) und in den Knollen der Roten Beete (Betacyanin, Betaxanthin). Der Chlorophyllgehalt in den Blättern von Raps und Rote Beete stieg mit steigender N-Düngung an, während bei der Ringelblume weder die N- noch die S-Düngung einen Einfluss auf die Chlorophyllgehalte zeigte. Änderungen in der S-Versorgung zeigten keinen Einfluss auf die Pigmentgehalte der Roten Beete. Die N-Versorgung hatte den stärksten Einfluss auf den Betalaingehalt der Roten Beete und auf die Farbintensität der Blüten von Raps und Ringelblume. Im Falle des Rapses wurde eine Interaktion zwischen der S- und der N-Versorgung ermittelt. Die Farbintensität der Rapsblüten stieg mit steigender S-Versorgung, während ein höherer N-Status die Pigmentgehalte reduzierte.

Introduction

In recent years, more and more synthetic pigments were replaced by natural food colorants as some synthetic substances were suspected to cause allergic, toxic and carcinogenic effects when used in human nutri-

tion (PICCAGLIA & al. 1997), while natural pigments have a high nutraceutical value. A well-balanced diet rich in natural pigments is the key to prevention and therapy for a range of chronic diseases (LILA 2004). Pigments per definition are molecules that adsorb light and are important for plant growth. The best example is chlorophyll. Pigments determine the colour of the plant tissue by selectively absorbing certain wavelengths of light and reflecting others (BUCHANAN & al. 2000). Different plant pigments occur in nature and based on their chemical structures they can be divided into four families, i.e. tetrapyrroles (e.g. chlorophyll), carotenoids, polyphenolic compounds (e.g. anthocyanins), and alkaloids (e.g. betalains). Chlorophyll molecules contain a porphyrin structure similar to that found in the heme prosthetic group of haemoglobin and the cytochromes. Hence, they contain a distinct amount of N. The function of photosynthetic pigments is to transform light energy during photosynthesis into chemical energy available for plant metabolism. The carotenoids operate as accessory pigments in photosynthesis. Carotenoids are tetraterpenes consisting of carbonhydrates and oxygen.

Flavonoid pigments (C_6-C_3-C_6 skeleton) consist of the anthocyanins, which are red, blue or purple pigments, the yellow anthoxanthins, and the colourless catechins and leuco-anthocyanins (RANGANNA 1986). Beside of flavonoids, carotenoids are important flower pigments responsible for petal colours in the yellow to red range (KISHIMOTO & OHMIYA 2009).

The red and yellow betalains show a very different chemical structure. They are indole-derived compounds synthesised from tyrosine. In contrast to anthocyanins, they contain N (NILSSON 1970). Beetroot pigments consist of red-violet betacyanins and yellow betaxanthins. There are still uncertainties about the functions of betalains in plants. There is some evidence that they may have fungicidal properties (KIMLER 1975).

Two plants, which are important in the production of natural food colorants, are marigold (*Calendula officinalis*) and red beetroot (*Beta vulgaris* subsp. *vulgaris*) (PICCAGLIA & al. 1997, PICCAGLIA & VENTURI 1998). Marigold is well known for its pharmacological activities. The petals contain two classes of pigments, flavonoids and carotenoids, used as yellow and orange natural colours (PICCAGLIA & al. 1997). The betalains of beetroots are responsible for the deep red colour of the tubers and they are mainly used as food colorants (E 162) in particular for dairy products like yoghurts and ice creams (PICCAGLIA & VENTURI 1998).

Growth conditions such as seed density, water supply, or fertilisation are crucial for yield and pigment content of such agricultural crops (SCHOEFS 2004). Several studies were undertaken to quantify these relationships, but still many questions are open with view to the impact of the nutritional status on pigment contents.

Severe sulphur (S) deficiency caused strong symptoms in leaves as well as in flowers of oilseed rape indicating a disruption in pigment bio-

synthesis (SCHNUG & HANEKLAUS 1994). The leaves show a characteristic marbling with dark green areas around the veins and much lighter areas in between. The flowers change in size and colour from big bright yellow up to little pale yellow. SCHNUG & HANEKLAUS 2005 explained the shift in flower colour by an overload of petal cells with carbohydrates due to disorders in the protein metabolism. They suggested that plants produce colourless leuco-anthocyanins to avoid excessive accumulation of free sugars in cells. With additional nitrogen (N) fertilisation, these symptoms became even stronger (HANEKLAUS & al. 2006).

These flower symptoms of S deficiency are unique in oilseed rape and so far, only little is known about the impact of the S supply on the pigment biosynthesis of different crops in general. Both, increasing the pigment content of plants, used as natural colorants and the quantification of the relationship between fertilisation and pigmentation, are of prime relevance for industries.

It was the aim of this study to investigate the impact of S and N fertilisation on selected pigments in different crop plants and plant organs.

Material and Methods

Experimental Design

A bi-factorial greenhouse experiment was conducted at the Julius Kühn-Institute in Braunschweig with 3 S (0, 50 and 250 mg S per pot as K_2SO_4) and 2 N levels (250 and 1000 mg N per pot as NH_4NO_3). S levels were chosen to adjust the supply to severe S deficiency, marginal supply and excess S; the N rates corresponded with severe N deficiency and excess N supply. Plants were grown in Mitscherlich pots, each filled with 8 kg of pure sand. S was fertilised before planting and potassium was balanced with KCl. Fertilisation with N and S was split into two equal doses. The first fertiliser rate was given before planting and the second one 6 weeks later. All other nutrients (P, Ca, K, Mg, Mn, Zn, Cu, Mo, Fe, B) were applied at physiologically optimum rates. Three different crops were grown: Summer oilseed rape (*Brassica napus*, cv. *"Topas"*), marigold (*Calendula officinalis*, cv. *"Porcupine"*), and beetroot (*Beta vulgaris*, cv. *"Monalisa"*). Young plants were grown in standard cultivation trays. At two-leaf stage, 9 plants were transferred to Mitscherlich-pots. Each treatment had four replicates. Plants were grown under a constant light regime of 1,500 Lx with a light/dark rhythm of 16h/8h and a temperature of 20° C at day and 15° C at night.

About 8 weeks after planting, when the main vegetative growth started, the number of plants was reduced to 5 plants per pot. In the harvested leaf material the mineral composition was determined (Table 1) and the contents of photosynthetic pigments were analysed (Table 2).

With the start of blooming flowers were collected two times per week during 11 weeks from oilseed rape and marigold. Leaf and flower samples were immediately shock-frozen in liquid N to prevent decomposition of pigments. Each time the number of harvested flowers was counted to determine the flower yield in relation to N and S fertilisation. The beetroot tubers were harvested 29 weeks after planting and

the whole fresh weight of the tubers per pot (from 5 plants) was calculated. The pigment content of the tubers was analysed directly after harvest in the fresh plant material.

Chemical Analysis

Mineral composition: Total N was determined employing the *Kjeldahl* method. For the mineral extraction of total S, calcium, magnesium, phosphorus and manganese 0.5 g dry plant material was digested with 4mL HNO_3 + 1mL H_2O_2 in a microwave oven (CEM/MDS-2100) at 950 ± 50 Watt for in total 57 minutes. A pressure of 1.37 bar was reached at 20% of the power in 15 minutes and was kept for another minute, then the pressure was further raised in 15 minutes to 2.96 bar at full power and kept again for 1 minute, and finally in 15 minutes to 9.99 bar at full power and kept for another 10 minutes. Then the samples were allowed to cool down for 20 minutes and afterwards the digest was filled up to 50 mL. The mineral contents were analysed by ICP-OES (Spectro Flame M120S, Kleve, Germany).

Photosynthetic pigments: Chlorophyll *a* and *b*, and carotenoids were determined according to LICHTENTHALER 1987a in leaf samples of all crops. Young, fully developed leaves were sampled at the beginning of main growth. 10 leaf discs (diameter of 6.5 mm) were directly grinded and extracted with acetone (100 %) whereby a pinch of $CaCO_3$ was added (ABADIA & ABADIA 1993). The sample was filled up with acetone to a final volume of 10 mL. The extract was centrifugated for 5 minutes at 5,900 rpm. Afterwards, the absorption of the supernatant was measured at different wavelengths (645, 662, 470, 730 nm) using a spectral photometer (Uvicon 931, Biotek Kontron).

The chlorophyll *a, b,* and carotenoid content were calculated according to LICHTENTHALER & WELLBURN 1983 by the following equations:

Chlorophyll *a* [µg/ml plant extract]: $C_a = 11.75 \cdot A_{662} - 2.35 \cdot A_{645}$

Chlorophyll *b* [µg/ml plant extract]: $C_b = 18.61 \cdot A_{645} - 3.96 \cdot A_{662}$

$$\text{Carotenoids [µg/ml plant extract]} = \frac{1000 \cdot A_{470} - 2.27 \cdot C_a - 81.4 \cdot C_b}{227}$$

Colour intensity of flowers and absorption spectrum: Flower colour and absorption spectra were determined in flowers of oilseed rape and marigold as a measure for the pigment content (flavonoids and carotenoids) according to the method described by ERNST 2002. The flower extract was prepared by extracting 25 petals of oilseed rape or about 30–50 mg fresh petals of marigold with 5 mL methanol (containing 1% HCl). The extraction procedure was repeated if the extracted flowers were still yellow. The flower extracts were filled up with methanol (1% HCl) to a final volume of 10 mL. The extract was measured in a UV-VIS spectrophotometer (Uvicon 931, Biotek Kontron) from 312 to 500 nm and the petal colour intensity was determined at the maximum extinction. For marigold, the maxima were 423 and 446 nm and oilseed rape showed maxima at 440 and 470 nm.

Determination of betacyanin and betaxanthin in beetroot according to NILSSON 1970, 1973:

The harvested tubers from one pot were washed and a piece from the middle of each tuber was cut and pooled for the sample. 300 g of the crushed sample was used for the analysis. After addition of water, corresponding to 80% of the weight of the sample, the samples were homogenized for 3 minutes and a fraction of 20 g was

centrifuged to separate the juice. 25 µL of the juice was diluted with 4 mL aqueous phosphate buffer (pH 6.5) and the absorbance was measured at 600, 538 and 476 nm by using a spectral photometer (Uvicon 931, Biotek Kontron). The average values of triplicate dilutions were used to calculate the absorbance components for red and yellow pigments with the formula described by NILSSON 1970.

Results and Discussion

N and S fertilisation distinctly increased the N and S content in leaves of oilseed rape, marigold and beetroot (Table 1).

Without S fertilisation oilseed rape and marigold displayed only low S contents in the leaves. However, only leaves of oilseed rape at the high N rate showed symptoms of severe S deficiency. S fertilisation had hardly any effect on S uptake of beetroot. A low level seemed to be sufficient to satisfy the S demand of this crop.

N and S metabolism are closely related as both nutrients are used in protein biosynthesis. In oilseed rape excess N rates increased S deficiency, expectedly. In case of beetroot and marigold excess N resulted in a higher S uptake.

N and S fertilisation affected the uptake of other essential plant nutrients, too. For example, in leaves of all three crops nearly twice of the amount of manganese was found when N was applied at the higher dose (Table 1).

Influence of N and S nutrition on photosynthetic pigments

The chlorophyll and carotenoid content was determined in leaf samples of all crops. The colour intensity of marigold and oilseed rape flowers was determined as a measure for the flavonoid and carotenoid content. Last, but not least the betalain (betacyanin and betaxanthin) content of beetroot was measured. Chlorophylls and betalains are N containing molecules, while carotenoids and flavonoids are polyunsaturated hydrocarbons without N or S.

Table 2 shows the effect of N and S fertilisation on the chlorophyll and carotenoid content in leaves of oilseed rape, marigold and beetroot. S fertilisation had no influence on the photosynthetic pigments of the investigated crops. Chlorophyll a increased only in marigold slightly with an increasing S supply. In water culture experiments, KASTORI & al. 2000 determined decreasing chlorophyll *a, b* and carotenoid contents in leaves of sugar beet when the S supply was set off during growth. They observed significantly lower photosynthetic pigment contents when the medium was S-free, however, not in relation to the S supply. Contrarily, other authors describe increasing chlorophyll contents in sugar beets due to an increasing S supply (THOMAS & al. 2000).

Table 1. Influence of graded N and S rates on the mineral composition in leaves of oilseed rape, marigold and beetroot at the start of main vegetative growth.

Treatment		Mineral content in leaves [mg g^{-1} dry weight]					
S [mg pot^{-1}]	N [mg pot^{-1}]	S[1]	N[2]	Ca[3]	Mg[4]	P[5]	Mn[6]
Oilseed Rape							
0	250	4.2	36.5	26.0	2.1	4.0	0.38
50	250	7.4	32.4	27.0	1.8	3.5	0.32
250	250	8.9	34.4	22.8	1.8	3.8	0.24
0	1000	2.8	57.6	19.9	2.2	6.1	0.54
50	1000	5.3	66.5	21.4	2.5	7.0	0.47
250	1000	12.6	65.1	21.8	2.6	6.9	0.40
Marigold							
0	250	2.0	24.7	38.2	5.5	9.1	0.78
50	250	4.0	27.0	39.0	5.5	9.5	0.63
250	250	7.1	34.2	33.3	5.0	8.6	0.53
0	1000	2.9	59.0	40.5	5.8	8.4	1.37
50	1000	5.4	57.6	41.6	5.8	8.0	0.96
250	1000	7.8	58.2	36.2	5.1	7.8	1.32
Beetroot							
0	250	3.9	31.0	20.1	9.7	10.9	1.10
50	250	3.7	33.2	17.4	9.2	8.6	1.08
250	250	3.8	26.2	16.9	7.9	15.6	1.01
0	1000	4.1	46.6	19.9	7.6	6.8	2.57
50	1000	4.0	47.7	22.2	7.8	6.5	2.39
250	1000	4.4	49.0	19.8	8.7	5.9	2.97

[1] sulphur, [2] nitrogen, [3] calcium, [4] magnesium, [5] phosphorus, [6] manganese.

N fertilisation affected the chlorophyll content of the crops strongest. The chlorophyll *a* and *b* content in leaves of oilseed rape and beetroot significantly increased with the N rate (Table 2). The chlorophyll content of marigold leaves slightly increased, too, but this difference was not significant (Table 2). Chlorophyll *a* and *b* generally occur in plants in a ratio of approximately 3:1 (Lichtenthaler 1987b). It is remarkable that the N supply changed the ratio between chlorophyll *a* and *b* as well as the ratio between chlorophylls and carotenoids significantly in marigold. The ratio between chlorophyll *a* to *b* decreased under the condition of an excess N supply in the same order of magnitude as in oilseed rape and beetroot. This indicates that the N level influenced the photosynthetic system even when single compounds showed no significant changes. The chlorophyll/carotenoid ratio is a good indicator of disturbances in plant metabolism caused by environmental factors (Hendry & Price 1993). For example, Duysen & Freeman 1974 found decreasing chlorophyll to carotenoid ratios when plants were exposed to moderate water deficiency. Roca & Mínguez-Mosquera 2001 described this effect in relation to olive fruit ripening.

Table 2. Influence of graded N and S rates on the concentrations of photosynthetic pigments (chlorophyll *a* and *b* and carotenoids) and their ratios in leaves of oilseed rape, marigold and beetroot at the start of main vegetative growth.

Treatment	Replicates	Chlorophyll *a*	Chlorophyll *b*	Carotenoids	Chl *a*[1]: Chl *b*[2]	(Chl *a*+Chl *b*) / Caro[3]
		- - - [µg ml^{-1} plant extract] - - -				
Oilseed rape						
S-0	n=8	30.9	12.5	6.0	2.5	7.3
S-50	n=8	31.0	12.6	6.2	2.5	7.2
S-250	n=8	28.8	11.6	5.7	2.6	7.0
LSD$_{5\%}$		*3.0*	*2.3*	*0.8*	*0.2*	*0.5*
N-250	n=12	27.0	10.1	5.9	2.7	6.4
N-1000	n=12	33.4	14.3	6.0	2.4	7.9
LSD$_{5\%}$		*2.5*	*1.8*	*0.7*	*0.2*	*0.4*
N x S		ns	ns	**	ns	**
Marigold						
S-0	n=8	26.9	9.9	4.5	2.8	8.2
S-50	n=8	30.1	11.3	5.1	2.7	8.1
S-250	n=8	30.6	11.8	5.1	2.6	8.3
LSD$_{5\%}$		*3.7*	2.5	*0.7*	*0.2*	*0.3*
N-250	n=12	28.8	10.3	5.0	2.8	7.8
N-1000	n=12	29.5	11.6	4.8	2.6	8.6
LSD$_{5\%}$		*3.1*	*2.0*	*0.6*	*0.2*	*0.3*
N x S		ns	ns	ns	ns	ns
Beetroot						
S-0	n=8	34.9	17.5	8.2	2.1	6.5
S-50	n=8	35.8	20.4	9.1	1.8	6.2
S-250	n=8	35.3	19.8	7.9	1.9	7.1
LSD$_{5\%}$		*1.1*	*4.0*	*1.1*	*0.3*	*0.9*
N-250	n=12	34.7	15.7	7.5	2.3	6.8
N-1000	n=12	35.9	22.7	9.3	1.6	6.5
LSD$_{5\%}$		*0.9*	*3.3*	*0.9*	*0.3*	*0.8*
N x S		ns	ns	ns	ns	ns

[1] Chl *a*: chlorophyll *a*; [2] Chl *b*: chorophyll *b*; [3] Caro: carotenoids; Two factorial ANOVA was used to analyse the results and the means were compared by the Tukey test at 5% probability level. Significance levels were given for the interaction between N and S (N x S) and coded in the following way: ns not significant; * significant, $p < 0.05$; ** highly significant, $p < 0.01$; *** very highly significant, $p < 0.001$.

Thus, it may be summarised that stress and senescence may reduce chlorophyll to carotenoid ratios.

Portable chlorophyll SPAD instruments are recommended to evaluate the N status of a crop as they suppose a close relationship between the

chlorophyll content of plants and the N status (REIS & al. 2009). Based on these data recommendations for variable N rates are made (HANEKLAUS & SCHNUG 2006). The data in table 2 demonstrate that this relationship is not generally valid for all crops. No such relationship was verified for instance for marigold. Noteworthy is further that oilseed rape revealed an interaction between N and S supply and the carotenoid content. Another obstacle is the chlorosis of shoots under conditions of severe S deficiency (SCHNUG & HANEKLAUS 2005), which would be very likely misinterpreted as N deficiency by chlorophyll sensors like the SPAD instrument. Finally, the presented results were obtained under controlled conditions. On production fields innumerable, other factors may change absorption characteristics.

MASONI & al. 1996 found that S, iron, magnesium and manganese deficiency caused decreasing chlorophyll a and b contents and concluded that modifications in leaf spectral properties were not characteristic. Thus, changes of the spectral signature can only be assumed to be caused by a variation of the N supply in the soil under *ceteris paribus* conditions, which in fact never occur under field conditions (HANEKLAUS & SCHNUG 2006).

Influence of the N and S supply on the pigment content of flowers of oilseed rape and marigold

Graded N and S rates increased the flower yield of oilseed rape and marigold. This increase was more pronounced and only significant in relation to the N level (Table 3). The higher N rate yielded a two times higher quantity of flowers of oilseed rape and marigold.

The flowers of oilseed rape showed symptoms of S deficiency in the treatments without S fertilisation while marigold showed no visible differences in relation to N or S fertilisation.

In general, chemically very different compounds are responsible for the colour of petals such as flavones, betaxanthine, anthocyanins and carotenoids. More than 700 naturally occurring carotenoids have been identified (BRITTON & al. 2004). KULL & PFANDER 1995 detected in the petals of oilseed rape at least 80 different carotenoids. Thus, the determination of all relevant pigments in flowers is a time-consuming and labour-intensive work. The determination of the petal colour intensity, which was performed in this study, is a simple diagnostic tool to measure the relative pigmentation of flowers and it is easy to perform also for large sample sets. The sampling of oilseed rape and marigold flowers two times per week over 10 to 11 weeks resulted in about 1,000 measurements of the colour intensity. The results show that S fertilisation increased and N fertilisation decreased the colour intensity of oilseed rape flowers (Table 3). A significant interaction was found between N and S showing that the effects of S deficiency on the colour intensity are even more pronounced under con-

ditions of a high N supply (Table 3). A similar interaction between N and S has already been described in previous studies (SCHNUG & HANEKLAUS 1994, 2005).

Table 3. Influence of graded N and S rates on flower yield and colour intensity of petals of oilseed rape (*Brassica napus*) and marigold (*Calendula officinalis*) (flowers were collected within 10–11 weeks).

Treatment	Replicates	Number of flowers per plant (within 10–11 weeks)	Petal colour intensity at 440 nm [Ext. unit g^{-1} fresh weight]	470 nm
Oilseed rape				
S-0	n=8	216.1	7.2	6.5
S-50	n=8	262.9	8.0	7.4
S-250	n=8	260.9	8.3	7.7
$LSD_{5\%}$		*81.5*	*1.0*	*0.9*
N-250	n=12	143.4	9.4	8.6
N-1000	n=12	349.8	6.3	5.8
$LSD_{5\%}$		66.6	2.3	0.8
N x S		ns	**	*
			423 nm	446 nm
Marigold			[Ext. unit g^{-1} fresh weight]	
S-0	n=8	2.6	21.3	23.0
S-50	n=8	3.7	22.0	23.5
S-250	n=8	3.9	21.0	22.3
$LSD_{5\%}$		*1.4*	*2.0*	*2.7*
N-250	n=12	2.1	23.4	25.5
N-1000	n=12	4.7	19.5	20.3
$LSD_{5\%}$		*1.1*	*1.7*	*2.2*
N x S		ns	ns	ns

Two factorial ANOVA was used to analyse the results and the means were compared by the Tukey test at 5% probability level. Significance levels were given for the interaction between N and S (N x S) and coded in the following way: ns not significant; * significant, $p<0.05$; ** highly significant, $p<0.01$; *** very highly significant, $p<0.001$.

Only the N nutrition affected the petal colour of marigold with a decreasing colour intensity of petals together with higher N rates.

The colour intensity of oilseed rape and marigold petals varied during flowering stage. In case of oilseed rape the colour intensity of petals correlated with the time of flower formation; these relationships proved to be significant for both N rates. Colour intensities decreased linear when the flowers opened later and when N was supplied at the lower dose (250 mg pot^{-1} N, Fig. 1). Also in case of marigold, the colour intensity was lower at

the higher N rates but the colour intensity of the flowers increased by trend when the flowers opened later.

A high N supply resulted in an increased formation of flowers, but it also reduced their colour intensity. In comparison, S fertilisation increased

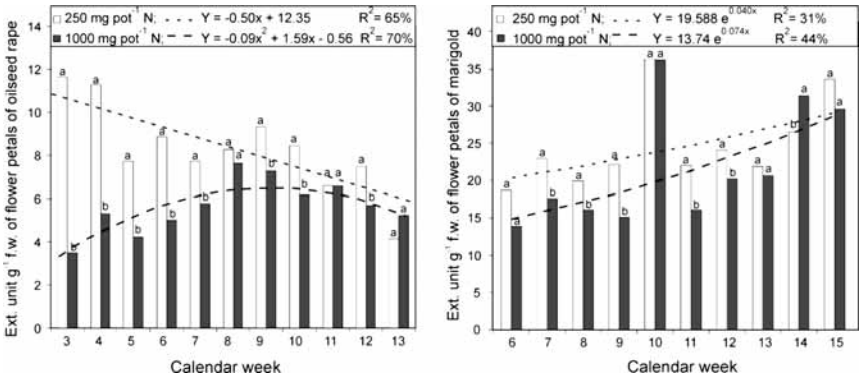

Fig. 1. Change in colour intensity of petals of oilseed rape and marigold over time in relation to N rate [Extinction (Ext.) was measured in fresh petals of oilseed rape at 470 nm and in marigold at 446 nm. Different letters denote significant differences between the N fertiliser treatments per week at the 5% level by t-test; n=12; f.w. = fresh weight].

the colour intensity of oilseed rape flowers except that of marigold. The results reveal that the plants in the presented study reacted differently to changes in the N and S supply, most likely due to different pigment compositions. Thus, it can be concluded that the influence of N and S on pigment formation needs to be investigated crop-specifically.

The strong relationships between the different plant pigments and the flower yield of oilseed rape and marigold clearly reflect the influence of N and S fertilisation on pigment formation in different plant organs (Fig. 2). From the correlation matrix, it is obvious that the colour intensity was negatively correlated with the chlorophyll *a* and *b* content in leaves and the flower yield of oilseed rape. Higher N rates increased the chlorophyll content and the number of flowers, but resulted also in less flower pigments. Marigold revealed similar results. In marigold, a decreased colour intensity of flowers was associated with an increased flower yield, which points to a dilution effect. The close relationship between flower yield and chlorophyll/carotenoid ratio indicate that the flower formation of both crops is directly linked to the chlorophyll content of the plant, as an indicator for the nutritional status of the crop.

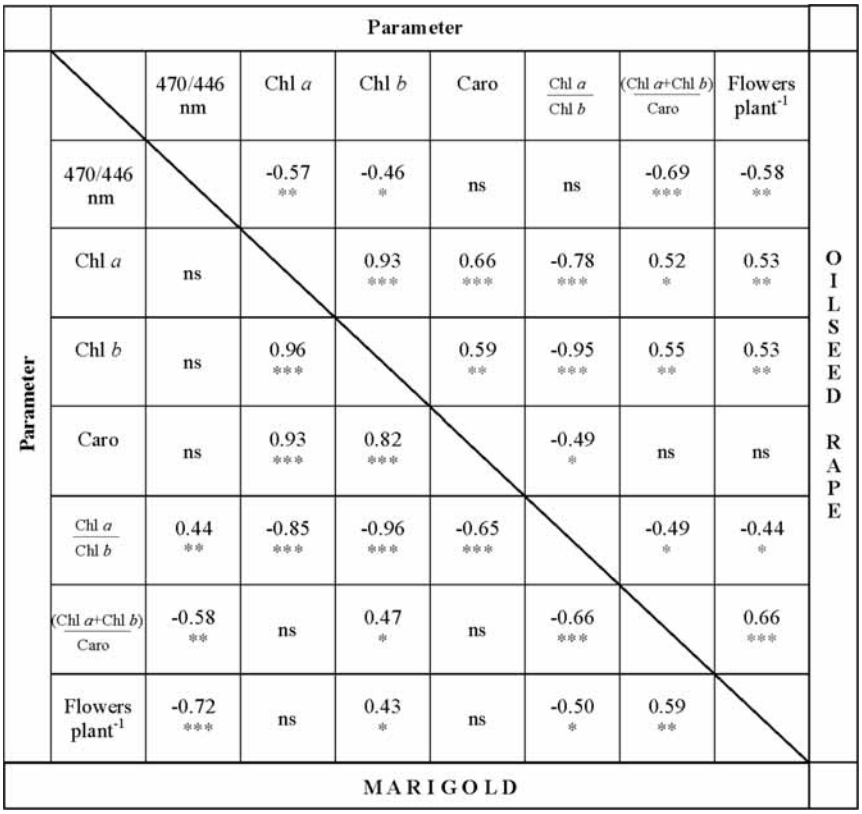

Fig. 2. Correlation coefficients (r) between different pigments in leaves [chlorophyll *a* (Chl *a*) and *b* (Chl *b*) and carotenoids (Caro) and their ratios], flowers [absorption at 470 nm (oilseed rape) or 446 nm (marigold)] and flower yield of oilseed rape and marigold. [Significance levels were given for the correlation between the different parameters and coded in the following way: ns not significant; * significant, $p < 0.05$; ** highly significant, $p < 0.01$; *** very highly significant, $p < 0.001$].

Influence of graded N and S rates on the betalain content of beetroot

Tubers of beetroot were harvested 29 weeks after planting, the fresh weight of the tubers and the betalain content in the fresh material were determined (Table 4). Beetroot growth in the control (250 mg N and 0 mg S per pot) was low as were yields. N fertilisation increased tuber yield by more than 100% and S fertilisation improved tuber yield, too. S fertilisation increased the betalain content but this effect was not significant. N fertilisation decreased both, the betacyanin and betaxanthin content. Similar effects were determined for the petal colours of oilseed rape and

marigold. MICHALIK and co-workers 1995 obtained similar results for the influence of N fertilisation on pigmentation and yield of beetroot. WEICHMANN 1987 determined decreasing betacyanin contents with increasing tuber weight and he found a higher betacyanin contents in plants, which received low fertiliser rates compared to higher doses.

The ratio between the yellow betaxanthin and the deep red betacyanin content is a measure for the colour intensity and by this a quality measure of the beetroot tubers. According to WEICHMANN 1987, a ratio between 0.36 and 0.63 indicates an intensive red colour of tubers, while a ratio between 0.13 and 0.25 reflects to low betacyanin contents. The ratios (x/c) shown in table 4 indicate that the colour intensity of the tubers significantly increased with higher N fertilisation despite of the fact that the betacyanin and betaxanthin contents decreased. This stresses the fact that colour intensity of a plant organ does not allow conclusions on its pigment contents.

Table 4. Influence of graded N and S rates on yield and betalain content of beetroot tubers 29 weeks after planting.

Treatment	Replicates	Yield pot^{-1} (5 plants) [g fresh weight]	Betalain content [mg 100g^{-1} fresh weight] Betacyanin (c)	Betaxanthin (x)	x/c
Beetroot					
S-0	n=8	35.2	148.5	53.0	0.38
S-50	n=8	44.3	169.7	64.3	0.40
S-250	n=8	41.4	161.5	60.6	0.40
$LSD_{5\%}$		*10.9*	*37.6*	*13.7*	*0.11*
N-250	n=12	26.2	189.7	62.1	0.32
N-1,000	n=12	54.4	130.1	56.5	0.46
$LSD_{5\%}$		8.9	30.7	11.2	0.09
N x S		ns	ns	ns	ns

Two factorial ANOVA was used to analyse the results and the means were compared by the Tukey test at 5% probability level. No interaction between N and S (N x S) was determined; ns = not significant.

Conclusions

The significance of fertilisation for pigment formation in different plant organs is well known. For instance, chlorosis of plants is usally associated with reduced chlorophyll contents. Another example is the enrichment of anthocyanins because of N, S and P deficiency or other stress parameters (DAVIES 2004, SCHNUG & HANEKLAUS 2005). Nevertheless, the diversity of different pigments and classes of plant pigments makes it difficult to draw general conclusions for fertiliser practices in agricultural production.

The N supply proved to be of high relevance for all pigments by increasing the photosynthetic pigments distinctly but by decreased pigmentation of oilseed rape and marigold flowers and of beetroot tubers (Table 5). In this study, N fertilisation generally decreased the pigment content of crops with the exception of the chlorophyll content of leaves, which increased in relation to the N supply. Excess N decreased the betalain content despite of the fact that betalains contain a distinct amount of N. In table 5 the effect of graded N and S rates on relative changes of pigment contents are summarised. The data show that S nutrition was of minor importance for pigment synthesis in the selected crops. An exception were the flowers of oilseed rape, where the intensity of the colour increased by about 18 % with S fertilisation. In contrast to the other two crops, oilseed rape contains glucosinolates, which are S and N containing secondary metabolites. Therefore, the higher need for these elements pronounced the interaction of these two nutrients in oilseed rape.

A sufficient N supply that exceeds the physiological demand is required for a high biomass production. If the target is a high pigment content, then medium N levels will supposedly yield the best results. In case of *Brassica* crops N and S rates should be balanced because of their interactions, which also imply pigment synthesis.

Table 5. Relative significant increase (+) or decrease (–) of different classes of plant pigments in different plant organs in relation to N and S fertilisation calculated from the results in table 2–4.

	Crop	Relative change [%] in pigment content from 0 to 250 mg pot^{-1} S and from 250 to 1000 mg pot^{-1} N					
		Porphyrins[1]		Caro-tenoids[1]	Antho-cyanins[2]	Betalains[3]	
		Chl. *a*	Chl. *b*			Betacyanin	Betaxanthin
S	Oilseed rape	–6.8^{ns}	–7.2^{ns}	–5.0^{ns}	+18.5*		
n=8	Marigold	–13.8*	+19.2^{ns}	+13.3^{ns}	–3.0^{ns}		
	Beetroot	+1.2^{ns}	+13.1^{ns}	–3.7^{ns}		+8.8^{ns}	+14.3^{ns}
N	Oilseed rape	+23.7***	+41.6***	+1.7^{ns}	–32.6***		
n=12	Marigold	+2.4^{ns}	+12.6^{ns}	–4.0^{ns}	–20.4***		
	Beetroot	+3.5**	+44.6***	+24.0***		–31.4***	–9.0^{ns}

[1] in leaves at the start of main vegetative growth; [2] in flowers of oilseed rape measured at 470 nm and marigold measured at 446 nm; [3] in tubers of beetroot. Significance levels were coded in the following way: ns not significant; * significant, $p < 0.05$; ** highly significant, $p < 0.01$; *** very highly significant, $p < 0.001$.

The pigment content of flowers, tubers and leaves is a major quality parameter, for example for the production of nutraceuticals and phytopharmaceuticals (Piccaglia & Venturi 1998). For food quality the car-

otenoid content of vegetables is for instance of prime importance for human and animal nutrition as it is converted into vitamin A in the body (RANGANNA 1986). Flavonoids possess various biological activities that might contribute to cancer protection and reduce mortality from myocardial infarction (GERHÄUSER 2001).

Plant colorants such as betacyanin contents of beetroot tubers and carotenoid contents of marigold flowers are main quality parameters of these crops and actually of high interest for colouring foods, textiles, cosmetics and pharmaceutical preparations (PICCAGLIA & VENTURI 1998).

Additionally, the content of flower pigments, primarily that of carotenoids and anthocyanins, has ecological relevance. Previous investigations revealed that the S nutritional status has an important impact on the number of honeybees and other flower-visiting insects. Among others this effect seems closely related to petal colour and shape (SCHNUG & HANEKLAUS 2005), and scent (BLOEM & al. 2010). Another ecological aspect is the possible mismatch of N rates and N demand of crop plants if calculations are based on reflection data (see above). The common practice to use SPAD data may lead to an oversupply with N, which is not transformed into biomass because of other mineral nutrient deficiencies. Here, it is recommended to study the influence of essential plant nutrients on the pigment content in the canopy in relation to crop type and with special view to chlorophyll.

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