

Aus dem Institut für Pflanzenernährung und Bodenkunde  
der Christian-Albrechts-Universität zu Kiel

**Importance of nutrient supply (N, P, K) for yield formation and  
nutrient use efficiency of safflower (*Carthamus tinctorius* L.)  
compared to sunflower (*Helianthus annuus* L.) including an  
assessment to grow safflower under north German conditions**

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**Dedicated to**

**MY FAMILY**

**&**

Prof. D. Burkhard Sattelmacher, who passed us away on 21<sup>st</sup> November 2005

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## Abbreviations

$A_{\max}$	Maximum yield potential in yield response curve using Michaelis-Menten equation
CAL	Calcium Acetate Lactate
$C_{\min}$	Minimal nutrient accumulated or concentrated to trigger yield formation in yield response curve using Michaelis-Menten equation
d.a.s.	Days after sowing
DM	Dry Matter
EEA	European Environment Agency
ER	Efficiency Ratio
FAO	Food and Agriculture Organization
GLM	General Linear Models
G×E	Genotype Environment interaction
GYP	Grain Yield Production
HI	Harvest Index
ICP	Inductively Coupled Plasma
KER	Potassium Efficiency Ratio
$K_m$	Nutrient accumulated or concentration to produce half maximum yield potential in Michaelis-Menten yield response curve
NUE	Nutrient Use Efficiency
NE	Nutrient Efficiency
NER	Nitrogen Efficiency Ratio
NHI	Nitrogen Harvest Index
PER	Phosphorus Efficiency Ratio
RCBD	Randomised Complete Block Design
RH	Relative Humidity
SD	Standard Deviation
SAM	Single Achene Mass
TAM	Thousand Achene Mass
TKG	Tausend Korn Gewicht
UI	Utilization Index
VC	Variation Coefficient
YMB	Young Mature Blade

### General Introduction

Humanity relies on a diverse range of cultivated species (at least 6000); such species are used for a variety of purposes. It is often stated that only a few staple crops produce the majority of the food supply. This might be correct but the important contribution of many minor species should not be underestimated. Agricultural research has traditionally focused on these staples, while relatively, particularly scientists in developed countries have given little attention to minor (or under utilized or neglected) crops. Such crops have, therefore, generally failed to attract significant research funding. Unlike most staples, many of these neglected species are adapted to various marginal growing conditions. Furthermore, many crops considered neglected at a global level are staples at a national or regional level, contribute considerably to food supply in certain periods or are important for a nutritionally well-balanced diet (Li and Mündel, 1997). In addition, world population is expected to increase to 8.5 billion by 2025, intensifying pressure on world's natural resources to achieve higher food production for increasing demand (Baligar et al., 2001) including vegetable oils. This must enhance researchers to search for alternative, nutrient efficient oil crops to expand the global acreage.

Safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.), both belonging to the *Asteraceae*, are important oil crops, but safflower remains a minor crop compared to sunflower (Li, and Mündel, 1997). The parental species of the cultivated specie *Carthamus tinctorius* are *C. oxyacanthus* and *C. persicus*, and the progenitor of these two species is the wild specie *Carthamus palaestinus* restricted to the Palestinian desert and western Iraq (Ashri and Knowels 1960). The cultivated safflower species is a highly branched, herbaceous, thistle-like annual, 30-150 cm tall plant with globular flower heads (capitula), characterized by a strong taproot, which enable it to thrive in dry climates and can access and utilize nutrients from below the root zone of cereal crops. It is one of humanity's oldest crops, and has been grown in Egypt in 2000 BC. Its cultivation was reported in Baghdad (c. AD 1000) and in Arabia (AD 1562), and was expanded in Europe in the fifth and sixth centuries through the western expansion of the Arabs. Safflower was grown for its flowers, which were used in making dyes for clothing and food, but nowadays grown mainly for its oil (Weiss, 2000). Safflower oil has been produced commercially and for export for about 50 years, first as an oil source for the paint industry, now as edible oil for cooking, margarine and salad oil in over 60 countries, but over half is produced in India, and the production in the U.S.A., Mexico, Ethiopia, Argentina and Australia comprises most of the remainder (Li,

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and Mündel, 1997). The oil crop sunflower, however, is much taller, usually un-branched, lacks a tap root, originated in the south west United States-Mexico area (Heiser, 1976). It was introduced to Europe in the sixteenth century, then became an international oil crop (Weiss, 2000), and is usually been considered high demanding in its nutrient and water (Diepenbrock and Pasda, 1995).

The oil derived from some safflower varieties is high in linoleic acid contains nearly 75% of total fatty acids, which is considerably higher than sunflower, corn, soybean, cottonseed, peanut or olive oils (Knowels, 1989; Kumar, 2000). It is used primarily for edible oil products such as salad oils and soft margarines, high in polyunsaturated fatty acids, help to decrease blood cholesterol and related heart and circulatory problems making it "high quality" edible oil (Li and Mündel, 1997, Kumar, 2000). Other safflower varieties are high in oleic acid (75 to 80%) and may serve as heat-stable, cooking oil used to fry potato chips and French fries (Fernández-Martínez et al., 1993). According to Smith (1996), safflower oil contains a total of 92% unsaturated fatty acids compared to 89% for sunflower and 86% for olive oil. Safflower oil contains moderate levels of saturated fatty acids (aprox. 10%), compared with higher contents in sunflower (aprox. 12%), and soybean (aprox. 16%), but lower contents in canola (aprox. 6%) (Padley et al., 1994).

Globally, safflower and sunflower represents a share of 0.07 and 7.3 % of the vegetable oil production, respectively. Although Europe contributes in 74.9 % of sunflower achenes produced globally, the production of safflower is not yet reported at the global scale (FAO, 2007a,b), except some trials that are running now (Reinbrecht et al., 2005). As safflower has a wide adaptation to be grown from 45°S to 60°N, it can be cultivated also in regions with a temperate climate (Weiss, 2000). It was cultivated as a potential oil crop in Germany before the Second World War (Scheibe and Yekta, 1934, Scheibe, 1938, Sessous, 1940) and held worthy to produce edible oil competed well with other oil crops developed at that time. Nowadays winter oil seed rape became the major oil crop in Germany (Reinbrecht et al 2005), but organically grown oilseed rape is highly susceptible to a wide range of pests (Reinbrecht et al., 2004), and since a Californian study has shown that safflower yielded similar in conventional as well as in organic systems (Clark et al., 1999), it is tempting to speculate that safflower could be grown successfully in organic farming in Central Europe.

In organic farming, where application of inorganic fertilizers is not permitted, the nutrient availability is not easily increased. In addition in developing countries, where the proportion of less fertile soils is particularly high, it may be difficult to fulfil the nutritional requirements of high-yielding crops (Sauerbeck and Helal, 1990; Marschner, 1995). Accordingly,

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fertilizer application represents an important measure to correct nutrient deficiencies replacing elements removed in the products harvested. Specifically, application of N fertilizer has been shown to be particularly effective with respect to yield formation in safflower (Steer and Harrigan, 1986) and sunflower (Connor and Sadras, 1992), however, recovery of fertilizer N is often as low as 50 %, with serious ecological consequences (Scheiner et al., 2002). Application of P fertilizer affects yield formation in safflower (Mündel et al., 1997) and sunflower (Connor and Sadras, 1992). Although many soils have large reserves of total P, but only a small fraction is immediately available. Because P is highly fixed in some types of soil, many agricultural areas are P deficient (Sanchez and Salinas, 1981) due to chemical immobilization in the soil (Sample et al., 1980) causing the recovery of fertilizer P below 15% (Roy et al., 2006) leading to depletion of P resources (Cathcart, 1980) and serious ecological and economical consequences of contaminating the environment (EEA, 1999, EEA, 2001, Chardon and Withers, 2003). Application of K fertilizer has been shown to be affecting yield formation in safflower (El-Nakhlawy, 1988) and sunflower (Grove and Sumner, 1982). Large amounts of K are required by high-yielding crops, and although total K contents of most soils are rather high, availability is usually low due to enclosure of K in silicates and strong adsorption by K-specific binding sites (Marschner, 1995; Russel, 1988), making its recovery is about 40% only (Baligar et al., 2001).

Several studies suggest that high-yielding varieties are more sensitive to reduced nutrient availability than the varieties with lower yield potential (Fischbeck, 1988; Dambroth and El Bassam, 1990) although contrary findings are also reported (Baligar et al., 2001). A similar response may be hypothesized for safflower vs. sunflower, in which the latter is known for its comparatively high nutrient demand (Diepenbrock and Pasda, 1995; Connor and Sadras, Grove and Sumner, 1982). Consequently, identification of plants species or cultivars that are efficient in absorption and utilization of nutrients greatly enhance the efficiency of applied fertilizers, reducing cost of inputs, and preventing losses of nutrients to ecosystems, and will offers considerable promise for increasing the production potential on marginal land and will contribute to sustainable agricultural systems that protect and promote soil, water and air quality (Baligar et al., 2001). The term ‘nutrient efficiency’ has been used widely as a measure of the capacity of a plant to acquire and utilize nutrients (Gourley et al., 1994), and may be broken down mechanistically into the ability to acquire nutrients from the soil (uptake efficiency) and the ability to utilize accumulated nutrients for biomass production or yield formation (use efficiency) (Sattelmacher, 1994). Definitions of nutrient efficiency vary greatly (Sattelmacher, 1994), and in some cases may be even misleading in the quest for

identifying mechanisms for enhanced nutrient acquisition and utilization (Gourley et al., 1994).

Although both safflower and sunflower crops thrive in similar environments, direct comparisons of their response to increasing NPK availability with respect to yield and nutrient efficiency are not available. In particular the putatively lower NPK requirement of safflower is not sustained. However, safflower was reported to accumulate 5 kg N ha<sup>-1</sup> to produce 100 kg of achene (Mündel, 2004), while the same figure for sunflower is only 3.7 kg N ha<sup>-1</sup> (Merrien, 1992). In addition, N recovery in safflower was found very variable, for reasons still to be determined (Weiss, 2000). It was also believed that the phosphate requirements of safflower are moderate (Esendal, 1997), and consistent response to P is obtained only when a soil test is in the low to very low range (Armah-Agyeman et al., 2002). In addition, safflower was reported to accumulate 0.52 kg P to produce 100 kg of achene (Mündel, 2004), while the corresponding figure for sunflower is 1.1 kg P (Merrien, 1992). With respect to K, Safflower was reported to accumulate only 3.2 kg K to produce 100 kg achenes (Mündel, 2004), while sunflower needs three times more K for the same purpose (Merrien, 1992). Indeed, Kumar (2000) argued that, the cost benefit ratio in safflower is about 40% of that obtained in sunflower due to the comparatively lower cost of its cultivation.

Accordingly, there is no clear data realizing the low requirement of safflower as compared to other oil crops. Therefore, the aim of the greenhouse work of this study is to directly compare the impact of NPK supply on growth, yield and nutrient use efficiency of safflower as compared to sunflower under semi-controlled conditions by attending two years pot experiments using soil mixture concerning the effect of increasing N, P, and K supply separately (non-orthogonal design) on the performance of both crops in term of yield and nutrient efficiency. Special attention was given to phosphorus according to the results of the two years pot experiments showing the high requirement of safflower for P compared to sunflower therefore a third year nutrient solution experiment was done to explain the failure of safflower to be a low input crop in term of P compared to sunflower. Accordingly; the response of both crops concerning each nutrient under study (N, P, and K) was discussed in seven chapters. The response of both species according to N, P, and K in terms of growth, yield and yield components were discussed in Chapter 1, 3, and 6 for mentioned nutrients respectively. The response of both plants to N and K in terms of nutrient use efficiency was discussed in chapters 2 and chapter 7 respectively. Because P was given more attention and the nutrient solution experiment was conducted to confirm the response of both plants in

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term of P according to results obtained from the pot experiment using soil; two chapters (4, and 5) discussed the response of both crops to P supply in terms of P use efficiency.



Figure 1: Effect of N, P, and K supply on growth and achenes yield of safflower and sunflower in the pot experiment in 2004.



Figure 2: Screening ten safflower cultivars for the growth in northern part of Germany in the field experiment in 2005.

In addition, it was argued that, safflower may have a certain production potential under German conditions, particularly in organic farming, therefore a two-year field experiment was conducted under low input organic farming conditions to assess the cultivation of safflower under north German conditions, results of which are discussed in chapter 8.

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## Chapter 1

### Effects of N Supply on Growth, Yield and Yield Components of Safflower and Sunflower

#### Abstract

Safflower represents an important oil crop internationally and may have a certain production potential under German conditions, particularly in organic farming where the putatively low nutrient requirement is highly welcomed. However, current knowledge regarding the nutrient requirements of safflower as compared to similar oil crops is limited. It was thus the aim of this study to determine the growth and yield response of safflower (*Carthamus tinctorius* L.) as compared to sunflower (*Helianthus annuus* L.) with respect to N supply in pot experiments. Initially N levels were the same for both species (0.25, 0.5, 1.0, 1.5, 2.0 g per pot), but were adjusted for sunflower in the second year (0.5, 1.0, 2.0, 3.0, 4.0 g per pot). Three safflower and two sunflower plants were individually cultivated in a mixture of equal volumes of sand, nutrient-poor limed soil, and perlite in 6 L Mitscherlich pots.

Both species responded strongly to increasing N supply with respect to plant growth and yield. Growth and yield of safflower increased up to 1 g N per pot for safflower, while the optimum for sunflower was 2.0 g N per pot. Safflower out-yielded sunflower at low N supply, while at high N level the opposite was observed. Pearson coefficients indicate that in safflower, yield is tightly correlated with the number of capitula per plant and the mass per achene, while these two yield components are tightly correlated to each other. To the contrary, sunflower yield was most tightly correlated with the number of achenes per capitulum, followed by the mass per achene. Path coefficient analysis considering the most important traits determining oil yield revealed that in sunflower, the number of achenes per capitulum exerts a strong direct effect on oil yield, and compensatory effects with the number of capitula per plant and the number of achenes per capitulum are not to be taken into consideration. Even more pronounced than for safflower direct effects of the achene and leaf N content as well as the leaf dry matter are small, and mediated principally via the indirect effect on the number of achenes per capitulum.

#### 1. Introduction

The oil derived from safflower and sunflower represents a share of 0.07 and 7.3 % of the global production, respectively, while corresponding figures for Europe are 0 and 74.9 % of safflower and sunflower achenes produced globally (FAO, 2007a,b). As possibilities to

expand planted acreage are limited on a global scale, appropriate production intensities are required to meet the increasing global demand for vegetable oil. In developing countries, where the proportion of less fertile soils is particularly high, it may be difficult to fulfil the nutritional requirements of high-yielding crops (Sauerbeck and Helal, 1990; Marschner, 1995). Fertilizer application represents an important measure to correct nutrient deficiencies and to replace elements removed in the products harvested, and N fertilization has been shown to be particularly effective with respect to yield formation (Connor and Sadras, 1992, Steer et al., 1984). However, recovery of fertilizer N is often as low as 50 %, which has serious ecological consequences (Scheiner et al., 2002).

Safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.), both belonging to the *Asteraceae*, are important oil crops in tropical areas. Safflower is a highly branched, herbaceous, thistle-like annual, 30-150 cm tall with globular flower heads (capitula), characterized by a strong taproot, which enable it to thrive in dry climates and can access and utilize nutrients from below the root zone of cereal crops (Li and Mündel, 1997). The oil crop sunflower, however, is much taller, usually un-branched, lacks a tap root, and is usually been considered more demanding in terms of nutrient and water requirements (Diepenbrock and Pasda, 1995). However, safflower was reported to accumulate 5 kg N ha<sup>-1</sup> to produce 100 kg of achene (Mündel, 2004), while the same figure for sunflower is only 3.7 kg N ha<sup>-1</sup> (Merrien, 1992). In addition, N recovery in safflower is very variable, for reasons still to be determined (Weiss, 2000).

The impact of N supply on the performance of sunflower has been extensively characterized, and oil yield was found to be determined by the number of achenes per capitulum and single achene mass (Diepenbrock and Pasda, 1995; Steer and Hocking, 1984; Hocking et al., 1987; Hocking and Steer, 1989; Connor and Sadras, 1992), while oil concentration is considered to be of minor importance (Connor and Sadras, 1992). Oil yield was also correlated with the plant dry matter (Steer and Hocking, 1984), particularly the number of leaves per plant (Hocking et al., 1987; Hocking and Steer, 1989; Connor and Sadras 1992), the leaf area (Diepenbrock and Pasda, 1995; Connor and Sadras, 1992), and the duration of the vegetative period (Diepenbrock and Pasda, 1995).

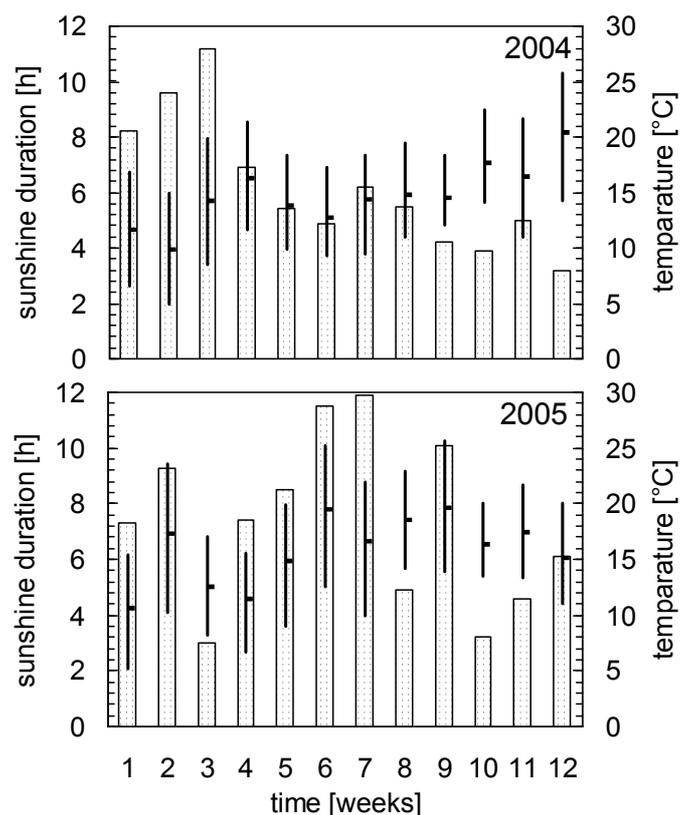
Although both crops thrive in similar environments, direct comparisons of their response to increasing N availability with respect to yield and quality are not available. In particular the putatively lower N requirement of safflower is not sustained. Therefore, the aim of this study is to directly compare the impact of N supply on growth, yield and quality of safflower as

compared to sunflower under semi-controlled conditions. Special emphasis was given to the response of yield components.

### 2. Materials and Methods

#### 2.1. Experimental conditions

Experiments using safflower (*Carthamus tinctorius* L., variety ‘Sabina’) and sunflower (*Helianthus annuus* L., variety ‘Salut R M’) were conducted in the period from May to October 2004 and 2005 under semi-controlled conditions. In 2004 five N levels (0.25, 0.5, 1.0, 1.5, 2.0 g per pot) were used in four replicates for both species. Other nutrients added were 0.72, 2.0, and 1.0 g per pot of P, K, and Mg respectively. In 2005, based on the information obtained in the first experimental year, the N levels were adjusted to 0.5, 1.0, 2.0, 3.0, and 4.0 g per pot for sunflower only, and P supply was increased to 2.0 g per pot for both species and K to 3.0 and 4.0 g per pot for safflower and sunflower, respectively. Three safflower and two sunflower plants per pot (6 L) were cultivated in a mixture of equal volumes of sand, soil, and perlite in Mitscherlich pots. Pots were randomised and maintained outdoors until the end of flowering stage (middle of August), followed by greenhouse cultivation with additional lighting (day and night temperatures were adjusted to 28°C and 15°C, respectively). Before being used in the experiment, soil (limed nutrient-poor Ferrasol containing 9.6 mg g<sup>-1</sup> organic matter, pH(CaCl<sub>2</sub>) 5.9), sand (0.4-0.6 mm diameter) and perlite (Perligran®; Germany, pH(CaCl<sub>2</sub>) 7, 95 % pores volume) were analysed using the calcium acetate lactate (CAL) method according to Schüller (1969) and found to contain 2.8, 0.0, and 0.0 mg P, and 5.6, 0.8, and 0.0 mg K 100 g<sup>-1</sup> respectively. The final soil mixture without fertilizer added contained 1.8 mg P, and 1.7 mg K 100 g<sup>-1</sup>. Total N in soil, sand and perlite was analysed using an isotope ratio mass spectrometer (Finnigan MAT Delta C) coupled to an elemental analyser 1108 (Carlo-Erba, Milan, Italy) and found to contain 1.0, 0.0, and 0.0 mg g<sup>-1</sup> respectively. Aphids were controlled with regular pesticide applications of Metasystox® (S-[2-Ethylsulfanyl) ethyl] O, O-dimethyl phosphorothioate), and infestations with *Perenospora sp.* were controlled by application of Amistar® (azoxystrobin) according to manufacturers recommendation. Daily air temperature and sunshine duration were calculated as weekly averages (Fig. 1.1).



**Figure 1.1:** Weekly means of daily sunshine duration (bars) and average, lowest and highest daily air temperatures during the experimental period of both years (15<sup>th</sup> May – 15<sup>th</sup> August).

## 2.2. Harvesting and analytical procedures

Growth and yield parameters were monitored along the growing period. Plant parts (achenes, capitula, leaves, stems) were separated at the end of the experiment, dried (except for achenes, which were dried at room temperature in a well-aerated area) at 70°C to constant weight, grinded to pass a 1.5 mm sieve of which, after thorough mixing, a sub-sample of 5 g was ball-milled to a fine powder. Oil was extracted from achenes using Soxhlet extraction method (SOX THERM 2000, S 206 AK/S2006A, Automatic from Gerhardt, Bonn, Germany). Directly after extraction oil samples were stored in the dark at -20 °C. The iodine value of the oil was measured according to Matissek et al. (1992). Total N of plant materials except achenes was measured as in soil, and achene N was determined as ammonium by a modified indophenol blue procedure using continuous flow analysis (Bran&Lübbe AutoAnalyzer II, Hamburg, Germany) after Kjeldal digestion (Novozamsky et al., 1983).

### 2.3. Statistics and yield component analysis

All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means with respect to the influence of N supply were carried out using the GLM procedure considering a fully randomised block design, treating years (2004 and 2005) separately. Where appropriate, data were log transformed to maintain homogeneity of variance. The Bonferoni procedure was employed with multiple t-tests in order to maintain an experimentwise  $\alpha$  of 5 %.

Oil yield may be broken down into its components and expressed in a multiplicative fashion as:

$$\text{Oil yield [g oil plant}^{-1}] = (\text{capitula plant}^{-1}) \times (\text{achenes capitulum}^{-1}) \times (\text{mass achene}^{-1}[\text{g}] \times (\text{oil concentration [g g}^{-1}])$$

Initially Pearson correlations were calculated to test the relation between individual yield components and oil yield. The NOMISS option was used in order to obtain results consistent with subsequent multiple regression studies. In order to quantify the impact of individual yield components multiple regression analysis is biased as the mathematical product (or the sum in case log-transformed data are used), rather than a statistical relationship, of all individual yield components represents the oil yield. Hence, a yield component analysis according to Piepho (1995) was employed, allowing the contribution of individual components to be quantified. This approach assumes that the SD of log-transformed yield is close to the coefficient of variance of the yield, and uses the log-transformed component data and interprets values of  $C_i = \text{Cov}[\log(\text{yield}), \log(\text{component}_i)]$  as an aggregate measure of the  $i^{\text{th}}$  component's contribution to the variability in yield.

Finally, the association of selected individual traits with respect to oil yield was evaluated. Most significant traits were selected by backward elimination, excluding *a priori* achene yield per pot and oil concentration, as their product defines the yield arithmetically. Secondary traits (harvest index, nutrient concentrations) were also excluded to avoid redundancy in the statistical model. Similarly, tests were run either with data for individual plant fractions (stems, leaves, capitula, achenes) or their partial or total sums (straw, total shoot). In order to characterize the direct and indirect effects of variation of individual traits to the variation of oil yield, path coefficient analysis was performed for the identified most important traits as described in detail by Dewey and Lu (1959).

### 3. Results and discussion

#### 3.1 Effect of N supply on growth and morphology

In both years, safflower and sunflower plants responded strongly to increasing N supply with respect to growth and morphology (Table 1.1, Table 1.2). In agreement with Hocking and Steer (1983a, 1989) low N supply substantially delayed the onset of floret initiation in both plant species (data not shown).

Growth and yield of safflower increased up to 1 g N per pot in both years. Growth improvement was accompanied by strong responses of all parameters reported, while plant height and stem diameter responded only at very low N supply (Table 1.1). In agreement with previous findings the number of branches was significantly increased by increasing N supply (Mündel et al., 1997; Weiss, 2000). In addition, Mündel et al. (1997) reported that N supply increases safflower leaf number significantly, while others stressed that the number of branches per plant are major effects of increasing N supply (Günel and Arslan, 1997). Shoot/root dry matter ratio generally increases as N supply increases (Levin et al., 1989), and the mobilization of N in mature leaves for retranslocation to areas of new growth upon N limitation (Marschner, 1995) was also observed here, though less pronounced in safflower. Sunflower growth and yield parameters were continuously increased by increasing N supply in the first experimental year (2004) and the optimum N supply could not be derived for this crop. In 2005, N supply covered a wider range and growth and achene yield was maximized at 2.0 g N per pot. In agreement with previous reports (Hocking et al. 1987; Hocking and Steer, 1982) leaf area, as indicated by leaf DM, and the dry matter of all plant parts responded particularly strongly to the altered N supply (Table 1.1). A strong response of the number of leaves has been frequently observed (Nawaz et al., 2003; Hocking and Steer, 1982; Hocking and Steer, 1989). Dry matter of the stem, known for its function as a temporary storage compartment for photosynthates (Hocking and Steer, 1983b) and reduced N during periods of ample supply (Hocking and Steer, 1982), was positively affected by N supply (Steer and Hocking, 1984; Table 1.1).

## Chapter 1 Effect of N on growth and yield

**Table 1.1:** Effect of N supply on stem diameter and dry matter (DM) of safflower and sunflower in 2004 and 2005<sup>1)</sup>.

N supply (g per pot)	Stem diameter (mm)	Leaf DM (g pot <sup>-1</sup> )	Stem DM (g pot <sup>-1</sup> )	Capitula DM (g pot <sup>-1</sup> )	TDM (g pot <sup>-1</sup> )
Safflower (2004)					
0.25	5.6±0.2 B	4.2±0.3 C	9.9±0.9 C	8.7±0.9 C	33.4±1.5 C
0.50	6.6±0.4 A	6.9±0.9 B	16.8±1.8 B	12.9±1.5 B	53.1±3.3 B
1.00	6.8±0.5 A	9.3±1.2 A	21.3±1.5 A	20.7±2.1 A	81.1±4.8 A
1.50	6.7±0.2 A	9.6±0.9 A	21.0±2.4 A	22.5±1.8 A	84.1±6.9 A
2.00	6.3±0.2 A	9.9±1.5 A	21.6±2.8 A	23.4±2.7 A	83.7±7.5 A
Safflower (2005)					
0.25	6.1±0.3 B	4.9±0.4 D	9.9±1.1 D	8.4±0.1 C	32.2±4.7 D
0.50	7.0±0.2 A	7.0±0.6 C	15.0±0.6 C	13.9±1.0 B	53.4±1.7 C
1.00	7.1±0.3 A	8.6±0.5 B	18.9±1.3 B	23.4±0.9 A	78.2±4.6 B
1.50	7.1±0.4 A	11.6±0.4 A	20.3±0.3 B	24.9±1.6 A	89.6±4.5 BA
2.00	7.2±0.5 A	12.3±1.7 A	23.1±0.9 A	26.5±3.2 A	96.6±4.8 A
Sunflower (2004)					
0.25	12.1±0.2 B	7.0±0.6 E	16.6±0.6 E	11.8±1.4 D	40.4±1.8 E
0.50	14.4±0.8 A	12.8±0.4 D	25.0±1.6 C	15.6±0.8 C	63.6±2.8 D
1.00	15.1±0.3 A	16.0±1.0 C	36.4±0.2 B	20.8±1.2 B	98.4±1.2 C
1.50	15.1±0.9 A	21.6±1.4 B	40.6±2.8 AB	26.4±1.4 A	120.5±5.6 B
2.00	15.7±0.7 A	30.2±1.8 A	44.8±4.2 A	31.4±3.8 A	141.9±3.6 A
Sunflower (2005)					
0.50	13.2±0.3 C	14.0±0.5 D	25.4±1.2 C	19.0±0.5 D	73.0±1.8 D
1.00	15.2±0.3 B	20.2±1.1 C	38.6±2.2 B	25.4±1.0 C	117.4±2.9 C
1.50	16.3±0.5 A	28.3±2.0 B	44.0±2.3 B	32.9±1.3 B	163.6±7.8 B
2.00	17.0±0.6 A	33.7±1.2 AB	53.1±3.3 A	37.3±1.7 A	207.1±6.6 A
4.00	16.8±0.5 A	36.0±1.8 A	51.6±1.5 A	32.9±1.1 B	208.0±6.5 A

<sup>1)</sup> for a given species and a given year means within each column followed by the same letter are not significantly different,  $p < 0.05$ ,  $n = 4$

**Table 1.2:** Effect of N supply on achene yield and yield components of safflower and sunflower in 2004 and 2005<sup>1)</sup>.

N supply (g pot <sup>-1</sup> )	Capitula per plant	Achenes per capitulum	SAM <sup>2)</sup> [mg achene <sup>-1</sup> ]	Achene yield [g pot <sup>-1</sup> ]	Oil yield [g pot <sup>-1</sup> ]	Oil conc. [g (100 g <sup>-1</sup> )]
Safflower (2004)						
0.25	4.9±0.2 C	21.4±1.0 B	30.8±2.7 D	9.9±0.6 C	2.1±0.2 C	21.0±1.2 B*
0.50	5.9±0.4 B	28.0±1.6 A	35.0±1.8 CD	16.8±0.9 B	4.0±0.3 B	23.6±0.3 A*
1.00	8.7±0.3 A	29.7±1.8 A	38.6±1.4 CB	29.7±1.5 A	7.2±0.6 A	24.1±0.8 A*
1.50	8.5±0.8 A	29.8±2.9 A	41.9±0.9 B	31.8±2.4 A	7.3±0.5 A	23.0±0.6 A*
2.00	9.6±1.5 A	25.2±5.6 AB	47.6±4.1 A	31.2±3.9 A	6.8±1.3 A	23.1±1.3 AB*
Safflower (2005)						
0.25	5.6±0.9 C	14.2±1.5 A	40.3±3.1 B	10.8±1.2 C	2.5±0.3 C	22.7±0.5 A
0.50	10.1±0.3 B	13.0±2.0 A	43.2±2.7 AB	17.4±1.3 B	3.8±0.5 B	21.9±1.3 A*
1.00	14.4±1.1 A	12.5±1.0 A	49.2±3.4 A	28.8±1.4 A	5.8±0.2 A	20.0±1.3 A*
1.50	16.1±1.2 A	13.0±1.6 A	49.7±1.3 A	31.2±4.2 A	6.4±1.0 A	20.6±1.4 A
2.00	16.0±1.7 A	15.0±0.6 A	47.2±1.1 A	33.7±4.3 A	7.1±1.4 A	20.9±1.8 A*
Sunflower (2004)						
0.25	1	151±19.5 E	17.6±3.1 B	5.2±0.4 E	2.3±0.3 E	44.7±1.7 AB
0.50	1	219±13.4 D	26.1±2.2 A	11.4±0.6 D	5.5±0.3 D	46.5±2.6 AB
1.00	1	466±27.3 C	25.2±3.0 A	25.0±1.0 C	11.3±0.3 C	45.6±0.5 A
1.50	1	619±4.0 B	25.9±1.4 A	32.0±2.6 B	13.6±1.6 B	42.5±1.8 BC
2.00	1	825±25.6 A	26.3±1.0 A	44.2±2.0 A	17.7±0.9 A	39.8±1.0 C
Sunflower (2005)						
0.50	1	381.6±40.1 D	19.1±0.7 D	14.6±1.7 D	7.0±0.8 D	47.8±0.6 A
1.00	1	654.6±49.2 C	25.4±1.6 C	33.2±1.3 C	16.1±0.9 C	48.5±0.7 A
1.50	1	1047±77.4 B	26.9±0.6 BC	58.4±2.5 B	27.3±1.7 B	46.8±2.4 A
2.00	1	1344 ±55.6 A	30.4±0.7 BA	81.7±2.5 A	33.4±1.4 A	40.9±1.7 B
4.00	1	1295 ±31.2 A	33.0±2.4 A	80.4±4.7 A	29.9±2.1 A	37.4±3.8 B

<sup>1)</sup> for a given species and year means within each column followed by the same letter are not significantly different,  $p < 0.05$ ,  $n = 4$ .

<sup>2)</sup> Single achene mass

### 3.2. Effect of N supply on yield and yield components

The increase in achene yield in both years in response to increasing N supply was accompanied by an increasing number of capitula per plant in safflower, while in sunflower the number of achenes per capitulum were mainly affected (Table 1.2). Single achene mass (SAM)

was increased with increasing N supply in both species. Oil yield increased merely as a result of increasing achene yield, while in agreement with previous reports response of oil concentration was small (Table 1.2; Steer et al., 1984; Connor and Sadras, 1992). As expected, sunflower had significantly higher oil concentrations than safflower irrespective of N supply (Kumar, 2000; Roth and Kormann, 2005). In agreement with previous reports (Haby et al., 1982, Nasr et al., 1978, Sharma and Verma, 1982) oil concentration of sunflower achenes was significantly reduced at higher N supply resulting from the dilution of oil in heavier achenes produced under high N nutrition, but this reduction didn't offset the advantage that large N supply has on increasing oil yield so that sunflower oil yield remains positively related to N application even at high supply (Connor and Sadras, 1992).

Pearson coefficients showing overall correlations between yield and yield components under the influence of different N supply indicate that in safflower yield is tightly correlated with the number of capitula per plant and the mass per achene, while these two yield components are tightly correlated to each other (Table 1.3). Significant negative correlations of around -0.5 were observed between the number of achenes per capitulum on the one hand and either the number of capitula per plant or the mass per achene, which agrees with previous observations (Camas et al., 2005, Tunctürk et al., 2005). Correlations do not allow the major traits responsible for the yield response to increasing N supply to be elucidated. Therefore  $c_i$  coefficients, based on the covariance of the log-transformed yield components with the log-yields, were calculated to quantify the contribution of each component to the yield variability (Piepho, 1995). The major effect of increasing oil yield in safflower in response to increasing N supply was due to a higher number of capitula per plant (Table 1.4), which agrees well with numerous previous studies (Steer and Harrigan, 1986; Gilbert and Tucker, 1967; Workhoven et al., 1968; Günel and Arslan, 1997; Weiss, 2000; Ibrahim, 1994; Esendal, 1997). The number of achenes per capitulum and the mass per achene had a smaller impact (Workhoven et al., 1968; Günel and Arslan, 1997; Ibrahim, 1994; Esendal, 1997). Obviously the oil concentration of the achenes is of limited significance with respect to the plant's response, which again agrees with previous studies (Zaman and Das, 1991; Souda et al., 1983; Kolsarici and Eda, 2002). Soil fertility trials show N application increases the yield and the protein concentration of safflower achene, consequently lowers the oil concentrations as a consequence of the dilution of oil in heavier achene produced under high N supply (Table 1.2; Table 1.3; Mündel, 2004).

To the contrary, the effect of N supply on sunflower yield is most closely associated with the number of achenes per capitulum, followed by the mass per achene, presumably due to the fixed number of capitula per plant (Table 1.3). In agreement, oil yield of sunflower was often found to be correlated with the number of achenes per capitulum (Steer and Hocking, 1984; Steer et al., 1984; Hocking et al., 1987; Hocking and Steer, 1989; Connor and Sadras, 1992) and the single achene mass (Hocking et al., 1987; Connor and Sadras, 1992), while it was only weakly associated with the oil concentration (Table 1.3; Connor and Sadras, 1992). Interestingly, the oil concentration was negatively correlated to the mass per achene in both crops, and it was significantly negatively related to the number of achenes per capitulum in sunflower, while the opposite holds true for safflower.

**Table 1.3:** Pearson coefficients between oil yield and yield components of safflower (above the diagonal) and sunflower (below the diagonal).

Variables	Oil yield plant <sup>-1</sup>	Capitula plant <sup>-1</sup>	Achenes capitulum <sup>-1</sup>	Mass achene <sup>-1</sup>	Oil concentration
Oil yield plant <sup>-1</sup>	-	0.694***	0.272	0.556***	0.163
Capitula plant <sup>-1</sup>	-	-	-0.497**	0.793***	-0.361*
Achenes capitulum <sup>-1</sup>	0.979***	-	-	-0.517***	0.602***
Mass achene <sup>-1</sup>	0.836***	-	0.720***	-	-0.398*
Oil concentration	-0.432**	-	-0.525***	-0.419**	-

(combined analysis of 2004 and 2005 data, variables were transformed to the natural logarithm, significance indicated as \* for  $p < 0.05$ , \*\* for  $p < 0.01$ , and \*\*\* for  $p < 0.001$ ,  $n = 38$  for safflower,  $n = 37$  for sunflower).

## Chapter 1 Effect of N on growth and yield

**Table 1.4:** Estimates of variation coefficients (VC) of yield, SD and variance of log-yields, and  $c_i$  coefficients for yield components of safflower and sunflower (data were transformed to the natural logarithm, calculations according to Piepho, 1995).

Crop and year	VC	SD	Variance	$C_i$ for yield component			
	Oil yield (%)	log-yield ( $\times 100$ )	log-yield ( $\times 100$ )	Capitula plant <sup>-1</sup> ( $\times 100$ )	Achenes capitulum <sup>-1</sup> ( $\times 100$ )	Mass achene <sup>-1</sup> ( $\times 100$ )	Oil concentration ( $\times 100$ )
Safflower							
2004	40.91	50.99	26.00	13.01	4.23	6.18	2.06
2005	40.43	47.72	22.77	18.82	1.11	3.95	-1.11
Pooled	40.48	48.92	23.93	14.44	4.95	4.33	0.62
Sunflower							
2004	53.21	72.97	53.24	-	44.27	11.52	-2.55
2005	44.56	59.96	35.96	-	28.61	11.30	-3.95
Pooled	60.69	78.14	61.06	-	50.91	13.48	-3.33

### 3.2.1. Evaluation of the most important parameters determining achene and oil yield

Several traits are frequently discussed in relation to the yield performance of sunflower, namely the produced biomass (Steer and Hocking, 1984; Hocking and Steer, 1989), the leaf area (Connor and Sadras, 1992) or the number of leaves per plant (Steer and Hocking, 1983; Hocking and Steer, 1989). In order to identify the most important traits determining oil yield in the current experiment Pearson correlations were calculated followed by path coefficient analysis. Variables considered were selected using backward elimination for model building as specified in the Material and Methods section. Interestingly, the same variables were identified in both species as being most significant, except for the leaf N content in sunflower that was included for reasons of uniformity (Table 1.5). Oil yield was tightly correlated with the amount of N accumulated in achenes, followed by the leaf N content and the leaf DM, indicating that a strong assimilating capacity is a prerequisite for high oil yield (Steer et al., 1985; Hocking and Steer, 1982; Vrebalov, 1974). As the oil concentration has little impact on oil yield of both crops (Table 1.4) a similar conclusion can be derived with respect to achene yield (data not shown). In agreement, achene yield of both safflower (Steer and Harrigan, 1986; Gilbert and Tucker, 1967) and sunflower (Steer and Hocking, 1984; Hocking and Steer, 1989; Connor and Sadras, 1992) was associated with high leaf area (leaf DM) and leaf N content. While oil yield was strongly associated with the number of achenes per capitulum and thus per plant in sunflower, the number of achenes per capitulum was only weakly associated with oil yield of safflower. Instead, the number of capitula per plant made a significant contribution to the variation of oil yield, and this agrees well with reports by Steer and Harrigan (1986), who concerned the number of capitula per plant as the major oil yield component in safflower responsible to increase number of achenes per plant. The most striking negative associations were found for the relationship between the number of achenes per capitulum on the one hand, and the number of capitula per plant ( $r = -0.50$ ) and the mass per achene ( $r = -0.52$ ), while the association between the number of capitula per plant and the mass per achene was highly positive ( $r = 0.79$ ). This indicates strong compensatory relations between these yield components, which has also been observed by Camas et al. (2005) and Tunctürk et al. (2005). As the sunflower variety used carries only one capitulum per plant, these compensatory effects are not observed in this crop.

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**Table 1.5:** Pearson coefficients among variables determining oil yield of safflower (above the diagonal) and safflower (below the diagonal) as identified by backward elimination.

Variables	Oil yield	Capitula plant <sup>-1</sup>	Achenes capitulum <sup>-1</sup>	Mass per achene	Achene N content	Leaf N content	Leaf DM
Oil yield [g pot <sup>-1</sup> ]	-	0.675***	0.272	0.552***	0.952***	0.772***	0.853***
Capitula plant <sup>-1</sup>	-	-	-0.497**	0.793***	0.551***	0.825***	0.823***
Achenes capitulum <sup>-1</sup>	0.979***	-	-	-0.517***	0.386*	-0.180	-0.088
Mass per achene	0.836***	-	0.733***	-	0.487**	0.770***	0.711***
Achene N content [mg pot <sup>-1</sup> ]	0.943***	-	0.934***	0.864***	-	0.750***	0.770***
Leaf N content [mg pot <sup>-1</sup> ]	0.895***	-	0.915***	0.745***	0.898***	-	0.944***
Leaf DM [g pot <sup>-1</sup> ]	0.960***	-	0.966***	0.773***	0.913***	0.963***	-

(Both years considered, all variables were transformed to the natural logarithm, significance indicated as \* for  $p < 0.05$ , \*\* for  $p < 0.01$ , and \*\*\* for  $p < 0.001$ ,  $n = 38$  for safflower,  $n = 36$  for sunflower). Years were pooled as backward elimination rendered its influence on oil yield of safflower and sunflower either marginal ( $p = 0.078$ ) or absent ( $p = 0.541$ ), respectively.

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**Table 1.6:** Direct (underlined) and indirect effects of selected variables determining oil yield of safflower and sunflower.

Variable	Pathway (via)						r with oil yield (total effect)
	Capitula plant <sup>-1</sup>	Achenes capitulum <sup>-1</sup>	Mass per achene	Achene N content	Leaf N content	Leaf DM	
Safflower							
Capitula plant <sup>-1</sup>	<u>0.714</u>	-0.342	0.243	0.091	-0.270	0.239	0.675
Achenes capitulum <sup>-1</sup>	-0.355	<u>0.688</u>	-0.158	0.064	0.059	-0.026	0.272
Mass per achene [g achene <sup>-1</sup> ]	0.566	-0.356	<u>0.307</u>	0.081	-0.252	0.206	0.552
Achene N content [mg N pot <sup>-1</sup> ]	0.394	0.265	0.149	<u>0.166</u>	-0.246	0.223	0.952
Leaf N content [mg N pot <sup>-1</sup> ]	0.589	-0.124	0.236	0.124	<u>-0.328</u>	0.274	0.772
Leaf DM [g pot <sup>-1</sup> ]	0.588	-0.061	0.218	0.128	-0.310	<u>0.290</u>	0.853
Sunflower							
Achenes capitulum <sup>-1</sup>	-	<u>1.025</u>	0.270	-0.239	-0.114	0.037	0.979
Mass per achene [g achene <sup>-1</sup> ]	-	0.751	<u>0.369</u>	-0.221	-0.093	0.030	0.836
Achene N content [mg N pot <sup>-1</sup> ]	-	0.957	0.319	<u>-0.256</u>	-0.112	0.035	0.943
Leaf N content [mg N pot <sup>-1</sup> ]	-	0.938	0.275	-0.230	<u>-0.125</u>	0.037	0.895
Leaf DM [g pot <sup>-1</sup> ]	-	0.989	0.285	-0.234	-0.120	<u>0.039</u>	0.960

(Both years considered, including year as additional variable; all variables were transformed to the natural logarithm, n = 38 for safflower and n = 36 for sunflower).

However, Pearson correlations may be misleading, as the causal structure of the observed relationship is not considered. For example, oil yield of sunflower was highly correlated with virtually all selected traits (Table 1.5). Path coefficient analysis is used to partition the relative contribution of several independent variables (here traits) on a dependent variable (here oil yield) by splitting the correlation coefficients (total effects in Table 1.6, representing Pearson correlations) into direct and indirect effects considering standardized regression coefficients (Dewey and Lu, 1959). This analysis reveals that in safflower oil yield is principally controlled by the number of capitula per plant followed by the number of achenes per capitulum, as their direct effects on oil yield are high (Table 1.6). The moderate total effect of the last trait stems from the negative association with the number of capitula per plant (Table 1.5). The other traits considered have only small direct effects, sometimes even negative, despite highly significant associations with oil yield (Table 1.5). It is evident, that this is principally mediated by the indirect effect via the number of capitula per plant. This is particularly obvious with respect to the achene N content, for which the high correlation with oil yield resides almost completely with the correlation between achene yield and achene N content per plant ( $r = 0.95$ , data not shown), which in turn is associated with the number of capitula per plant and the number of achenes per capitulum (Table 1.5, Table 1.6). Thus, the close relationship between oil yield and leaf N content as observed by Steer and Harrigan (1986) and Gilbert and Tucker (1967) is merely indirect, while the reported association of the number of capitula per plant with the oil yield (Steer and Harrigan, 1986) is genuine.

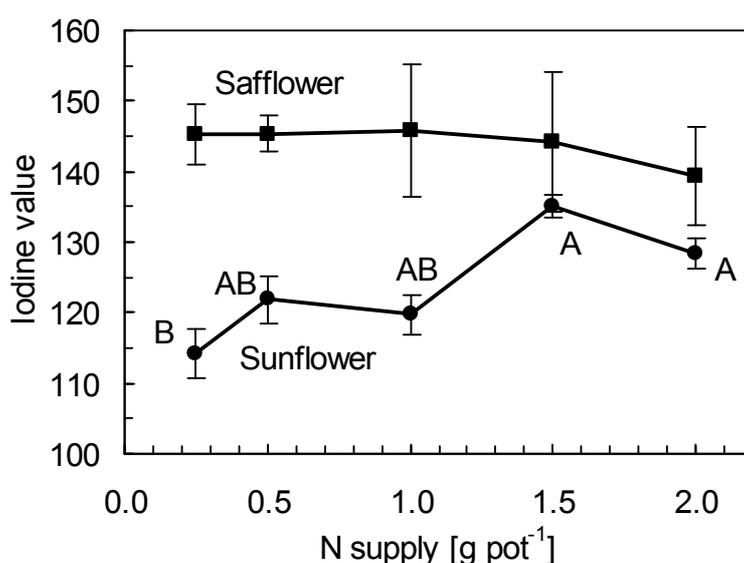
In agreement with previous reports achene yield of both safflower (Steer and Harrigan, 1986; Gilbert and Tucker, 1967) and sunflower (Steer and Hocking, 1984; Hocking and Steer, 1989; Connor and Sadras, 1992) was associated with high leaf area (leaf DM) and leaf N content (Table 1.5). While oil yield was strongly associated with the number of achenes per capitulum and thus per plant in sunflower, the number of achenes per capitulum was only weakly associated with oil yield of safflower. Instead, the number of capitula per plant made a significant contribution to the variation of oil yield, and this agrees well with recent reports (Tunctürk et al., 2005).

In sunflower, the number of achenes per capitulum exerts a strong direct effect on oil yield, and compensatory effects with the number of capitula per plant and the number of achenes per capitulum are not to be taken into consideration as discussed before (Table 1.6). Even more pronounced than for safflower the direct effects of the achene and leaf N content as

well as the leaf DM are small, and mediated principally via the indirect effect on the number of achenes per capitulum. Thus, the association of the leaf area (Connor and Sadras, 1992) or the number of leaves per plant (Steer and Hocking, 1983; Hocking and Steer, 1989) with oil or achene yield is merely indirect by providing photosynthetic capacity for yield formation.

### 3.3. Effect of N on quality parameters

Iodine numbers indicating the frequency of unsaturated fatty acids in the oil were consistently higher in safflower than in sunflower (Fig. 1.2). The values indicated here for both species fall within the range reported by Kumar (2000). In fact, the high iodine value of safflower oil is well documented (Padley et al., 1994, Yau et al., 1999), making it most valuable for human nutrition (Velasco et al., 2002). Overall, influence of N supply on oil quality was small, and in safflower only an insignificant trend towards lower iodine values with increasing N supply was observed. In sunflower values were more heterogeneous, and although no clear trend was observed, lowest values were reported at low N supply, which agrees with Steer and Seiler (1990) who found that the effect of N supply on sunflower oil quality is small. Actually, the proportion of oleic and linoleic acids depends merely on genotype and environmental conditions during oil formation (Talha and Osman, 1975). To the contrary, more significant positive relationships between N supply and iodine values were demonstrated for cotton seeds (Sawan et al., 2006) and oilseed rape (Narang et al., 1993).



**Figure 1.2:** Effect of N supply on iodine value of safflower and sunflower in 2004. Means assigned the same letter are not significantly different at  $p < 0.05$  ( $n = 4$ ).

### 4. Conclusion

Although only limited information on the nutrient requirement under field conditions may be obtained from pot experiments, it appears that – in terms of achene yield formation – safflower is performing better than sunflower under N limited conditions. In sunflower, the number of achenes per capitulum exerts a strong direct effect on oil yield. Even more pronounced than for safflower the direct effects of the achene and leaf N content as well as the leaf DM are small, and mediated principally via the indirect effect on the number of achenes per capitulum.

### Acknowledgment

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## Chapter 2

### Nitrogen Use Efficiency of Safflower as Compared to Sunflower

#### Abstract

Safflower's putatively NUE as compared to similar oil crops is not sustained. It was thus the aim of this study to determine the nitrogen use efficiency of safflower as compared to sunflower in pot experiments. Safflower out-yielded sunflower at low N supply, while at ample supply the opposite was observed. Both species accumulated similar amounts of N per pot at equivalent N supplies, but safflower was better N accumulator (concentrator) than sunflower due to safflower's less dry matter. Safflower utilizes absorbed N more efficiently than sunflower to produce achene yield at suboptimal N supply in terms of efficiency ratio and utilization index, but the opposite holds true at optimal and high supply. The efficiency to use accumulated N for dry matter and achene production interpreted in terms of Michaelis-Menten kinetics reveals the low  $C_{min}$  and  $K_m$  value in safflower compared to sunflower. It can be concluded that in terms of N availability safflower represents a low input crop and outperforms sunflower with respect to achene yield on soils low in available nitrogen.

#### 1. Introduction

Application of N fertilizer has been shown to be particularly effective with respect to yield formation in safflower (Steer and Harrigan, 1986) and sunflower (Connor and Sadras, 1992), but in organic farming, where application of inorganic N fertilizers is not permitted, the N availability is not easily increased. In addition, in developing countries, where the proportion of less fertile soils is particularly high, it may be difficult to fulfil the nutritional requirements of high-yielding crops (Sauerbeck and Helal, 1990; Marschner, 1995). Furthermore, even with inorganic N fertilizers the recovery of fertilizer N is often as low as 50 %, with serious ecological consequences (Scheiner et al., 2002). It is thus desirable to aim for efficient use of N, both in view of resource limitations and environmental constraints. Identification of crops species or cultivars with greater tolerance to suboptimal nutrient availability offers considerable promise for increasing the production potential on marginal land (Baligar et al., 1990; Gourley et al., 1994; Föhse et al., 1988).

The term 'nutrient efficiency' has been used widely as a measure of the capacity of a plant to acquire and utilize nutrients (Gourley et al., 1994), and may be broken down mechanistically into the ability to acquire nutrients from the soil (uptake efficiency) and the ability to utilize

accumulated nutrients for biomass production or yield formation (use efficiency) (Sattelmacher, 1994). Definitions of nutrient efficiency vary greatly (Sattelmacher, 1994), and in some cases may be even misleading in the quest for identifying mechanisms for enhanced nutrient acquisition and utilization (Gourley et al., 1994). They generally can be divided into those emphasizing productivity and those emphasizing the internal nutrient requirement of the plant. With regard to yield parameters, nutrient efficiency has been defined as the ability to produce a relatively high yield on a substrate that would otherwise limit the production of a standard line (Buso and Bliss, 1988; Graham, 1984). Other definitions of nutrient efficiency, also referred to as ‘agronomic efficiency’ include plant production of shoots, or harvestable products, per unit of nutrient applied (Blair and Cordero, 1978; Sauerbeck and Helal, 1990). The ‘external nutrient requirement’ refers to the amount of nutrient in the media required to achieve a given percentage of maximum yield (Föhse et al., 1988; Fox, 1981). Yield response per unit of added nutrient has also been used as a measure of nutrient efficiency (Blair, 1983; Baligar et al., 1990).

Safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.), both belonging to the *Asteraceae*, are important oil crops in tropical areas. Safflower is a highly branched, herbaceous, thistle-like annual, 30-150 cm tall with globular flower heads (capitula), characterized by a strong taproot, which enable it to thrive in dry climates, and which can access and utilize nutrients from below the root zone of cereal crops (Li and Mündel, 1997). The oil crop sunflower, however, is much taller, usually un-branched, lacks a taproot, and is considered more demanding in terms of nutrients and water (Diepenbrock and Pasda, 1995). Although both crops thrive in similar environments, direct comparisons of their response to increasing N availability with respect to N use efficiencies are limited, and the putatively higher N use efficiency of safflower is not sustained. Therefore, the aim of this study is to directly compare the N use efficiency of safflower as compared to sunflower under semi-controlled conditions.

## 2. Materials and Methods

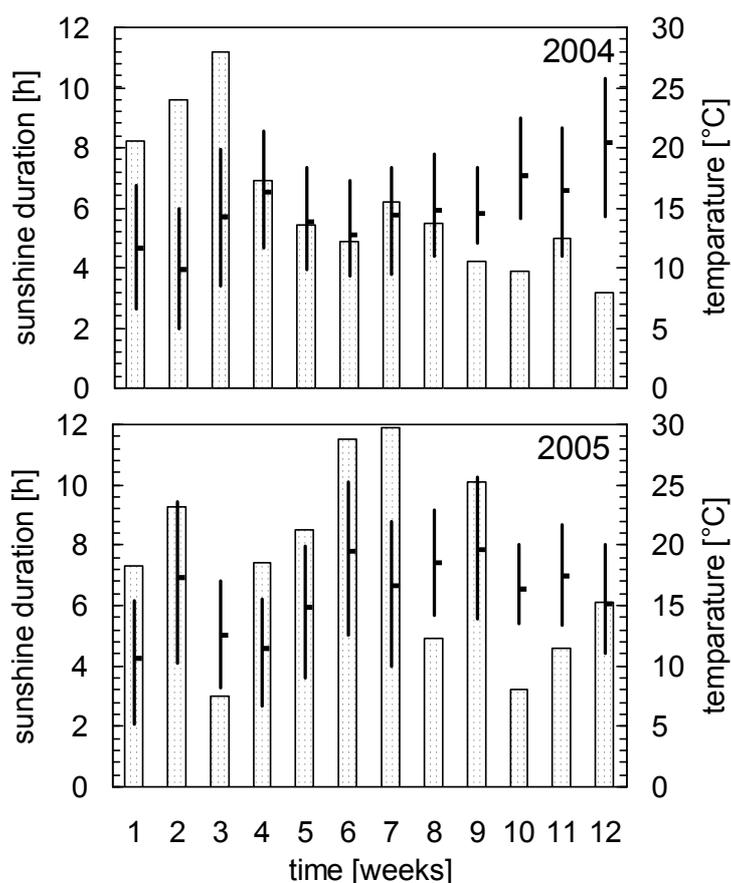
### 2.1. Experimental conditions

Experiments using safflower (*Carthamus tinctorius* L., variety ‘Sabina’) and sunflower (*Helianthus annuus* L., variety ‘Salut R M’) were conducted in the period from May to October 2004 and 2005 under semi-controlled conditions. In 2004 five N levels (0.25, 0.5, 1.0, 1.5, 2.0 g per pot) were used in four replicates for both species. Other nutrients added were 0.72, 2.0, and 1.0 g per pot of P, K, and Mg, respectively. In 2005, based on the infor-

mation obtained in the first experimental year, the N levels didn't change for safflower, but were adjusted to 0.5, 1.0, 2.0, 3.0, and 4.0 g per pot for sunflower, and P supply was increased to 2.0 g per pot for both species and K to 3.0 and 4.0 g per pot for safflower and sunflower, respectively. Three safflower and two sunflower plants per pot (6 L) were cultivated in a mixture of equal volumes of sand, soil, and perlite in Mitscherlich pots and were randomised under normal climatic conditions until the end of flowering (middle of August), followed by greenhouse cultivation (day and night temperatures were adjusted to 28°C and 15°C, respectively) with additional lighting (intensity of light supplementation at canopy level was 260  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Before being used in the experiment, soil (limed nutrient-poor Ferrasol containing 9.6 mg  $\text{g}^{-1}$  organic matter, pH(CaCl<sub>2</sub>) 5.9), sand (0.4-0.6 mm diameter) and perlite (Perligran®, 0-6 mm, pH(CaCl<sub>2</sub>) 7.0, 95 % pore volume) were analysed using the calcium acetate lactate (CAL) method according to Schüller (1969) and found to contain 2.8, 0.0, and 0.0 mg P, and 5.6, 0.8, and 0.0 mg K 100  $\text{g}^{-1}$  respectively. The final soil mixture without fertilizer added contained 1.8 mg P, and 1.7 mg K 100  $\text{g}^{-1}$ . Total N in soil, sand and perlite was analysed using an isotope ratio mass spectrometer (Finnigan MAT Delta C) coupled to an elemental analyser (Carlo-Erba 1108, Milan, Italy) and found to contain 1.0, 0.0, and 0.0 mg N  $\text{g}^{-1}$  respectively. Aphids were controlled with regular pesticide applications of Metasystox® (S-[2-(ethylsulfinyl) ethyl] O, O-dimethyl phosphorothioate), and infestations with *Perenospora sp.* were controlled by application of Amistar® (azoxystrobin) according to manufacturers recommendation. Daily air temperature and sunshine duration was obtained from another institution for the experimental area and are presented as weekly averages (Fig. 2.1).

### 2.2. Harvesting and analytical procedures

Plant parts (achenes, capitula, leaves, stems) were separated at the end of the experiment, dried at 70°C to constant weight (except for achenes, which were dried at room temperature in a well aerated area), grinded to pass a 1.5 mm sieve of which, after thorough mixing, a sub-sample of 5 g was ball-milled to a fine powder. Oil was extracted from achenes using Soxhlet extraction (SOX THERM 2000, S 206 AK/S2006A, Automatic from Gerhardt, Bonn, Germany). Total N of plant materials except achenes was measured as in soil, and achene N was determined as ammonium by a modified indophenol blue procedure using continuous flow analysis (Bran & Lütbe AutoAnalyzer II, Hamburg, Germany) after Kjeldal digestion (Novozamsky et al., 1983).



**Figure 2.1:** Weekly means of daily sunshine duration (bars) and average, lowest and highest daily air temperatures during the experimental period of both years (15th May – 15th August).

### 2.3. Statistical analysis and calculations

All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means with respect to the influence of N supply were carried out using the GLM procedure considering a fully randomised design, treating years separately. Where appropriate, data were log transformed to maintain homogeneity of variance. With multiple t-tests the Bonferoni procedure was employed in order to maintain an experimentwise  $\alpha$  of 5 %.

Response curves for DM and achene yield production were derived from the relationship between each yield parameter ( $\text{g pot}^{-1}$ ) and the amount of N accumulated in the plants ( $\text{mg N total plant}^{-1} \text{ pot}^{-1}$ ) using the following Michaelis-Menten-type equation

$$\text{Yield parameter} = (A_{\max} \times (\text{mg N} - b)) / (c + (\text{mg N} - b))$$

with ' $A_{\max}$ ' as an estimate of maximum yield, 'b' as the minimum N accumulation required for achene yield formation corresponding to the  $c_{\min}$  in enzyme kinetics, and 'c' as the N accumulation required for half maximum yield, corresponding to the  $K_m$  in Michaelis-Menten kinetics. The term 'b' was omitted in the case of DM, as there is no N-free biomass. The Michaelis-Menten equation proved superior to the Mitscherlich curve and has been widely used to describe nutrient efficiency (Gourley et al., 1994, Steingrobe and Claassen, 2000). Curve fitting was carried out using the procedure NLIN in SAS, employing the Gauss-Newton algorithm. In order to maintain homogeneity of variance calculations were carried out using inverted data for mg N and yield, as known from Lineweaver-Burk plots (Rao et al., 1993). However, untransformed data were used for plotting graphs of more familiar appearance. The internal N requirement of the two species is defined as 'c', the mg N accumulated to produce 50 % of predicted maximum yield (Föhse et al., 1988).

### 3. Results and discussion

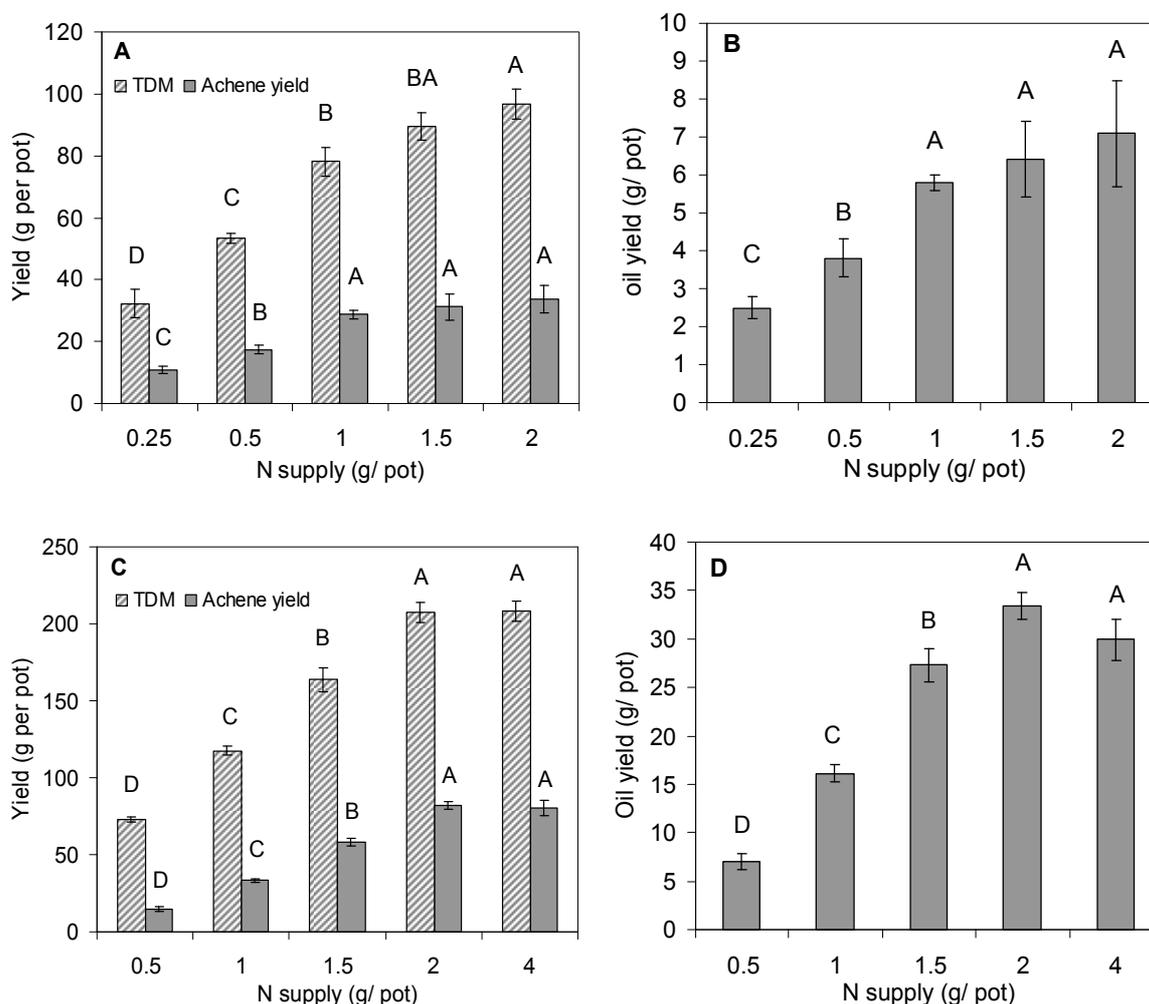
#### 3.1. Effect of N supply on N accumulation and uptake efficiency

Both crops responded strongly to increasing N supply with respect to growth and yield (Fig. 2.2). Growth and yield of safflower increased up to 1 g N per pot, while in sunflower, optimal growth and yield was achieved at 2 g N pot<sup>-1</sup> in the experiment of 2005, and data for 2004 are not shown because the optimal N supply was not reached in sunflower.

Both species accumulated similar amounts of N per pot in whole shoots at low N supplies in both years (Table 2.1), but safflower exceeded sunflower at the high N supplies in 2004, while the difference was not significant in 2005. Considering that 1 g and 2 g N per pot allowed for maximal growth and yield of safflower and sunflower, respectively (Fig. 2.2), sunflower accumulated significantly more total N in their tissues than safflower at their optimum N supply. In both years, safflower N concentration (mg N (g DM)<sup>-1</sup>) in the whole plant was higher than that of sunflower in all equivalent N supplies as a result of less DM produced per pot than sunflower causing a concentration effect (Table 2.1). N concentration increased in both species as N supply increased, but the differences in N concentration between N supplies were more pronounced in safflower than sunflower. It can be concluded that, safflower is more efficient than sunflower in concentrating N in their shoots at low and high equivalent N levels and also in terms of total N accumulation at high N supply.

Nitrogen uptake efficiency (Moll et al., 1982) was the same in both species at equivalent low and high N supplies, but safflower was more efficient in N uptake than sunflower at 1.0 and 1.5 g N pot<sup>-1</sup> in 2004 and 1.0 g pot<sup>-1</sup> in 2005 (Table 2.3). Comparing both crops at their

optimal N supplies (1.0, 2.0 g/pot for safflower and sunflower, respectively) safflower evidently absorbs a significantly higher proportion of the N supplied than sunflower.



**Figure 2.2:** TDM and achene yield (A, C) and oil yield (B, D) of safflower (A, B) and sunflower (C, D) over a range of N supply in 2005. Bars assigned the same letter are not significantly different (means  $\pm$  SD,  $p < 0.05$ ,  $n = 4$ ).

Hocking and Steer (1989) observed increasing N concentrations in sunflower with increasing N supply in all organs except achenes, which agrees with the experiments presented here (data not shown). As Vose (1987) noted that cultivars may be classified as ‘efficient’ or ‘inefficient’ for uptake or translocation, or ‘accumulators’ or ‘non-accumulators’ of certain elements. The uptake efficiency is defined as the total N absorbed in relation to N available in the soil (Moll et al., 1982). Because efficiency ratios (a measure for nutrient utilization discussed later) are calculated on the basis of the amount of N accumulated in the plant, this implies that the efficient species absorb more N than the inefficient one (Gerloff and Gabelman, 1983). However, higher elemental concentrations in cultivars could also be

## Chapter 2 Nitrogen use efficiency

attributed to lower DM accumulation (Baligar et al., 1990) as found here in terms of safflower compared to sunflower.

**Table 2.1:** Effect of N supply on the total N accumulated, N concentration, and plant N uptake efficiency of safflower and sunflower <sup>1)</sup>.

N supply (g per pot)	Safflower		Sunflower	
	total N accumulated (mg N pot <sup>-1</sup> )			
	2004	2005	2004	2005
0.25	298.2±17.2 E <sup>n.s</sup>	290.3±20.8 E	273.7±13.7 E	-
0.50	481.3±47.4 D <sup>n.s</sup>	497.9±31.7 D <sup>n.s</sup>	458.6±19.4 D	507.3±29.9 E
1.00	1018.5±45.9 C*	954.6±114.0 C*	809.1±70.0 C	823.3±13.1 D
1.50	1379.6±74.5 B*	1301.5±115.0 B	1191.0±52.3 B	-
2.00	1769.7±97.3 A*	1542.2±102.0 A*	1359.0±187.5 A	1433.3±43.3 C
3.00	-	-	-	2114.4±85.4 B
4.00	-	-	-	2775.9±170.2 A
	N concentration (mg N (g DM) <sup>-1</sup> )			
0.25	8.9±0.7 D*	7.9±1.0 D	6.8±0.4 C	-
0.50	9.1±0.7 D*	9.3±0.7 CD*	7.2±0.1 C	6.9±0.3 D
1.00	12.6±1.0 C*	12.2±0.9 BC*	8.7±0.5 B	7.0±0.1 D
1.50	16.4±0.8 B*	15.1±0.8 AB	9.9±0.2 A	-
2.00	20.6±0.9 A*	16.1±2.3 A*	11.0±1.3 A	8.8±0.3 C
3.00	-	-	-	10.2±0.2 B
4.00	-	-	-	13.7±0.5 A
	N uptake efficiency (mg N accumulated (mg N provided) <sup>-1</sup> )			
0.25	1.19 ± 0.07 A <sup>n.s</sup>	1.2 ± 0.09 A	1.09 ± 0.06 A	-
0.50	0.96 ± 0.10 AB <sup>n.s</sup>	1.0 ± 0.07 AB <sup>n.s</sup>	0.92 ± 0.04 B	1.01 ± 0.06 A
1.00	1.02 ± 0.05 AB*	0.95 ± 0.11 B *	0.81 ± 0.07 BC	0.82 ± 0.01 B
1.50	0.92 ± 0.05 B *	0.87 ± 0.08 BC	0.79 ± 0.03 C	-
2.00	0.86 ± 0.07 B <sup>n.s</sup>	0.77 ± 0.05 C <sup>n.s</sup>	0.73 ± 0.13 C	0.72 ± 0.02 C
3.00	-	-	-	0.70 ± 0.03 C
4.00	-	-	-	0.69 ± 0.04 C

<sup>1)</sup> Different letters within each column indicate significant difference (means ± SD,  $p < 0.05$ ,  $n = 4$ ). Significant and insignificant differences between species in a given year indicated by \* and n.s., respectively ( $p < 0.05$ ).

### 3.2. Effect of N supply on N utilization efficiencies

To characterize different genera, species or genotypes for nutrient use efficiency (NUE), researchers include many criteria such as; presence or absence of deficiency symptoms (e.g. Jensen et al., 1986), absolute growth at a limiting nutrient level (e.g. Chisholm and Blair, 1988), relative growth obtained by comparing growth at limiting and adequate nutrient levels (e.g. Coltman et al., 1985; Gerloff, 1987), efficiency ratio or amount of biomass produced per unit of nutrient present in the tissues (e.g. Coltman et al., 1985; O'Sullivan et al., 1974), and utilization coefficient as the inverse of the whole plant nutrient concentration (e.g. Lonergan and Asher, 1967). The utilization index, which is defined as biomass produced per unit of tissue nutrient concentration, was proposed by Siddiqui and Glass (1981) as an improved measure that, unlike the efficiency ratio, takes differences in the amount of produced biomass into consideration. Agronomic efficiency denotes the shoot biomass, or harvestable product, produced per unit of nutrient applied (Blair and Cordero, 1978; Moll et al., 1982). While such measures are relatively easily obtained, they may not reflect, or be correlated with economic NUE. Despite the considerable differences in the utilization of absorbed nutrients, there is as yet little understanding of the underlying mechanisms causing these differences (Gourley et al., 1994; Svecnjak and Rengel, 2006).

#### 3.2.1. Effect of N supply on agronomic N efficiency

An agronomic definition of nutrient efficiency (NE) relates plant productivity to nutrient supply (Sauerbeck and Helal, 1990; Moll et al., 1982). Nutrient efficiency has also been calculated on the basis of the amount of nutrient available (Gerloff and Gabelman, 1983), and the term 'external nutrient requirement' refers to the amount of nutrient in the media required to produce a given percentage of maximum yield (Föhse et al., 1988; Fox, 1981). According to all these interpretations for NUE we adopted a calculation that defines the required external N quantity (g) to produce 1 kg of achenes. It was found that safflower's external N requirement decreases with reduced N supply in both years, while sunflower's requirement only marginally responded to the N supply (Table 2.2). In both years safflower required less N than sunflower to produce 1 kg of achenes at low N supplies indicating that safflower can tolerate N deficiency more efficiently than sunflower. As a genotype that is able to realize a yield above average under conditions of suboptimal nutrient supply has been named nutrient efficient (Graham, 1984, Sattelmacher et al., 1994), safflower can be regarded a low input oil crop as compared to sunflower. At their optimal supplies, both species required the same amount of N to produce a given quantity of achenes, and

safflower's external N requirements exceeded that of sunflower at the highest N supply. This indicates that both species have the same external N efficiency for achene production at their respective optimum N levels.

**Table 2.2:** Effect of N supply on the external N requirement and on the N utilization index for achene production according to Siddiqi and Glass (1981) for the production of achenes of safflower and sunflower.<sup>1)</sup>

N supply (g/pot)	Safflower		Sunflower	
	2004	2005	2004	2005
External N requirement for achene production (g N applied (kg achenes) <sup>-1</sup> )				
0.25	25.4 ± 1.3 D *	23.3 ± 2.4 D	48.1 ± 3.9 A	-
0.50	30.0 ± 1.7 C *	28.8 ± 2.1 D *	44.0 ± 2.4 A	34.6 ± 2.8 B
1.00	33.7 ± 1.7 C *	34.8 ± 1.7 C <sup>n.s</sup>	41.1 ± 2.4 A	32.0 ± 1.1 B
1.50	47.6 ± 3.4 B <sup>n.s</sup>	48.8 ± 6.6 B	47.0 ± 3.8 A	-
2.00	67.5 ± 8.8 A <sup>n.s</sup>	60.0 ± 7.8 A *	51.1 ± 11.6 A	34.3 ± 1.5 B
3.00	-	-		36.7 ± 1.2 B
4.00	-	-		49.9 ± 3.0 A
N utilization index for achene production (g achene / (g N (g DM) <sup>-1</sup> ))				
0.25	11.1 ± 1.0 C *	12.2 ± 1.7 B	7.7 ± 0.3 C	-
0.50	18.5 ± 1.7 B *	18.8 ± 2.6 A <sup>n.s</sup>	15.8 ± 0.7 B	21.0 ± 1.9 D
1.00	23.8 ± 3.3 A <sup>n.s</sup>	21.86 ± 2.7 A *	28.0 ± 2.6 A	47.3 ± 2.2 C
1.50	19.32 ± 2.2 AB *	20.76 ± 3.6 A	32.4 ± 2.4 A	-
2.00	14.6 ± 2.2 BC *	21.47 ± 5.1 A *	35.0 ± 5.1 A	66.6 ± 4.7 B
3.00	-	-	±	80.1 ± 3.8 A
4.00	-	-	±	58.8 ± 5.1 B

<sup>1)</sup> Different letters within each column indicate significant difference (means ± SD,  $p < 0.05$ ,  $n = 4$ ). Significant and insignificant differences between species in a given year indicated by \* and n.s., respectively ( $p < 0.05$ ).

### 3.2.2. Effect of N supply on N utilization efficiency

To illustrate the concept of efficiency in nutrient utilization and to facilitate comparisons of the utilization of the same nutrient by different species or cultivars, investigators used calculated efficiency ratio, which is the mg of plant DM produced per mg element accumulated in

the plant. This indicator has been used extensively to describe the internal nutrient requirement of many crops (e.g. Godwin and Blair, 1991; Gourley et al., 1994), as it represents a valuable parameter in differentiating plants into efficient and inefficient utilizers of the absorbed nutrients (Baligar et al., 1990). Here, we expressed the nutrient ERs as g plant DM, achene, or oil produced per g element accumulated, which facilitates comparisons of efficiencies of nutrient utilization between the two species (Table 2.3).

In terms of DM production, sunflower had significantly higher N efficiency ratio (N ER) values than safflower at the same level of N supply including their respective optimal supply levels in both years, indicating that sunflower is more efficient in utilising N to produce DM than safflower. Indeed, previous studies reported that the N UE of sunflower was relatively high as compared to other species (Eckstein and Karlsson, 1997; Nakamura et al., 2002). However, in terms of achene production, safflower was more efficient than sunflower at low N supply, while the opposite holds true at optimal and high N supply (Table 2.3). This difference in efficiencies in terms of DM and achene production among the two species can be explained by the higher HI in safflower as compared to sunflower especially at low N supply (compare Fig. 2.2). In terms of oil production the two species had the same ER at low N supply, while sunflower was more efficient than safflower to produce oil at optimal and high N levels. The differences in N ERs response between achene and oil production in the two species is explained by the largely different oil concentration and because safflower reached a lower oil concentration than typically documented.

Nitrogen ER of both crops with respect to DM increased with decreasing N supply. In terms of achene production, the N ER in safflower increased under deficient N supply while sunflower was more efficient at intermediate N levels and its N ER decreased significantly at lower and higher levels, the same response was observed with respect to oil yield in both species and both years. The high utilization efficiency at low nutrient supply can be due to the very low nutrient concentration present in the plant, not to the utilization efficiency itself. Therefore, the utilization index (UI) was adopted (Siddiqi and Glass, 1981) to overcome this problem and to take the differences in biomass produced into consideration, not only the amount of accumulated nutrients. Under low N supplies (2004), N UI in terms of achene production was higher in safflower than in sunflower, but the opposite holds true at high N levels in both years (Table 2.3). Comparing the two species at their optimal supplies (1 and 2 g pot<sup>-1</sup> for safflower and sunflower, respectively), N UI was higher in safflower than in sunflower in both years. Both species showed highest values of N UI at levels near their respective optimal N supplies indicating that the high UE of safflower compared to

sunflower expressed as ER at low N supply is genuine and not stemming from a dilution effect.

In agreement with our findings, the results of Yulan et al., (2005) concerning sunflower showed that N UE represented as N ER decreased with increasing N availability, and this pattern has been found in other studies (Pastor and Bridgham 1999; Aerts and Chapin, 2000), but this response was not always the same (Yasumura et al., 2002). Therefore, plants adapt to N-poor environments simply by enhancing their N UE (Yulan et al., 2005). A major response of N-stressed sunflower plants was a more economical use of each unit of N acquired into total DM as was reported by Hocking and Steer (1989). Both the uptake and the utilization efficiency contribute to the genetic variation in nutrient use efficiency. For N, it was observed that, at low N supply, N UE was of overriding importance, but variation in uptake efficiency gained importance with increasing N supply (Moll et al., 1982).

It was argued that a proper evaluation of N use strategies requires data at the whole plant level, as patterns of aboveground N UE are not necessarily similar to that of the whole-plant (Aerts and Chapin 2000). The amount of N in roots in this study was not evaluated owing to the difficulty in measuring belowground biomass and nutrients. In the present study, neither safflower nor sunflower use the roots as storage for growth in the next season and, consequently, allocate smaller amounts of biomass to the roots. It is thus assumed that the plant's ranking based on our results would not be affected if roots were included in the analysis (Yulan et al., 2005).

According to our results, safflower is more efficient in utilizing absorbed N to produce achenes under low N supply. It can thus be regarded a low input species with respect to N.

## Chapter 2 Nitrogen use efficiency

**Table 2.3:** Effect of increasing N supply on Nitrogen Efficiency Ratios (NER) for the production of dry matter, achenes, and oil (g DM, achenes, oil g<sup>-1</sup> N) in 2004 and 2005.<sup>1)</sup>

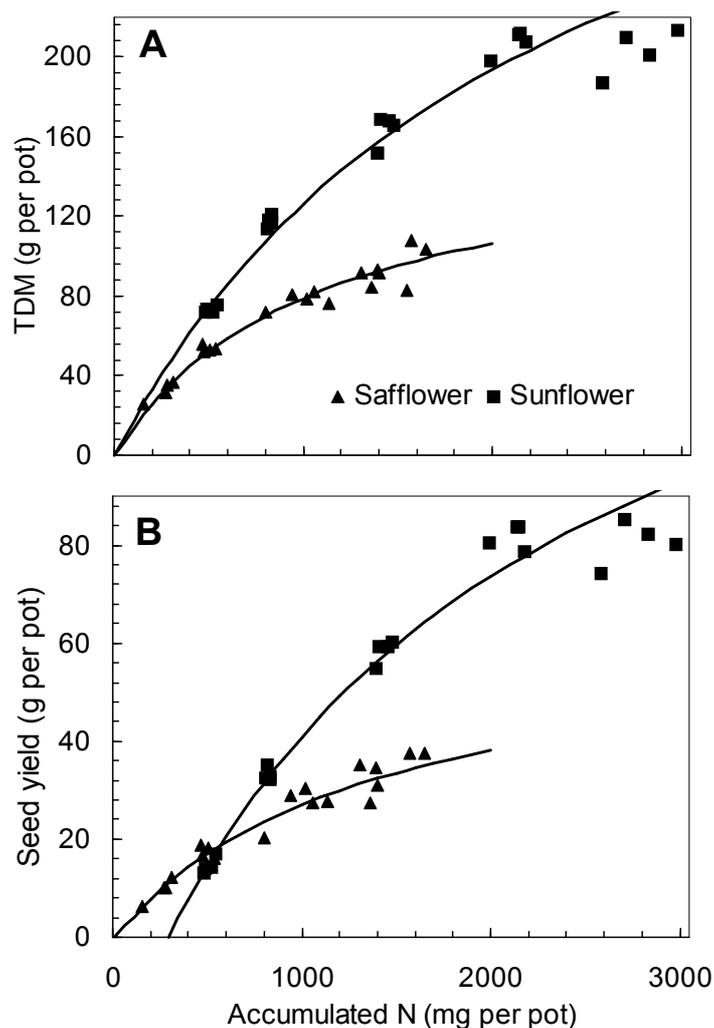
N supply (g/pot)	NER dry matter		NER achenes		NER oil	
	Safflower	Sunflower	Safflower	Sunflower	Safflower	Sunflower
2004						
0.25	112.4±8.5 A*	147.9±8.9 A	33.1±1.5 AB*	19.1±0.8 C	7.0±0.5 A	8.4±0.6 D
0.50	110.8±8.2 A*	138.7±1.4 A	34.9±3.1 A*	24.9±1.5 B	8.2±0.7 A*	12.1±0.3 B
1.00	79.7±6.7 B*	116.9±5.0 B	29.2±2.4 B	30.3±1.4 A	7.1±0.8 A*	13.8±0.7 A
1.50	60.9±3.0 C*	101.2±2.2 C	22.9±0.8 C*	26.9±1.2 B	5.3±0.2 B*	11.4±1.0 BC
2.00	48.7±2.1 D*	91.8±10.6 C	17.5±1.1 D*	24.6±3.0 B	3.9±0.5 C*	9.7±1.3 CD
2005						
0.25	118.3±4.9 A	-	37.9±1.8 A	-	8.6±0.4 A	-
0.50	107.7±8.5 AB*	144.2±6.5 A	35.2±4.2 A*	28.7±1.9 B	7.3±1.3 A*	13.7±0.7 B
1.00	82.5±6.3 CB*	142.5±2.3 A	27.9±2.6 B*	40.3±1.9 A	5.6±0.5 B*	19.6±1.1 A
1.50	66.4±3.3 C	-	24.0±2.8 B	-	4.9±0.8 B	-
2.00	62.7±1.8 C*	114.1±4.5 B	21.9±2.2 B*	40.7±1.1 A	4.6±0.7 B*	19.0±0.7 A
3.00	-	98.0±2.0 C	-	38.7±1.9 A	-	15.8±1.2 B
4.00	-	73.1±2.9 D	-	29.0±1.9 B	-	10.9±1.4 C

<sup>1)</sup> Different letters within each column indicate significant difference (means ± SD,  $p < 0.05$ ,  $n = 4$ ). Significant and insignificant differences between species in a given year indicated by \* and n.s., respectively ( $p < 0.05$ ).

### 3.2.3. Yield response curves based on accumulated N

The functional relationship between nutrient supply and yield parameters may be described in several ways. Polynomial functions are easily applied, but do not allow interpreting their coefficients in a straightforward fashion. The classical Mitscherlich equation has often been used to describe yield responses, but in order to characterise nutrient efficiency the Michaelis-Menten equation has been more frequently employed (e.g. Föhse et al., 1991, Gourley et al., 1994, Steingrobe and Claassen, 2000). As the data analysis requires variation in both dimensions, it is best applied to parameters describing efficiency ratios (Fig. 2.3). In analogy to enzyme kinetics, the N accumulation required to produce 50 % of the predicted maximum yield (term 'c') corresponds to the  $K_m$  in Michaelis-Menten kinetics and essentially describes the curvature of the graph. It is thus a good indicator of the sensitivity of a crop to reduced nutrient supply, hence its nutrient efficiency. However, this approach requires a well-defined response curve from which the yield maximum can be deduced. As the optimum nutrient supply for sunflower was not reached in 2004, this data analysis is confined to the data of 2005.

Characterising nutrient efficiency according to this approach indicates that safflower is less sensitive to inadequate N supply and thus more N efficient than sunflower with respect to yield parameters (TDM, achenes), as the term 'c' is always lower for safflower (Fig. 2.3). As expected, the response curve concerning TDM does not require an additional term to account for unproductive N accumulation, as N-free biomass is not to be considered (Fig. 2.3A). The minimal accumulated N per pot to trigger yield formation of achenes (term 'b'), corresponding to the  $c_{min}$  in enzyme kinetics, was lower in safflower than in sunflower indicating the capability of the former to realize achene yield at marginal N accumulation as compared to sunflower (Fig. 2.3B). This striking difference in the threshold value 'b' and the N response (term 'c') results in the two response curves intersecting, and illustrates nicely difficulties arising from different definitions of nutrient efficiency as discussed by Sattelmacher et al. (1994). Based on data by Thiraporn et al. (1987) they argued that two genotypes, of which one outperforms the other at low nutrient availability, while the opposite holds true at high nutrient supply, could both be considered nutrient efficient, as the more responding genotype converts applied nutrients more efficiently into yield. As we follow the definition that a more efficient genotype is able to realize a yield above average under conditions of suboptimal nutrient supply (Graham, 1984, Sattelmacher et al., 1994), safflower can be considered a more efficient crop in terms of the ability to utilize internal N to produce agronomic yield under suboptimal N supply.



**Figure 2.3:** Yield (TDM, seeds) response curves for safflower and sunflower in 2005 based on the total N accumulated in the above-ground biomass per pot. Michaelis-Menten-type equations are given as: Yield parameter =  $(A_{\max} \times (\text{mg N accumulated} - b)) / (c + (\text{mg N accumulated} - b))$ , with 'mgN' representing the N accumulated in the above-ground biomass per pot, 'c' the  $K_m$ , 'A' the maximal yield potential, and 'b' the minimal N accumulation required for yield formation.  $TDM_{\text{sunflower}} = (416 \times \text{mgN}) / (2303 + \text{mgN})$ ,  $TDM_{\text{safflower}} = (163^* \times \text{mgN}) / (1071^* + \text{mgN})$ ,  $\text{Seed yield}_{\text{sunflower}} = (168 \times (\text{mgN} - 297)) / (2194 + (\text{mgN} - 297))$ ,  $\text{Seed yield}_{\text{safflower}} = (64^* \times (\text{mgN} - 9.5^*)) / (1363 + (\text{mgN} - 9.5^*))$ . '\*' indicates that coefficients for safflower are significantly different from those for sunflower based on the 95% confidence limits.

### 3.2.4. Effect of N supply on N harvest index

While the harvest index is used to quantify the partitioning of dry matter, the nutrient harvest index is widely used to quantify the partitioning of nutrients towards the harvested organ. The N harvest index thus indicates what proportion of the N accumulated is actually removed by the harvested organ, while the N left behind may ultimately be lost to the environment. Here, the N harvest index (NHI) of safflower was significantly higher than that of

sunflower at all equivalent N supplies in the first experiment (Table 2.4), while in 2005, where N supplies were extended for sunflower, safflower had higher NHI at low N supply than sunflower while the opposite holds true at high N supply. Concerning the optimal N supplies for both species (1 g and 2 g N per pot for safflower and sunflower, respectively), both plants performed very similar in terms of the NHI. The consideration of a high NHI represents an important, though rarely discussed, aspect in environmental protection, as cultivars or species of high NHI enable removal of as much of the applied N in their harvestable plant parts as possible, thus reducing environmental contamination.

**Table 2.4:** Effect of N supply on nitrogen harvest index (NHI) of safflower and sunflower in 2004 and 2005 <sup>1)</sup>.

N supply (g per pot)	Safflower		Sunflower	
	2004	2005	2004	2005
0.25	65.8±2.9 B *	56.0±3.4 A	49.2±3.6 B	-
0.50	67.8±2.1 B *	57.0±5.2 A *	55.1±7.5 AB	45.5±2.6 C
1.00	73.5±2.4 A *	53.0±7.7 A n.s	64.0±1.9 A	56.1±2.3 B
1.50	71.1±0.5 A *	50.9±4.5 A	62.1±1.6 A	-
2.00	64.4±5.6 B *	47.4±5.0 A *	50.3±5.2 B	57.9±2.3 B
3.00	-	-	-	66.8±1.7 A
4.00	-	-	-	59.4±4.0 B

<sup>1)</sup> Different letters within each column indicate significant difference (means ± SD,  $p < 0.05$ ,  $n = 4$ ). Significant and insignificant differences between species in a given year indicated by \* and n.s., respectively ( $p < 0.05$ ).

#### 4. Conclusion

Although only limited information on the nutrient requirement under field conditions may be obtained from pot experiments, it is evident that – in terms of achene yield formation – safflower can be regarded a low input crop in terms of its N requirement. Safflower is more efficient than sunflower in utilizing absorbed N at low N levels only, but accumulates the same amount of N in the above-ground biomass and even exceeds sunflower with increasing N supply. The agronomic use efficiency (N supply required to produce 1 kg of achenes) points in a similar direction. Safflower maintained a higher N concentration (mg N (g DM)<sup>-1</sup>) than sunflower irrespective of N supply and can be regarded a good N

accumulator. Although most efficiency indicators provide a similar impression in this study, they should not be considered superfluous, as several investigations have shown remarkable differences between widely used indicators (e.g. Siddiqi and Glass, 1981; Gourley et al., 1994). The use of Michaelis-Menten kinetics provides a useful tool for studying and interpreting nutrient response curves and further sustains the rating of safflower as a truly N-efficient crop.

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### Chapter 3

#### Effects of Phosphorus Supply on Growth and Yield of Safflower (*Carthamus tinctorius* L.) as compared to Sunflower (*Helianthus annuus* L.)

##### Abstract

Safflower represents an important oil crop internationally and may have a certain production potential under German conditions, particularly in organic farming where the putatively low nutrient requirement is highly welcomed. However, current knowledge regarding the nutrient requirements of safflower as compared to similar oil crops is limited. It was thus the aim of this study to determine the P requirement for safflower (*Carthamus tinctorius* L.) as compared to sunflower (*Helianthus annuus* L.) with respect to P supply. Three safflower and two sunflower plants were cultivated in 6 L Mitscherlich pots. Both species responded strongly to increasing P supply with respect to plant growth and yield. Growth and yield of safflower increased up to 1 g P per pot, while the optimum for sunflower was 0.5 g P per pot only. Supply of P affected safflower yield mainly through increasing number of capitula per plant in both years and number of achenes per capitulum was reduced at very low P supply, while sunflower yield in both years was improved with increasing P supply through increased number of achenes per capitulum only. Yield component analysis reveals that, oil yield in safflower was affected by P deficiency mainly due to number of capitulum per plant followed by the number of achenes per capitulum followed by single achene mass (SAM), but contribution of oil content to overall yield variation was insignificant. As unbranched plant, sunflower major yield component influencing oil yield was number of achenes per plant, followed by SAM without a significant contribution of the oil content. Pearson correlations, followed by path coefficient analysis indicate that in sunflower only total N accumulated was found to be most important, while in safflower the total amount of P and N accumulated showed the highest standardised partial regression coefficient. It may be concluded that safflower is more sensitive to inadequate P supply than sunflower.

##### 1. Introduction

Many soils have large reserves of total P, but only a small fraction is immediately available. In fact, many agricultural areas are P deficient (Sanchez and Salinas, 1981), and application of fertiliser P represents an important measure to correct nutrient deficiencies and to replace elements that have been removed in the products harvested (Dambroth and El Bassam,

1990). In fact, P is the nutrient being most limiting for crop growth and yield in many regions of the world (Constant and Sheldrick, 1991; Rodríguez et al., 1999). However, due to chemical immobilisation in the soil (Sample et al., 1980) recovery of fertiliser P in the first year is often below 15% and hardly reaches 50 % after 30 years (Roy et al., 2006). In view of limited P resources (Cathcart, 1980) and serious ecological and economical consequences of contaminating the environment (EEA, 1999, EEA, 2001, Chardon and Withers, 2003) a considerate use of P is mandatory. Apart from fine-tuning fertilising strategies the cultivation of more efficient genotypes that are less sensitive to low P availability represents a promising option (Buso and Bliss, 1988).

The use of alternative oil crops that differ in their response to P is another possibility to meet the increasing global demand for vegetable oil, as possibilities to expand planted acreage on a global scale are limited. The oil derived from safflower and sunflower represents a share of 0.07 and 7.3 % of the global production of oil achenes, respectively (FAO, 2007 a, b). Safflower, *Carthamus tinctorius* L., a member of the *Asteraceae*, is a highly branched, herbaceous, thistle-like annual, 30-150 cm tall with globular flower heads (capitula), characterised with a strong taproot which enables it to thrive in dry climates and can access and utilise nutrients from below the root zone of cereal crops (Li and Mündel, 1997). The oil crop sunflower, however, is much taller, usually unbranched, lacks a tap root, and is usually been considered more demanding in terms of nutrient and water requirements (Diepenbrock and Pasda, 1995). It is believed that phosphate requirements of safflower are moderate (Esendal, 1997), and consistent response to phosphorus is obtained only when a soil test is in the low to very low range (Armah-Agyeman et al., 2002). In agreement, safflower was reported to accumulate 5.2 mg P to produce 1 kg of achene (Mündel, 2004), while the corresponding figure for sunflower is 11 mg P (Merrien, 1992). Although both crops thrive in similar environments, direct comparisons of their response to increasing P availability with respect to growth and yield formation are not available. In particular the putatively lower P requirement of safflower is not sustained.

Although the requirement for P is smaller than for either K or N (Marschner, 1995), P plays important roles as a structural component (e.g. DNA, RNA, phospholipids), and in the conversion and transfer of energy for a wide range of biochemical processes, as a very large number of enzymatic reactions depend on phosphorylation. Phosphorus plays a pivotal role in photosynthesis, in assimilate transport and metabolic regulation (starch, sucrose biosynthesis) (Bisson, 1994). Consequently, P plays a crucial role in yield formation (Marschner, 1995, Russel, 1988). In agronomic terms, yield is broken down into its components (capitula

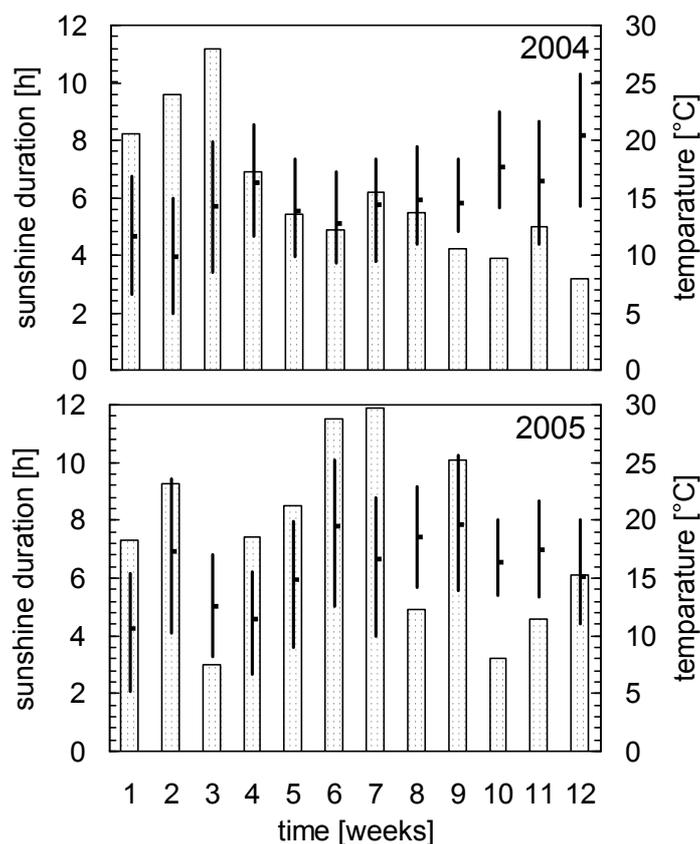
per plant, achenes per capitula, single achene mass, and oil concentration) that are linked in a multiplicative fashion to give the final yield. As these yield components are manifested along plant development, and as the two crops differ in their yield components, the P availability may affect yield formation by differential influence on individual yield components. However, little is known on this response in safflower as compared to sunflower (Kumar, 2000). Therefore, the aim of this study is to directly compare the impact of P supply on growth, yield and yield formation of safflower as compared to sunflower under semi-controlled conditions. Special emphasis was given to the response of yield components.

## 2. Materials and Methods

### 2.1. Experimental conditions

Experiments using safflower (*Carthamus tinctorius* L., variety 'Sabina') and sunflower (*Helianthus annuus* L., variety 'Salut R M') were conducted in the period from May to October 2004 and 2005 under semi-controlled conditions. In 2004 four P levels (0.04, 0.08, 0.24, 0.72 g per pot), were used in four replicates for both species. Other nutrients added were 2.0, 2.0, and 1.0 g per pot of N, K, and Mg, respectively. In 2005, based on the information obtained in the first experimental year, the P levels were adjusted to 0.25, 0.5, 1.0, and 2.0 g per for both species, N supply was not changed in safflower but was increased to 4 g N per pot in sunflower, and K supplies were increased to 3.0 and 4.0 g per pot for safflower and sunflower respectively. Three safflower and two sunflower plants per pot (6 L) were cultivated in a mixture of equal volumes of sand, soil, and perlite in Mitscherlich pots and were placed under normal climatic conditions till the end of flowering stage (middle of August), followed by greenhouse cultivation with additional lightning (intensity of light supplementation at canopy level was  $260 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Day night temperature was adjustment to 28°C, and 15°C respectively. Before be used in the experiment, soil (limed nutrient-poor Ferrasol, containing 9.6 mg g<sup>-1</sup> organic matter, pH (CaCl<sub>2</sub>) 5.9), sand (0.4-0.63 mm diameter) and perlite (Perligran®; Germany, pH (CaCl<sub>2</sub>) 7, 95% pores volume) were analysed using the Calcium Acetate Lactate (CAL) method according to Schüller (1969) and found to contain 28, 0, 0 mg P, and 56, 8, 0 mg K kg<sup>-1</sup>, respectively. Total N in soil, sand and perlite was analysed using an elemental analyser 1108 (Carlo-Erba, Milan, Italy) coupled to an isotope ratio mass spectrometer (Finnigan MAT Delta C) and found to contain 1.0, 0.0, and 0.0 mg g<sup>-1</sup> respectively. The final soil mixture without fertilizer added contained 18, 17, and 56 mg available P, available K, and total N kg<sup>-1</sup> respectively. Aphids were

controlled with regular applications of Metasystox® pesticide (S-[2-Ethylsulfinyl] ethyl] O, O-dimethyl phosphorothioate) and infestations with *Perenospora sp.* were controlled with application of Amistar® fungicide (azoxystrobin) according to manufacturers recommendation. Daily air temperature and sunshine duration was obtained from another institution for the experimental area and calculated as weekly average (Fig. 3.1).



**Figure 3.1:** Weekly means of daily sunshine duration (bars) and average, lowest and highest daily air temperatures during the experimental period of both years (15<sup>th</sup> May – 15<sup>th</sup> August).

## 2.2 Harvesting and analytical procedures

At physiological maturity plant parts (achenes, capitula, leaves, stems) were separated, dried (except achenes which were dried at room temperature in a well aerated area) at 70°C to constant weight, grinded to pass a 1.5 mm sieve of which, after thorough mixing, a subsample of 5 g was ball-milled to a fine powder. Oil was extracted from the achenes using Soxhlet extraction method (SOX THERM 2000, S 206 AK/S2006A, Automatic from Gerhardt, Bonn, Germany). Extracted oil samples were stored directly after extraction in nontransparent polypropylene tubes away from light in the freezer (-20°C). The iodine value of the oil was measured according to Matissek et al. (1992). For mineral nutrient determina-

tion samples were prepared by dry ashing (Dechassa, 2001) in which 50 mg of dried samples were ashed in a crucible at 450°C in a muffle furnace overnight. Then 1 ml of 0.35 M HNO<sub>3</sub> solution was added, and after swirling left for at least 10 minutes. After addition of 9 ml of purified water (18.2 MΩ cm<sup>-1</sup>), the sample is filtered through ashless filter paper (Whatman® #42, Whatman International Ltd, UK) into polypropylene tubes. Total P of the plant material was measured using inductively-coupled plasma optical emission spectrometry (Spectro Analytical Instruments GmbH, Kleve, Germany). Total N of plant materials except achenes was measured as in soil, and achene N was determined as ammonium by a modified indophenol blue procedure using continuous flow analysis (Bran & L bbecke Auto Analyzer II, Hamburg, Germany) after Kjeldal digestion (Novozamsky et al., 1983).

### 2.3. Statistics

All statistical analysis was carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means with respect to the influence of P supply were carried out using the GLM procedure considering a fully randomised block design, treating years (2004 and 2005) as separately. Where appropriate, data were log transformed to maintain homogeneity of variance. The Bonferoni procedure was employed with multiple t-tests in order to maintain an experimentwise  $\alpha$  of 5%.

Oil yield may be broken down into its components and expressed in a multiplicative fashion as:

$$\text{Oil yield [g oil plant}^{-1}] = (\text{capitula plant}^{-1}) \times (\text{achenes capitula}^{-1}) \times (\text{mass achene}^{-1}[\text{g}] \times (\text{oil [g g}^{-1}])$$

Initially Pearson correlations were calculated to test the relation between individual yield components and oil yield. The NOMISS option was used in order to obtain results consistent with subsequent multiple regression studies. In order to quantify the impact of individual yield components multiple regression analysis is biased as the mathematical product (or the sum in case log-transformed data are used), rather than a statistical relationship, of all individual yield components represents the oil yield. Hence, a yield component analysis according to Piepho (1995) was employed, allowing the contribution of individual components to be quantified. This approach assumes that the SD of log-transformed yield is close to the coefficient of variance of the yield, uses the log-transformed component data and interprets values of  $C_i = \text{Cov}[\log(\text{yield}), \log(\text{component}_i)]$  as an aggregate measure of the  $i^{\text{th}}$  component's contribution to the variability in yield.

Finally, the association of selected individual traits with respect to oil yield was evaluated. Most significant traits were selected by backward elimination, using the most frequently mentioned traits influencing sunflower and/or safflower yield in the literature. In order to characterise the direct and indirect effects of variation of individual traits to the variation of oil yield, path coefficient analysis was performed for the identified most important traits as described in detail by Dewey and Lu (1959).

### 3. Results and discussion

#### 3.1. Effect of P supply on growth and morphology

In both years, safflower and sunflower responded strongly to increasing P supply with respect to plant growth and morphology (Table 3.1). In the first experimental year the optimum P supply could not be deduced as the highest level still resulted in a positive response in both species. In the second experimental year, where P supply covered a wider range, 1 g P per pot was regarded as the optimum for safflower while only 0.5 g P per pot was enough for optimal growth and yield production in sunflower. In agreement with Rossiter (1973), as well as Russell (1988), low P supply substantially delayed the onset of floret initiation and ripening in both plants species (data not shown).

Stem diameter of both species was reduced by low P supply in both years, and the reduction of biomass accumulation was even more pronounced. It was reported that P deficiency limits shoot growth of sunflower (Connor and Sadras, 1992, Abdul Sadiq et al., 2000, Grove and Sumner, 1982, Rodríguez et al., 1998), safflower (Zaman, 1988, Padmavathi et al., 2003), cotton plants (Hearn, 1981, Sawan et al., 2001). The reduction of leaf biomass was striking and, indeed, in plants suffering from P deficiency, reduction in leaf area (Freedman et al., 1989) and leaf number (Lynch et al., 1991) are the most striking effects. Leaf expansion is strongly related to cell extension, requiring appropriate provision of water. However, in P deficient plants the decrease of root hydraulic conductivity is among the earliest events (Radin, 1990, Radin and Eidenbock, 1984).

**Table 3.1:** Effect of P supply on stem diameter and plant dry matter (DM) of safflower and sunflower in 2004 and 2005 <sup>1)</sup>.

P supply (g pot <sup>-1</sup> )	Stem diameter (mm)	Leaf DM (g pot <sup>-1</sup> )	Stem DM (g pot <sup>-1</sup> )	Capitula DM (g pot <sup>-1</sup> )	TDM (g pot <sup>-1</sup> )
Safflower (2004)					
0.04	2.9±0.1 C	0.6±0.3 C	2.1±0.6 C	1.3±0.7 C	5.0±1.4 C
0.08	3.2±0.2 C	0.8±0.2 C	2.4±0.3 C	2.4±0.6 C	7.0±1.2 C
0.24	4.0±0.3 B	2.3±0.7 B	5.5±1.2 B	6.8±1.2 B	21.9±4.3 B
0.72	6.3±0.2 A	9.3±1.4 A	21.6±2.8 A	23.4±2.7 A	84.9±7.4 A
Safflower (2005)					
0.25	5.8±0.3 B	7.6±0.8 B	13.1±2.4 B	15.4±2.5 C	57.3±4.9 B
0.50	6.0±0.4 B	8.3±0.6 B	16.3±2.3 B	19.4±1.4 BC	64.1±5.3 B
1.00	7.1±0.5 A	12.6±2.0 A	21.9±3.4 A	24.3±1.0 BA	92.6±5.4 A
2.00	7.2±0.5 A	12.3±1.7 A	23.1±0.9 A	26.5±3.2 A	95.6±8.4 A
Sunflower (2004)					
0.04	9.3±0.8 C	11.8±0.5 C	16.0±0.4 C	14.4±1.0 C	57.6±2.6 C
0.08	9.9±0.9 C	13.5±0.2.1 C	17.8±0.4 C	15.9±2.8 C	62.6±1.0 C
0.24	13.1±0.6 B	18.9±2.1 B	35.6±3.6 B	24.3±3.2 B	117.3±11.0 B
0.72	15.7±0.7 A	26.1±1.6 A	44.8±4.2 A	30.3±1.8 A	144.9±6.5 A
Sunflower (2005)					
0.25	14.0±0.9 B	29.1±0.8 B	42.0±2.0 B	34.3±3.8 A	175.6±5.1 B
0.50	15.8±0.7 A	33.4±1.3 A	49.9±2.2 A	34.5±2.4 A	197.3±4.9 A
1.00	16.9±0.2 A	35.7±2.0 A	48.6±1.4 A	32.8±1.2 A	199.2±7.8 A
2.00	16.8±0.5 A	36.0±1.5 A	51.6±1.2 A	32.9±1.1 A	200.1±6.7 A

<sup>1)</sup> for a given species and a given year means within each column followed by the same letter are not significantly different,  $p < 0.05$ ,  $n = 4$ .

As a consequence of severely inhibited leaf expansion the contents of chlorophyll per unit leaf area are often increased under P deficiency (Rao and Terry, 1989), and leaves have a darker green colour as cell and leaf expansion are more retarded than chloroplast and chlorophyll formation (Hecht-Buchholz, 1967). However, photosynthetic rate per unit area is reduced, as reported for sunflower (Jacob and Lawlor, 1991; Rodríguez et al, 1998), suggesting that both effects (reduction of leaf area and reduction of net photosynthesis per unit of leaf area) may contribute to the final reduction of biomass production. However, several

authors have shown that plant growth under P deficiency is usually reduced before the photosynthesis rate per unit leaf area (Jacob and Lawlor, 1991; Qui and Israel, 1994; Rao and Terry, 1989). This suggests that the reduction of photosynthesis rate is not the major cause of growth reduction, at least in early P deficiency (Plénet et al., 2000). Concerning the second year experiment where P supply was optimal for the two species, safflower stem diameter and all dry matter parameters were reduced significantly in two low P levels while sunflower was affected at the lowest p supply only without affecting its capitula dry matter. In agreement, under field conditions, sunflower yield (GYP0/GYPmax) was found the less sensitive in the absence of P fertilization compared to maize, sorghum, and wheat (Colomb et al., 2007).

#### **3.2. Effect of P supply on yield and yield components**

Supply of P (Table 3.2) affected safflower achene yield in the first experimental year -where severe P deficiency levels were used- through increasing number of capitula per plant (Ahmed et al., 1985, El-Nakhlawy, 1991, Singh et al., 1995), in agreement with that found for cotton (Sawan et al., 2006, Russel, 1988), okra (Majanbu et al., 1985, Oluwatoyinbo et al., 2005), canola (Afridi et al., 2002), ears of wheat (Rodríguez et al.1999, Horst et al., 1993). Number of achenes per capitulum was improved as P supply increased in agreement with previous work on safflower (El-Nacklawy, 1991, Singh et al., 1995, Singh and Singh, 1989), canola (Afridi et al., 2002, Rajput, 1988), and number of kernels per ear in maize (Hajabbasi and Schumacher, 1994, Barry and Miller, 1989). Single achene mass (SAM) of safflower was affected as a response to P supply (El-Nacklawy, 1991, Singh et al., 1995, Zaman, 1988) and this response was found in cotton (Sawan et al., 2001, Sawan et al., 2006). In 2005, where P supply was largely extended, only the number of capitula per safflower plant was improved and unexpectedly SAM was reduced. Safflower yield components in both years shows that the number of capitula per plant is the major component affecting the yield of this plant, the SAM is sensitive to low and high P supply, and the number of achenes per capitulum decreased only under severe P deficiency but both don't affect yield substantially. Concerning that, the experiment in 2005 covered a wide range of P levels and the plant response can be considered more reliable, the possible explanation to decreasing SAM is that, with increasing P fertilization, protein content in the achenes decreases (Blamey and Chapman, 1981), so under P deficiency, and adequate N supply, more protein will be accumulated in the achene compensating with production of other yield components resulting in increase of SAM.

**Table 3.2:** Effect of P supply on oil yield and yield components of safflower and sunflower in 2004 and 2005 <sup>1)</sup>.

P supply (g pot <sup>-1</sup> )	Capitula pot <sup>-1</sup>	Achenes [capitulum <sup>-1</sup> ]	SAM <sup>2)</sup> [mg achene <sup>-1</sup> ]	Achene yield [g pot <sup>-1</sup> ]	Oil conc. [mg g <sup>-1</sup> ]	Oil yield [g pot <sup>-1</sup> ]
Safflower (2004)						
0.04	1.6±0.6 C	5.5±1.4 C	31.9±3.3 B	0.8±0.1 C	215±0 A	0.2±0.1 C
0.08	2.4±0.5 B	5.3±0.4 C	34.8±3.5 B	1.3±0.3 C	217±0 A	0.3±0.1 C
0.24	4.0±0.5 B	15.9±1.7 B	37.4±3.5 B	7.1±1.2 B	218±9 A	1.6±0.2 B
0.72	9.6±1.5 A	22.2±3.6 A	47.6±4.1 A	30.0±4.1 A	225±14 A	6.8±1.3 A
Safflower (2005)						
0.25	8.7±0.9 C	13.7±1.1 A	59.8±4.5 A	21.1±1.2 B	191±17 A	4.1±0.7 B
0.50	11.3±1.8 BC	11.4±1.2 A	55.0±5.0 AB	20.0±1.4 B	191±16 A	3.8±0.5 B
1.00	14.8±1.8 BA	16.2±1.7 A	47.6±6.5 BC	33.7±1.8 A	223±10 A	7.5±0.7 A
2.00	16.0±1.4 A	14.8±0.9 A	47.4±1.8 C	33.7±4.3 A	209±18 A	7.1±1.4 A
Sunflower (2004)						
0.04	-	274±73.5 C	26.8±4.1 B	14.4±1.8 C	376±41 A	5.4±0.3 B
0.08	-	340±34.6 C	25.2±1.1 B	17.2±2.0 C	378±40 A	6.5±1.4 B
0.24	-	541±29.2 B	33.3±1.3 A	36.0±3.0 B	418±18 A	15.1±1.0 A
0.72	-	840±36.6 A	26.3±1.0 B	44.2±2.0 A	398±10 A	17.7±0.9 A
Sunflower (2005)						
0.25	-	1030±36. C	33.5±1.5 A	70.3±2.7 B	383±35 A	27.0±3.4 A
0.50	-	1175±36.1 B	33.8±0.5 A	79.5±3.1 A	389±8 A	30.9±0.2 A
1.00	-	1297±75.3 A	31.6±1.0 A	82.0±4.4 A	378±8 A	31.1±1.7 A
2.00	-	1295±31.0 A	33.0±2.4 A	80.4±4.7 A	374±38 A	29.9±2.1 A

<sup>1)</sup> for a given species and year means within each column followed by the same letter are not significantly different,  $p < 0.05$ ,  $n = 4$ .

<sup>2)</sup> Single achene mass.

Sunflower achene yield (Table 3.2) as affected by P supply was increased in both years due to increased number of achenes per capitulum only, as a single-headed variety was used (Abdul Sadiq et al., 2000, Weiss, 1967). It is well known that by P limitation, the formation of plant's reproduction organs retarded, also the number of flowers decreased (Bould and Parfitt, 1973; Russel, 1988), and seed formation is restricted in particular (Barry and Miller, 1989). It was observed that the response of safflower with respect to achene yield to phosphorous fertilization was greater than that to nitrogen fertilization (Hoag et al., 1968), which makes safflower a high P required plant compared to sunflower as observed in our results.

The significant improvement of achene yield as influenced by P application might be due to the improvement in plant growth by P application leading to higher photosynthetic activity and translocation of photosynthates to the sink (Rodríguez et al., 1998, Russell, 1988, Marschner, 1995), which consequently resulted in a better development in yield components and finally in a higher achene yield. Oil yield of both species was significantly increased as a result of increasing P supply but achenes oil concentration didn't changed with increasing P supplies in both plants and both years in contradiction with what found concerning oil concentration in achenes of safflower (El-Nacklawy, 1991, Singh et al., 1995, Zaman, 1988), sunflower (Blamey and Chapman, 1981), and cottonseed (Sawan et al., 2001).

Pearson correlation between yield and yield components (Table 3.3) shows that safflower oil yield is highly and positively correlated with number of capitula per plant (Tunctürk et al., 2005, Tabrizi 2000, Malleshappa et al., 2003), number of achenes per capitula (Tunctürk et al., 2005, Camas et al., 2005), and single achene mass (SAM) (Camas et al., 2005). Number of capitulum per plant was highly correlated with number of achenes per plant (Camas et al., 2005) and mass of achene (Camas et al., 2005, Tabrizi, 2000) revealing the overall improvement of increasing P supply on plant development, but has insignificant negative correlation with oil content (Tabrizi, 2000, Malleshappa et al., 2003, Tunctürk et al., 2005). Number of achenes per capitulum had positive significant correlation with mass of achene (Camas et al., 2005, Malleshappa et al., 2003) but in contrary with Tunctürk et al. (2005), these two traits are not correlated with each other. Also number of achenes per capitulum was found to have insignificant correlation with oil content (Tunctürk et al., 2005) but in contrary with Camas et al. (2005), these two traits are positively and significantly correlated to each other. Mass of achene had significant negative correlation with oil content (Malleshappa et al., 2003). As a mono-headed plant, sunflower oil yield was correlated with number of achenes per capitulum and single achene mass (SAM) (Marinković, 1992, Tahir et al., 2002, Ahmad et al., 1991), and was not correlated with oil content (Ahmad et al., 1991), but in contrary with Marinković (1992), oil content is positively correlated with achene yield and the later had positive correlation with oil yield. Number of achenes per capitulum was highly correlated with SAM (Marinković, 1992, Tahir et al., 2002) and in contrary with Ahmad et al. (1991), a significant negative correlation between these two traits was recorded, but number of achenes per capitulum was not correlated with oil content which disagree with Ahmad et al. (1991) who found them negatively correlated to each other and with Marinković (1992) who found them positively correlated with each other. Single

achene mass was found to be not correlated with oil content in contrary with Tahir et al. (2002) and Ahmad et al. (1991) who both found a positive correlation between the two traits. Pearson correlations are obviously misleading, as they do not allow the quantitative contribution of the variation of a given yield component to final yield to be elucidated. Therefore  $c_i$  coefficients, based on the covariance of the log-transformed yield components with the log-yields, were calculated to quantify the contribution of each component to the yield variability (Piepho, 1995). The major effect of increasing oil yield in safflower in response to increasing P supply was mainly due to higher capitula per plant, followed by the number of achenes per capitulum followed by SAM, but contribution of oil content to overall yield variation was insignificant (Table 3.4). As unbranched plant sunflower major yield component influencing oil yield was number of achenes per plant, followed by SAM (Marinković, 1992, Steer et al., 1984) without a significant contribution of the oil content to the variation of yield. Since the number of achenes per capitulum is the major yield component of sunflower, it became obvious that special attention should be given to plant requirements from stage 5 to stage 8 (flowering) (Palmer and Steer, 1985).

**Table 3.3:** Pearson coefficients between yield and yield components of safflower (above the diagonal) and sunflower (below the diagonal) (pooled over both years)<sup>1)</sup>.

Variables	Oil yield pot <sup>-1</sup>	Capitula plant <sup>-1</sup>	Achenes capitulum <sup>-1</sup>	Mass achene <sup>-1</sup>	Oil concentration mg oil g achenes <sup>-1</sup>
Oil yield pot <sup>-1</sup>	-	0.962***	0.873***	0.775***	-0.080
Capitula plant <sup>-1</sup>	-	-	0.719***	0.770***	-0.164
Achenes capitulum <sup>-1</sup>	0.975***	-	-	0.530**	0.096
Mass achene <sup>-1</sup>	0.721***	-	0.574***	-	-0.482**
Oil concentration mg oil g achenes <sup>-1</sup>	0.140	-	-0.029	0.300	-

<sup>1)</sup> Variables were log-transformed. Due to largely different P supply in the two years. Significance indicated as \* for  $p < 0.05$ , \*\* for  $p < 0.01$ , and \*\*\* for  $p < 0.001$ ,  $n = 32$  for each crop.

**Table 3.4:** Estimates of variation coefficients (VC) of achene yield, SD and variance of log-yields, and  $c_i$  coefficients for yield components of safflower and sunflower<sup>1)</sup>.

Crop and year	VC Oil yield (%)	SD log-yield (×100)	Variance log-yield (×100)	$C_i$ for yield component			
				Capitula plant <sup>-1</sup> (×100)	Achenes capitulum <sup>-1</sup> (×100)	Mass achene <sup>-1</sup> (×100)	Oil concentration (mg oil g achene <sup>-1</sup> ) (×100)
Safflower							
2004	130.28	151.39	229.18	107.37	96.33	22.77	2.73
2005	34.03	34.35	11.80	7.64	4.55	-3.32	2.94
Pooled	75.72	143.36	205.57	116.98	64.2	25.26	-0.98
Sunflower							
2004	49.75	54.16	29.33	-	23.79	2.79	2.59
2005	8.83	9.34	0.87	-	0.41	0.13	0.34
Pooled	50.72	68.09	46.36	-	39.28	6.31	0.73

<sup>1)</sup> Calculations according to Piepho (1995). (n = 16 for each year, n = 32 when pooled).

### 3.2.1. Important traits determining oil yield

In order to identify the most important traits determining the oil yield in the current experiment, Pearson correlations were calculated followed by standardized partial regression coefficients, known as path coefficient analysis (Dewey and Lu, 1959). Variables considered were selected using backward elimination using the traits mentioned in the literature that may be important in influencing sunflower yield. For example, yield per plant was found to be positively and significantly correlated with plant height and stem diameter in sunflower (Ashok et al., 2000, Tahir et al., 2002) also Ahmad et al. (1991) reported that sunflower plant height had strong direct effect on achene yield. In addition main stem diameter was found as principle yield component in safflower (Digming and Yuguang, 1993) and plant height was found to have a positive direct effect on single plant yield (Tunctürk et al., 2005). The produced biomass has frequently been discussed in relation to the yield performance of sunflower (Steer and Hocking, 1984; Hocking and Steer, 1989), and safflower (Tabrizi, 1999) for which the shoot DM (without achene DM) was taken as a proxy. The leaf area (Connor and Sadras, 1992) or the number of leaves per plant (Steer and Hocking, 1983; Hocking and Steer, 1989) has also frequently been mentioned due to their importance for providing assimilates, for which the leaf DM was used as indicator. The stem DM was included to consider its importance as transient reservoir for assimilates, P, and N (Steer and

Harrigan, 1986). The total N accumulated was taken as indicator for overall yield capacity, due to its pivotal importance for yield formation (Connor and Sadras, 1992), while the amount of P available for plant growth was assessed by the total amount of P accumulated in the plants. Finally, the achene P concentration was taken as indicator of P utilisation efficiency with respect to oil yield. After backward elimination at SLSTAY = 0.2 level; leaves dry matter, P content in achenes, total accumulated N in the plants were selected in both species as important traits at that cut-off P-level in addition to total P accumulated in safflower only. For a reason of uniformity, the four traits were used for both species to be proved for significance using Pearson correlations followed by standardized partial regression analysis.

**Table 3.5:** Pearson correlations between achene yield and selected traits of safflower (above the diagonal) and sunflower (below the diagonal). For selection criteria see text. Significance indicated by \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ) and \*\*\* ( $p < 0.001$ )<sup>1)</sup>.

Variable	Oil yield	Leaf DM	Achene P concentration	Total N accumulated	Total P accumulated
Oil yield		0.979***	0.916***	0.988***	0.987***
Leaf DM	0.184		0.934***	0.983***	0.993***
Achene P concentration	0.392*	0.027		0.935***	0.949***
Total N accumulated	0.809***	0.059	0.229		0.152
Total P accumulated	0.816***	0.099	0.841***	0.607***	

<sup>1)</sup> All values LOG-transformed. Due to largely different K supply in the two years the lowest and highest K treatments of both crops were excluded from the calculations (n = 32).

Oil yield was tightly correlated with the leaf DM in safflower while in sunflower these two traits are not associated. Although area and number of leaves has been frequently discussed in relation to achene yield. Achene P content was highly correlated with oil yield in safflower ( $r^2 = 0.916$ ) compared to the weak correlation with sunflower oil yield ( $r^2 = 0.392$ ). Oil yield of both species were highly correlated with both total N and P accumulated in the plant but this correlation was higher in safflower than sunflower for both traits (Table 3.5)., Interestingly, in safflower the total P accumulated was tightly correlated to the leaf DM ( $r^2 \approx 0.99$ ), while this relationship was not exist in sunflower ( $r^2 \approx 0.10$ ), which may indicate that P is more crucial for safflower than sunflower. All traits tested for correlation in

safflower were highly correlated to each other except that of total P accumulated is not correlated with total N accumulated in the plant in contrary with the high correlation of these two traits in sunflower.

However, Pearson correlations may be misleading, as several traits that show a high correlation with achene yield are highly correlated to each other, resulting in substantial multicollinearity (Table 3.5). A highly significant multiple regression model considering selected traits as specified above exhibits an adjusted  $r^2$  of 0.99 and 0.96 for safflower and sunflower, respectively (Table 3.6). Standardised partial regression coefficients, allowing assessment of the direct effect of a certain trait on achene yield, reveal that, in sunflower only total N accumulated was found to be most important, while in safflower the total amount of P and N accumulated showed the highest standardised partial regression coefficient. It may be concluded that safflower is more sensitive to inadequate P supply than sunflower.

**Table 3.6:** Standardised partial regression coefficients for selected traits and achene yield and statistics for the multiple linear regression model in safflower and sunflower. For selection criteria see text<sup>1)</sup>.

Variable	Safflower	Sunflower
Leaf DM	-0.382	0.052
Achene P concentration	-0.248	-0.912
Total P accumulated	0.604	0.089
Total N accumulated	1.005	1.525
Regression model		
Adjusted $r^2$	0.987	0.964
Significance	$p < 0.0001$	$p < 0.0001$

<sup>1)</sup> All values LOG-transformed. Due to largely different (n = 32).

### 3.3. Effect of P on oil quality

Safflower is considered to be a source of the highest quality vegetable oils (Kaffka et al., 2001). As compared to sunflower and olive oil, it is particularly rich in unsaturated fatty acids (Li and Mündel, 1997) and has a higher hypocholesterolemic activity as compared to sunflower oil and other vegetable oils (Kumar, 2000). In agreement, iodine numbers indicating the relative abundance of polyunsaturated fatty acids in the oil were usually higher in safflower than in sunflower and for both species fall in the range of reported values (Kumar,

2000; Roth and Kormann, 2005). In safflower there was no difference in iodine values as P supply was increased, while in sunflower P deficiency resulted in an insignificant reduction of this oil quality parameter (Table 3.7). It was reported that, the application of P tended to increase iodine value, which indicates that oil quality characters seemed to be enzymatically controlled and may affect the seed oil fatty acids composition (Epstein, 1971). Gushevilov and Palaveeva (1991) studied the changes in sunflower oil contents of linoleic, oleic, stearic and palmitic acids due to P-application rate and found that oil quality remained high at high P-rate. Khan et al. (1997) indicated that, oleic acid increased by increasing levels of P added to rapeseed-mustard. Apparently, the oil quality is merely influenced by genotype, capitulum position (primary, secondary and tertiary branches), location and stage of maturity as found in safflower (Nagaraj and Reddy, 1997). In addition to genotype, the temperature during oil formation and water stress can affect oil quality as found in sunflower (Connor and Sadras, 1992)

**Table 3.7:** Effect of P supply on Iodine value of safflower and sunflower in 2004 <sup>1)</sup>.

P supply (g per pot)	Safflower	Sunflower
0.04	/	121.5±5.6 A
0.08	139.3±0.0 A*	118.1±15.2 A
0.24	138.6±2.9 A	137.0±12.2 A
0.72	139.4±2.1 A*	132.5±9.8 A

<sup>1)</sup> Figures within each column followed by the same letter are not significantly different,  $p < 0.05$ ,  $n = 4$ . \* Within the two plant species in the same parameter means significantly different,  $p < 0.05$ .

#### 4. Conclusion

Both crops responded strongly to varied P supply, resulting in reduced vegetative growth and yield under conditions of limited P supply, and the response of safflower was particularly strong. As a single-headed sunflower variety was used the variation in achene yield was merely associated with variations in the number of achenes per capitulum, followed by mass of achene, while in safflower the number of capitula per plant was the most responding yield component, followed by the number of achenes per capitula, followed by mass of achene. Several traits, which were selected on the basis of previous hypothesis concerning their association with yield formation, were evaluated concerning their importance. In sunflower,

only total N accumulated was found to be most important, while in safflower the total amount of P and N accumulated showed the highest standardised partial regression coefficient. Based on this analysis and in view of the relative yield in response to altered P supply it may be concluded that safflower is more sensitive to inadequate P supply than sunflower. The differential efficiency of safflower and sunflower with respect to P utilisation will be the focus of a forthcoming publication.

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### Chapter 4

#### Phosphorus Use Efficiency of Safflower (*Carthamus tinctorius* L.) and Sunflower (*Helianthus annuus* L.)

##### Abstract

Safflower represents an oil crop believed to have putatively low nutrient requirement and high nutrient efficiency, but current knowledge regarding its nutrient use efficiency (NUE) as compared to similar oil crops is limited. It was thus the aim of this study to determine the nutrient use efficiency (NUE) of safflower (*Carthamus tinctorius* L.) as compared to sunflower (*Helianthus annuus* L.) with respect to P supply in pot experiments. Safflower and sunflower were cultivated with increasing P supply in a mixture of equal volumes of sand, nutrient-poor limed soil, and perlite in 6 L Mitscherlich pots.

Sunflower accumulated much more P (mg/pot) than safflower at all equivalent P supplies especially at low levels, but both accumulated the same P amounts at their individual optimal supplies. Uptake efficiency ( $\text{mg P accumulated} (\text{mg P provided})^{-1}$ ) was higher in sunflower than safflower at all equivalent P supplies including their optimal levels. Sunflower contained higher P concentration ( $\text{mg P} (\text{g DM}^{-1})$ ) than safflower at low P supplies only; while safflower needed more P concentration in their tissues at optimal levels. Agronomic efficiency interpreted as g P required to produce fixed amount of achenes was higher in safflower than sunflower at optimal and suboptimal P supply indicating the superiority of sunflower in term of the efficiency to use external P supply to produce achenes than safflower. Sunflower was much more efficient at their optimal P supplies to utilize absorbed P than safflower in term of efficiency ratio ( $\text{g achene} (\text{g P accumulated})^{-1}$ ) and utilization index ( $\text{g achene} / (\text{g P} (\text{g DM}^{-1}))$ ). Safflower showed higher efficiency in utilizing P at the very severe P deficiency than sunflower in term of efficiency ratio but it was a dilution effect not efficiency itself when interpreted in term of utilization index, while sunflower was found better P utilizer than safflower under moderate P deficiency interpreting utilization efficiency in term of efficiency ratio and utilization index. Harvest Index in sunflower out-yielded that of safflower at low and optimal P supplies. It can be concluded that safflower has a high requirement for P with respect to growth and yield; sunflower is more efficient than safflower in term of uptake and utilization of P at optimal and sub-optimal P supplies indicating that safflower failed to be a low nutrient input crop in term of phosphorus.

### 1. Introduction

Application of P fertilizer has been shown to be particularly effective with respect to yield formation in safflower (Mündel et al., 1997) and sunflower (Connor and Sadras, 1992). Although many soils have large reserves of total P, only a small fraction is immediately available making many agricultural areas P deficient (Sanchez and Salinas, 1981), therefore application of fertiliser P represents an important measure to correct nutrient deficiencies and to replace elements that have been removed in the products harvested (Dambroth and El Bassam, 1990), but in organic farming, where application of inorganic P fertilizers is not permitted, the P availability is not easily increased. In addition, in developing countries, where the proportion of less fertile soils is particularly high, it may be difficult to fulfil the nutritional requirements of high-yielding crops (Sauerbeck and Helal, 1990; Marschner, 1995). However, due to chemical immobilization in the soil (Sample et al., 1980) recovery of fertiliser P in the first year is often below 15% and hardly reaches 50 % after 30 years (Craswell and Godwin, 1984; Roy et al., 2006). In view of limited P resources (Cathcart, 1980) and serious ecological and economical consequences of contaminating the environment (EEA, 1999, EEA, 2001, Chardon and Withers, 2003) a considerate use of P is mandatory. Apart from fine-tuning fertilising strategies, the cultivation of more efficient genotypes that are less sensitive to low P availability represents a promising option (Buso and Bliss, 1988). It is thus desirable to aim for efficient use of P, both in view of resource limitations and environmental constrains. Identification of crops species or cultivars with greater tolerance to suboptimal nutrient availability offers considerable promise for increasing the production potential on marginal land (Baligar et al., 1990; Gourley et al., 1994).

The term 'nutrient efficiency' has been used widely as a measure of the capacity of a plant to acquire and utilize nutrients (Gourley et al., 1994), and may be broken down mechanistically into the ability to acquire nutrient from the soil (uptake efficiency) and the ability to utilize accumulated nutrients for biomass production or yield formation (use efficiency) (Sattelmacher, 1994). Definitions of nutrient efficiency vary greatly (Sattelmacher, 1994), and in some cases may be even misleading in the quest for identification of mechanisms for enhanced nutrient acquisition and utilization (Gourley et al., 1994). They generally can be divided into those emphasizing productivity and those emphasizing the internal nutrient requirement of the plant. With regard to yield parameters, nutrient efficiency has been defined as the ability to produce a relatively high yield on a substrate that would otherwise limit the production of a standard line (Buso and Bliss, 1988; Graham, 1984). Other definitions of nutrient efficiency, also referred to as 'agronomic efficiency' include plant produc-

tion of shoots, or harvestable products, per unit of nutrient applied (Blair and Cordero, 1978; Sauerbeck and Helal, 1990). The 'external nutrient requirement' refers to the amount of nutrient in the media required to achieve a given percentage of maximum yield (Föhse et al., 1988; Fox, 1981). Yield response per unit of added nutrient has also been used as a measure of nutrient efficiency (Blair, 1983; Baligar et al., 1990).

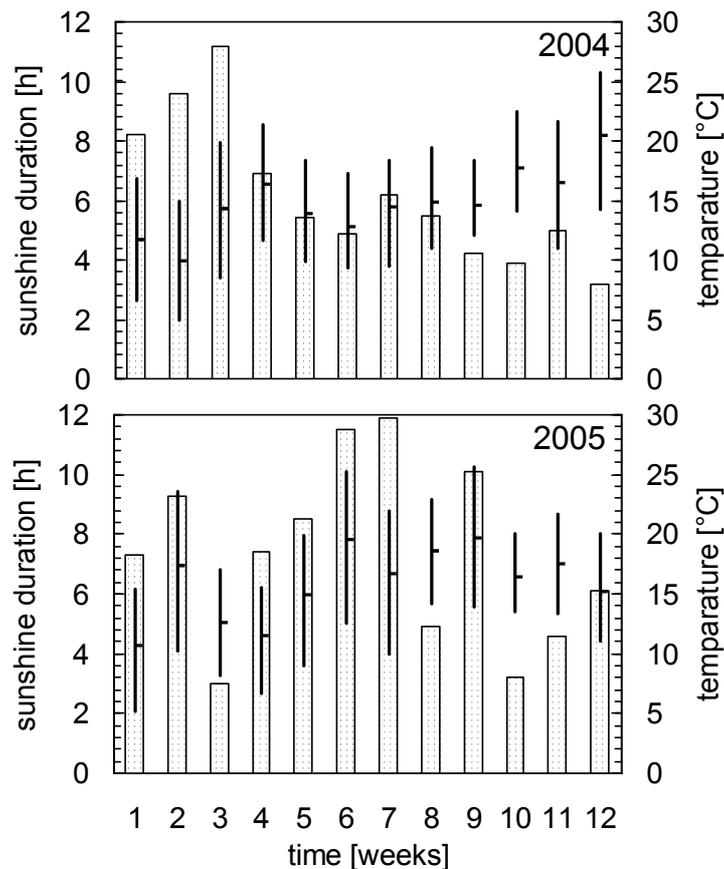
Safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.), both belonging to the *Asteraceae*, are important oil crops in tropical areas. Safflower is a highly branched, herbaceous, thistle-like annual, 30-150 cm tall with globular flower heads (capitula), characterised by a strong taproot, which enable it to thrive in dry climates and can access and utilize nutrients from below the root zone of cereal crops (Li and Mündel, 1997). The oil crop sunflower, however, is much taller, usually un-branched, lacks a taproot, and is considered more demanding in terms of nutrients and water (Diepenbrock and Pasda, 1995). Although both crops thrive in similar environments, direct comparisons of their response to increasing P availability with respect to P use efficiencies are limited, and the putatively higher P use efficiency of safflower is not proved. Therefore, the aim of this study is to directly compare the P use efficiency of safflower as compared to sunflower under semi-controlled conditions.

## 2. Materials and Methods

### 2.1 Experimental Conditions

Experiments using safflower (*Carthamus tinctorius* L., variety 'Sabina') and sunflower (*Helianthus annuus* L., variety 'Salut R M') were conducted in the period from May to October 2004 and 2005 under semi-controlled conditions. In 2004 four P levels (0.04, 0.08, 0.24, 0.72 g per pot), were used in four replicates for both species. Other nutrients added were 2.0, 2.0, and 1.0 g per pot of N, K, and Mg, respectively. In 2005, based on the information obtained in the first experimental year, the P levels were adjusted to 0.25, 0.5, 1.0, and 2.0 g per for both species, N supply was not changed in safflower but was increased to 4 g N per pot in sunflower, and K supplies were increased to 3.0 and 4.0 g per pot for safflower and sunflower respectively. Three safflower and two sunflower plants were cultivated in a mixture of equal volumes of sand, soil, and perlite in Mitscherlich pots (6L) individually and were placed under normal climatic conditions till the end of flowering stage (middle of August), followed by greenhouse cultivation with additional lightning (intensity of light supplementation at canopy level was  $260 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Day night temperature was

adjustment to 28°C, and 15°C respectively. Before be used in the experiment, soil (limed nutrient-poor Ferrasol, containing 9.6 mg g<sup>-1</sup> organic matter, pH (CaCl<sub>2</sub>) 5.9), sand (0.4-0.63 mm diameter) and perlite (Perligran®; Germany, pH (CaCl<sub>2</sub>) 7, 95% pores volume) were analysed using the Calcium Acetate Lactate (CAL) method according to Schüller (1969) and found to contain 28, 0, 0 mg P, and 56, 8, 0 mg K kg<sup>-1</sup>, respectively. Total N in soil, sand and perlite was analysed using an elemental analyser 1108 (Carlo-Erba, Milan, Italy) coupled to an isotope ratio mass spectrometer (Finnigan MAT Delta C) and found to contain 1.0, 0.0, and 0.0 mg g<sup>-1</sup> respectively. The final soil mixture without fertilizer added contained 18, 17, and 56 mg available P, available K, and total N kg<sup>-1</sup> respectively. Aphids were controlled with regular applications of Metasystox® pesticide (S-[2-Ethylsulfinyl] ethyl] O, O-dimethyl phosphorothioate) and infestations with *Perenospora sp.* were controlled with application of Amistar® fungicide (azoxystrobin) according to manufacturers recommendation. Daily air temperature and sunshine duration was obtained from another institution for the experimental area and calculated as weekly average (Fig. 4.1).



**Figure 4.1:** Weekly means of daily sunshine duration (bars) and average, lowest and highest daily air temperatures during the experimental period of both years (15th May – 15th August).

### 2.2. Harvesting and analytical procedures

Plant parts (achenes, capitula, leaves, stems) were separated at the end of the experiment, dried at 70°C to constant weight (except achenes which were dried at room temperature in a well aerated area), grinded to pass a 1.5 mm sieve of which, after thorough mixing, a sub-sample of 5 g was ball-milled to a fine powder. Oil was extracted from the achenes using Soxhlet extraction method (SOXTHERM 2000, S 206 AK/S2006A, Automatic from Gerhardt, Bonn, Germany). For mineral nutrient determination samples were prepared by dry ashing (Dechassa, 2001) in which 50 mg of dried samples were ashed in a crucible at 450°C in a muffle furnace overnight. Then 1 ml of 0.35 M HNO<sub>3</sub> solution was added, and after swirling left for at least 10 minutes. After addition of 9 ml of purified water (18.2 MΩ cm<sup>-1</sup>), the sample is filtered through ashless filter paper (Whatman® #42, Whatman International Ltd, UK) into polypropylene tubes. Total P of the plant material was measured using inductively-coupled plasma optical emission spectrometry (Spectro Analytical Instruments GmbH, Kleve, Germany).

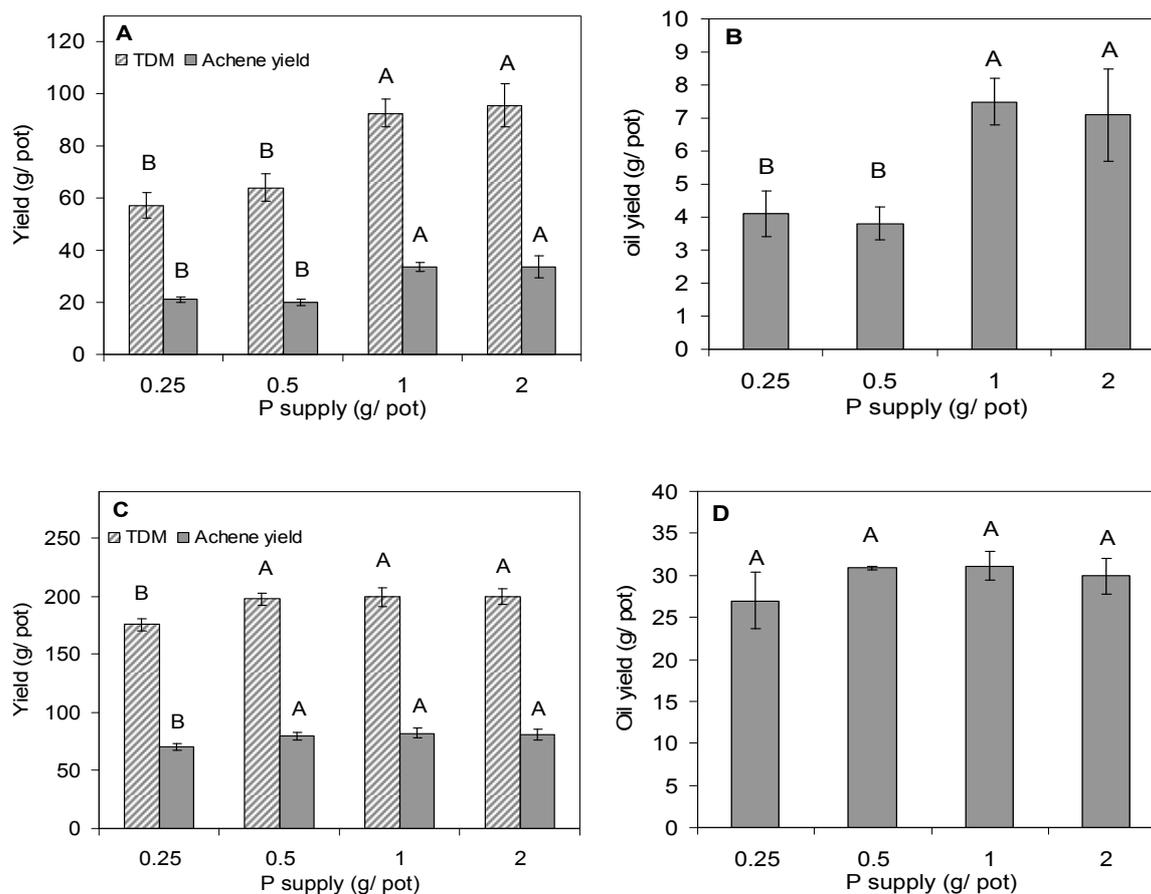
### 2.3. Statistics

All statistical analysis was carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means with respect to the influence of P supply were carried out using the GLM procedure considering a fully randomised block design, treating years separately. Where appropriate, data were log transformed to maintain homogeneity of variance. The Bonferoni procedure was employed with multiple t-tests in order to maintain an experimentwise  $\alpha$  of 5%.

## 3. Results and discussion

### 3.1 Effect of P supply on P uptake and accumulation

Both crops responded strongly to increasing P supply with respect to growth and yield (Fig. 4.2). Growth and yield of safflower increased up to 1.0 g P per pot, while in sunflower, optimal growth and yield was achieved at 0.5 g P per pot in the experiment of 2005, and data for 2004 was not shown because the optimal P was not reached in both species.



**Figure 4.2:** TDM and achene yield (A, C) and oil yield (B, D) of safflower (A, B) and sunflower (C, D) over a range of P supply. Bars assigned the same letter are not significantly different ( $p < 0.05$ ,  $n = 4$ ). Error bars:  $\pm$  SD

Sunflower accumulated much higher total mg P in whole plant per pot than safflower at all equivalent added P fertilizer levels in both years (Table 4.1). Total plant-P increased with increasing P supply in safflower in both years with continuous increase in P-accumulation responding to increasing external P supply, while sunflower accumulated constant values of total P per pot at the lower three P levels maintaining much higher accumulated-P in comparison with safflower indicating that sunflower can accommodate to low P supply much better than safflower, and respond to increasing added P in term of accumulation. Considering that 1 g and 0.5 g P per pot allowed for maximal growth and yield of safflower and sunflower, respectively (Fig. 4.2), both species accumulated the same total P in their tissues at their optimum P supply. Safflower had much less P concentration than sunflower at low P supplies, but out-yielded that of sunflower at their optimal levels, indicating the need of more P concentration in tissues of safflower to perform optimally compared to sunflower. In both years P concentration ( $\text{mg P (g DM)}^{-1}$ ) in whole plant of safflower increased significantly with increasing P fertilization, while sunflower tune its P concentration in a steady

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state under suboptimal P supply (year 2004) without a significant difference between different P supplies except on 0.24 g P per pot where P concentration was significantly lower than the lower and higher adjacent levels and can be discussed as dilution effect because the total accumulated P in that treatment was the same as the two lower levels but the dry matter was higher. Considering the optimal P supplies for both species, safflower had higher P concentration than sunflower to perform optimal growth and yield.

**Table 4.1:** Effect of P supply on the total plant P, P concentration, and P uptake efficiency of safflower and sunflower <sup>1)</sup>.

P supply (g/pot)	Safflower		Sunflower	
	2004	2005	2004	2005
Total P accumulated (mg P pot <sup>-1</sup> )				
0.04	2.8±0.7 C*	-	96.1±23.4 B	-
0.08	4.9±1.8 C*	-	97.7±15.0 B	-
0.24	27.6±6.1 B*	-	111.6±14.5 B	-
0.25	-	176.2±23.1 C*	-	242.8±6.2 D
0.50	-	190.5±35.2 C*	-	312.1±16.6 C
0.72	233.5±21.2 A*	-	296.9±28.5 A	-
1.00	-	313.0±49.4 B*	-	501.2±72.9 B
2.00	-	414.2±43.6 A*	-	953.1±62.7 A
P concentration (mg P (g DM) <sup>-1</sup> )				
0.04	0.6±0.1 C*	-	1.7±0.5 A	-
0.08	0.7±0.1 C*	-	1.6±0.3 A	-
0.25	1.3±0.1 B*	3.1±0.4 B*	1.0±0.1 B	1.4±0.1 C
0.50	-	3.0±0.6 AB*	-	1.6±0.1 C
0.72	2.8±0.1 A*	-	2.0±0.2 A	-
1.00	-	3.4±0.5 AB*	-	2.5±0.4 B
2.00	-	4.3±0.5 A	-	4.8±0.4 A
P uptake efficiency (mg P accumulated (mg P provided) <sup>-1</sup> )				
0.04	0.07 ± 0.02 C*	-	2.39 ± 0.31 A	-
0.08	0.06 ± 0.02 C*	-	1.27 ± 0.11 B	-
0.24	0.12 ± 0.02 B*	-	0.47 ± 0.06 C	-
0.25	-	0.70 ± 0.09 A*	-	0.97 ± 0.02 A
0.50	-	0.38 ± 0.07 B*	-	0.62 ± 0.04 B
0.72	0.33 ± 0.03 A*	-	0.44 ± 0.04 C	-
1.00	-	0.31 ± 0.03 B*	-	0.5 ± 0.07 C
2.00	-	0.21 ± 0.02 C*	-	0.48 ± 0.03 C

<sup>1)</sup> Different letters within each column indicate significant difference ( $p < 0.05$ ,  $n = 4$ ); ±SD: Standard deviation. \* indicates significance between species in a given year ( $p < 0.05$ ).

Phosphorus uptake efficiency (Moll et al., 1982) in safflower was much less than that of sunflower at low and high P supplies including their optimal P supplies (Table 4.1). At severe deficient P supplies, safflower taken up extremely low portion of external P compared to sunflower. The uptake efficiency decreased with increasing P supply in both species except in safflower in 2004 where P supply was extremely deficient and the uptake efficiency of this crop was extremely reduced.

In agreement with our findings, even with optimal placement, P uptake efficiency in safflower is generally less than 35% of added P (Kaffka and Kearney, 1998). It was observed that, most plants grow satisfactorily under variable conditions of soil nutrient supply and with wide ranges in nutrient concentrations within their tissues by inducing nutrient uptake capacity through modifications of uptake mechanisms as general response to nutrient stress (Läuchli 1976), and differences among cultivars in P uptake at low P supply (Steffens, 1984) can be explained, to a large degree, by differences in root growth (Sattelmacher et al., 1994). Unlike N, P is immobile in soil (Barber, 1984), therefore different P accumulation performance of different cultivars or species can be attributed to roots which allow them to explore the soil mass and present a large surface area appear to be important for absorption of P (Nye et al., 1965).

It is widely believed that the efficiency of the uptake system is of minor importance for P acquisition from soils because transport of P to the root surface rather than the uptake is the limiting step (Barber, 1984). Selection for an efficient P uptake system thus appears not very promising. Access particularly seems to be a problem in relation to P in deficient soils; diffusion and mass flow at times do not bring adequate P to root surfaces to meet plant needs. Under these conditions, plants undoubtedly benefit from the increased exposure to P provided by a rapidly developing and finely divided root system (Gerloff and Gabelman, 1983). It was found that, plant roots have the potential to take up phosphate faster than it can be supplied to the root by the soil solution (Klimashevsky, 1990) because the root surface supply of nutrients with low mobility in the soil, such as P, is mainly limited by solubilization / desorption from less available nutrient source (Sattelmacher et al., 1994). It was reported that differences in shoot dry matter production were related to differences in abilities to acquire P than differences in internal P utilization in tomatoes (Coltman et al., 1987) and maize (Furlani et al., 1984) grown in low-P stress conditions, and finally, Brück et al. (1992) observed that, at low P levels, the variation in phosphate efficiency was due to differences in uptake efficiency, whereas under higher P levels uptake and utilization efficiency became equally important.

### 3.2. Effect of P supply on P utilization efficiencies

To characterize different genera, species or genotypes for nutrient use efficiency (NUE), researchers include many criteria such as; presence or absence of deficiency symptoms (Diers and Fehr, 1989; Jensen et al., 1986), absolute growth at a limiting nutrient level (Chisholm and Blair, 1988; Schettini et al., 1987), relative growth obtained by comparing growth at limiting and adequate nutrient levels (Coltman et al., 1985; Gerloff, 1987), efficiency ratio or amount of biomass or yield produced per unit of nutrient present in the tissues (Coltman et al., 1985; Woodend, 1986), and utilization coefficient as the inverse of the whole plant nutrient concentration, the latter being expressed on a dry matter basis (Steenbjerg and Jakobsen, 1963; Lonergan and Asher, 1967). The utilization index (Siddiqi and Glass, 1981; Woodend, 1986), which is defined as biomass per unit of tissue nutrient concentration, was proposed by Siddiqi and Glass (1981) as an improved measure which, unlike the efficiency ratio, takes differences in amount of biomass produced into consideration. Agronomic efficiency; is the plant shoots, or harvestable product produced per unit of nutrient applied (Blair and Cordero, 1978; Moll et al., 1982). While such measures are relatively easy to obtain, they may not reflect, or be correlated with economic NUE. Despite the considerable differences in the utilization of absorbed nutrients, there is as yet little understanding of the underlying cause of these differences (Gourley et al., 1994; Svečnjak and Rengel, 2006).

#### 3.2.1. Effect of P supply on agronomic efficiency

An agronomic definition of nutrient efficiency (NE) relates plant productivity to nutrient supply (Sauerbeck and Helal, 1990; Moll et al., 1982). Nutrient efficiency has also been calculated on the basis of the amount of nutrient available (Gerloff and Gabelman, 1983), and the term 'external nutrient requirement' refers to the amount of nutrient in the media required to produce a given percentage of maximum yield (Föhse et al., 1988; Fox, 1981). According to all these interpretations for NUE we adopted a calculation that defines the required external P quantity (g) to produce 1 kg of achenes. Comparing the two species at their individual optimal supplies (1g, 0.5g P per pot for safflower and sunflower respectively), it was found that, safflower required 30 g P per pot to produce 1 Kg achenes, while sunflower required 6 g P per pot only (Table 4.2). This indicates the high P requirement in safflower compared to sunflower at optimal growth, and these requirements still less in sunflower than safflower under low and high P supplies. Safflower requirement of P supplies increase under low and high P levels compared to that at its optimal P supply to

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produce a fixed achene yield, while the requirement of sunflower to produce the same amount of achene decreased with decreasing P supply indicating that sunflower is more efficient in utilizing external P than safflower at suboptimal, optimal, and high P supplies to express achene yield.

**Table 4.2:** Effect of P supply on the external P requirement, harvest index and on the P utilization index of safflower and sunflower<sup>1)</sup>.

P supply (g/pot)	Safflower		Sunflower	
	2004	2005	2004	2005
External P requirement for achene production (g P applied (kg achenes) <sup>-1</sup> )				
0.04	52.0 ± 8.0 A *	-	3.0 ± 0.4 D	-
0.08	62.0 ± 11.0 A *	-	5.0 ± 0.5 C	-
0.24	35.0 ± 6.0 B *	-	7.0 ± 0.6 B	-
0.25	-	12.0 ± 1.0 C*	-	4.0 ± 0.2 D
0.50	-	27.0 ± 4.0 B*	-	6.0 ± 0.2 C
0.72	24.0 ± 3.0 C <sup>n.s</sup>	-	18.0 ± 4.0 A	-
1.00	-	30.0 ± 2.0 B*	-	12.0 ± 0.7 B
2.00	-	60.0 ± 8.0 A*	-	25.0 ± 2.0 A
Harvest index (g achene (g DM) <sup>-1</sup> )				
0.04	16.0 ± 1.7 B*	-	25.0 ± 2.5 B	-
0.08	18.7 ± 1.6 B*	-	27.4 ± 2.2 B	-
0.24	32.8 ± 2.4 A	-	30.8 ± 1.5 A	-
0.25	-	37.1 ± 3.1 A*	-	40.0 ± 1.0 A
0.50	-	31.4 ± 1.0 A*	-	41.3 ± 1.0 A
0.72	35.3 ± 2.4 A*	-	29.8 ± 1.2 A	-
1.00	-	36.5 ± 2.9 A*	-	41.2 ± 0.6 A
2.00	-	35.2 ± 1.5 A	-	40.0 ± 0.6 A
P utilization index for achene production (g achene / (g P (g DM) <sup>-1</sup> ))				
0.04	14.3 ± 4.6 C*	-	87.0 ± 3.5 D	-
0.08	19.5 ± 1.7 C*	-	112.1 ± 23.9 C	-
0.24	56.6 ± 9.2 B*	-	380.1 ± 33.3 A	-
0.25	-	69.4 ± 8.5 A*	-	509.2 ± 34.7 A
0.50	-	71.0 ± 24.4 A*	-	503.5 ± 32.8 A
0.72	108.3 ± 15.3 A*	-	208.0 ± 3.5 B	-
1.00	-	99.9 ± 14.0 A*	-	294.1 ± 6.0 B
2.00	-	78.8 ± 17.0 A*	-	170.5 ± 22.8 C

<sup>1)</sup> Figures within each column followed by the same letter are not significantly different,  $p < 0.05$ ,  $n = 4$ . \* Within the two plant species in the same parameter means significantly different,  $p < 0.05$ .

### 3.2.2. Effect of increasing P supply on P utilization efficiency

Many investigators widely use the calculated efficiency ratio for the comparisons of efficiency under moderate to severe nutrient deficiency stress (Gerloff and Gabelman, 1983),

which is a valuable parameter in differentiating plants into efficient and inefficient utilizers of the absorbed nutrients (Baligar et al., 1990), and has been used extensively to describe internal nutrient requirements in many agronomic species (Godwin and Blair, 1991; Gourley et al., 1994). As a convenient mean of expressing efficiency in element utilization we adopted calculation of nutrient efficiency ratios; the g of plant dry matter, achene, or oil produced per g element absorbed by plant species which facilitates comparisons of efficiencies of nutrient utilization between the two crops.

In both years, sunflower was found to be more efficient in utilizing absorbed P (in term of ER) than safflower at optimal and moderate P deficiency supplies for the production of all yield parameters (DM, achenes, oil) (Table 4.4). Phosphorus ER in safflower declined with increasing P supply in term of dry matter, achenes and to less extent oil yield indicating a decline in the internal utilization of P, while sunflower showed higher P ER at its optimal supply than at low and high levels. Under severe P deficiency (in year 2004), the very low P content ( $\text{mg P pot}^{-1}$ ) in safflower tissues lead to high value of ER and reflected that safflower is more efficient than sunflower to utilize absorbed P at the extreme low P availability to produce DM and achenes yield, but this response was found to be as an effect of dilution of P in plant tissue not as utilization efficiency itself when expressed in terms of utilization index. Therefore, to avoid the interpretation of the dilution effect under low P supply as utilization efficiency; utilization index was calculated (Siddiqi and Glass, 1981) which takes the concentration of P in the tissue (a function of biomass produced and total P accumulated) in consideration. In terms of utilization index (Table 4.2), sunflower was found much more efficient in utilizing internal P to produce achenes yield at all equivalent P levels including their optimal supply in both years and proved that the very high values of the efficiency ratio in safflower under extreme P deficient levels are not more than an effect of dilution of the very little absorbed P amount in the DM produced without any meaning as utilization efficiency compared to sunflower at that levels.

In terms of harvest index (HI) which indicates the partitioning of assimilates to economic sinks and also attributed to nutrient efficiency (Sattelmacher et al., 1994, Svecnjak and Rengel, 2006), sunflower out-yielded safflower at low P (in year 2004) and high P supplies (in year 2005) including their optimum P levels (Table 4.2). It can be observed that HI of both species is reduced only under very low P supplies, but safflower HI was highly reduced under severe P deficiency, while that of sunflower was less sensitive.

It was reported by Silver (1994) that P utilization efficiency of sunflower decreased with increasing P supply and higher P efficiency ratios are found at lower levels of applied

phosphorus. But this high efficiency ratios obtained at very low P could be as a reason of dilution effect because P concentration in the biomass tends to decrease and, therefore, this ratio express a dilution effect of P rather than an actual efficiency in P utilization when P is very limiting (Israel and Rufty, 1988), in the same line with what we found in safflower at severe P deficiency. It was stated that a nutrient is needed at a threshold concentration to reach maximum growth (Israel and Rufty, 1988); not threshold quantity, therefore, the utilization index is considered as a more reliable estimate of P use efficiency (Siddiqui and Glass, 1981).

**Table 4.3:** Effect of increasing P supply on Phosphorous Efficiency Ratios (PER) for the production of dry matter, achenes, and oil (g DM, achenes, oil g<sup>-1</sup> P) in 2004 and 2005<sup>1)</sup>.

P supply (g/pot)	PER dry matter		PER achenes		PER oil	
	Safflower	Sunflower	Safflower	Sunflower	Safflower	Sunflower
2004						
0.04	1826±457 A*	629±162 B	288±53 A*	154±31 B	62±11 AB	59±16.4 B
0.08	1524±354 B*	653±107 B	284±59 A*	175±18 B	62±13 A	67±14 B
0.24	795±64 C*	1057±76 A	260±13 A*	325±23 A	57±4 A*	135±8 A
0.72	355±16.8 D*	520±51 B	127±9.5 B	150±18.2 B	29±3.6 B*	60±8.6 B
2005						
0.25	328±45 A*	724±25.8 A	121±14 A*	290±13.1 A	23.1±2.2 A*	111±13.1 A
0.50	308±5.2 BA*	634±39.3 B	90±12.3 BA*	255±11.5 B	17±3.6 A*	100±4.1 A
1.00	272±12.6 BA*	366±8.5 C	96±5.2 BA*	150±2.1 C	20±2.9 A*	57±2.1 B
2.00	232±27.2 B	210±19.4 D	81±12.1 B	84±10.5 D	17±3.5 A*	31±4.2 C

<sup>1)</sup> Figures within each column followed by the same letter are not significantly different, p<0.05, n=4. \* Within the two plant species in the same parameter means significantly different, p<0.05.

The term ‘nutrient efficiency’, in some cases may be misleading in the quest for increased productivity and identification of mechanisms for enhanced nutrient acquisition and utilization (Sattelmacher et al., 1994; Gourley et al., 1994). As suggested by Gerloff and Gabelman, (1983); a genotype to be regarded as inefficient under stress should appear normal, and yield approximately the same as efficient genotypes under optimum supplies of the element. Also, it would be desirable to have a cultivar that grows well at low P levels and also responds to optimum levels of P (Buso and Bliss, 1988). As example, the grain yield of a modern wheat cultivar (Cosir) was higher at limiting and non-limiting P supply than a

traditional one (Peragis), therefore this cultivar can be considered as more P-efficient than the traditional cultivar (Horst et al., 1993), but also the best performing genotypes under high fertilizer supply do not necessarily perform best under conditions, where yields are limited by suboptimal uptake and utilization of nutrients (Sattelmacher et al., 1994).

The main factors contributing to the higher efficiency of modern cultivar are (i) efficient use of assimilates from root growth characteristics which enhance P acquisition (ii) efficient remobilisation of P from vegetative plant organs to grains, and (iii) lower P requirement for grain yield formation (Sattelmacher et al., 1994). Substantial genetic variation, however, has been reported in P utilization efficiency (Coltman et al., 1985); whether these variations relate to differences in P assimilation and / or compartmentation on the cellular level is not known (Sattelmacher et al., 1994). The intracellular acid phosphatases, present in the cytosol, plastids and vacuoles, are responsible for the P-hydrolysis from organic compounds, favouring P mobilization and translocation from senescent tissues (Duff et al., 1994). It has been suggested that lower acid P-ase activities in root or leaf would be related to adequate or sufficient P-tissue concentrations, even under low external P (Helal, 1990; Tadano et al., 1993). Such low P-ase activity in plants would be potentially adapted to low-P environments, which might be explained by a lower plant demand for P and a feedback regulation effect on enzyme activity (Mc Lachlan and De Marco, 1982); therefore, plants adapted to lower external P concentrations would have tissues adequately supplied in P and the lower P demand would inhibit P-ase activity. But since an extremely rapid turnover of the most important P fractions is a typical feature of P metabolism, the role of P assimilation in genetic variation of P utilization efficiency can be questioned (Sattelmacher et al., 1994). The failure of a particular cultivar to release  $P_i$ , or another element, as nutrient stress developed following a period of adequate supply, could result in an evaluation as inefficient in the utilization of that element and could be related to genotypic variation in P utilization efficiency (Gerloff and Gabelman, 1983; Bielecki, 1973). So, the prospects of increasing nutrient utilization efficiency in P through better utilization on the cellular level are considered to be rather poor (Sattelmacher et al., 1994), but can be improved through screening methods and breeding program between P deficiency tolerant traits (Da Silva et al., 1992).

### 4. Conclusion

It can be concluded that safflower has a high requirement for P with respect to growth and yield; Safflower is less P-efficient than sunflower in terms of uptake and utilization of P at

optimal and sub-optimal P supplies indicating that safflower failed to be a low nutrient input crop in terms of phosphorus compared to sunflower.

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## Chapter 5

### Phosphorus Use Efficiency of Safflower (*Carthamus tinctorius* L.) and Sunflower (*Helianthus annuus* L.) studied in nutrient solution

#### Summary

The oil crop safflower may have a certain production potential under low-input conditions (organic farming, developing countries), where the putatively low nutrient requirement is highly welcomed. However, current knowledge regarding the nutrient use efficiency of safflower as compared to similar oil crops is limited, and since a two-year pot experiment using soil mixture (chapter 3 and 4 in this dissertation) shows the high P requirement and low P UE of safflower compared to sunflower. Therefore, the aim of this study is to directly compare the P use efficiency of safflower (*Carthamus tinctorius* L.) as compared to sunflower (*Helianthus annuus* L.) in nutrient solution with increasing [P] (mM) supply under controlled conditions.

Total P supply ( $\text{mg P pot}^{-1}$ ) was different between species at equivalent [P] (mM), because P was added to each crop according to nitrate test for each crop separately (depletion of nitrate in the highest P level) to make sure that there is no other limiting nutrient rather than P and to follow the demand of each species independently on the another. Accordingly, response curves were applied using linear regression and Michaelis-Menten (MM) equations. Both species responded strongly to increasing P supply with respect to growth. The higher  $A_{\text{max}}$ , and  $K_m$  in sunflower response curves in terms of P accumulation (P accumulated ( $\text{mg P in DM pot}^{-1}$ ) based on P supply ( $\text{mg P pot}^{-1}$ )) compared to safflower, could be interpreted as, sunflower received more P than safflower at equivalent [P], and produced more DM hence accumulated more P in tissues not surely an efficiency of P uptake. Sunflower recovers more proportion of added P than safflower at low, optimal and high P supplies. Agronomic efficiency interpreted as g P required to produce a fixed amount of DM was higher in safflower than sunflower indicating the superiority of sunflower in terms of the efficiency to use external P supply to produce DM compared to safflower. The efficiency of a crop for dry matter production based on accumulated P ( $\text{mg P pot}^{-1}$ ) (efficiency ratio), and P concentration in DM ( $\text{mg P (g DM)}^{-1}$ ) (utilization index) may be interpreted in terms of Michaelis-Menten (MM) kinetics, specifically addressing the shape of the nutrient response curve.  $K_m$  constant in Michaelis-Menten equation was found to be lower in sunflower compared to safflower in term of utilization index, but both showed no significant difference in term of

efficiency ratio. Low  $K_m$  constant in terms of efficiency ratio indicates the low amount of accumulated P to produce 50% of potential maximum DM production consequently high efficient crop. In term of utilization index, low  $K_m$  constant indicates the less P concentration in tissues to produce half potential maximum DM production consequently high efficient crop. In accordance to the results of this experiment and the two year experiment conducted in soil, it can be concluded that safflower has a high requirement for P with respect to growth and yield; sunflower is more efficient than safflower in term of uptake and utilization of P at optimal and sub-optimal P supplies indicating that safflower cannot be considered a low nutrient input crop with respect to phosphorus.

### 1. Introduction

Although many soils have large reserves of total P, only a small fraction is immediately available making many agricultural areas P deficient (Sanchez and Salinas, 1981). The application of fertiliser P represents an important measure to correct nutrient deficiencies and to replace elements that have been removed in the products harvested (Dambroth and El Bassam, 1990). In developing countries, where the proportion of less fertile soils is particularly high, it may be difficult to fulfil the nutritional requirements of high-yielding crops (Sauerbeck and Helal, 1990; Marschner, 1995). However, due to chemical immobilization in the soil (Sample et al., 1980) recovery of fertiliser P is very low (Craswell and Godwin, 1984; Roy et al., 2006), causing serious ecological and economical consequences of contaminating the environment (EEA, 1999, EEA, 2001, Chardon and Withers, 2003). It is thus desirable to aim for efficient use of P, both in view of resource limitations and environmental constraints, through the identification of crops species or cultivars with greater tolerance to suboptimal P availability to increase the production potential on marginal lands (Baligar et al., 1990; Gourley et al., 1994).

The ability of cultivars to tolerate low P may be due to either high P absorption ability at low P concentrations and/or more efficient use of P for more yield production (Blair, 1993, Sattelmacher 1994). Efficient cultivars are of great importance to enable farmers to achieve reasonable yields with minimum input of P. However, cultivating P-efficient species or cultivars to improve yields or developing genotypes that are more P-efficient may be possible if phosphorus efficiency mechanisms are elucidated (Lynch and Beebe, 1995; Buso and Bliss, 1988). Overall nutrient use efficiency (NUE) in plant is a function of capacity of soils to supply adequate levels of nutrients, and the ability of plant to acquire nutrients, transport them in roots and shoot and to remobilise them to other parts of the plant, involving various

soil and plant mechanisms and processes that contribute to genetic variability in efficiency of uptake and utilization of nutrients (Baligar et al., 2001).

Safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.), both belonging to the *Asteraceae*, are important oil crops in tropical areas. Safflower is a highly branched, herbaceous, thistle-like annual, 30-150 cm tall with globular flower heads (capitula), characterised by a strong taproot, which enable it to thrive in dry climates and can access and utilize nutrients from below the root zone of cereal crops (Li and Mündel, 1997). The oil crop sunflower, however, is much taller, usually un-branched, lacks a taproot, and is considered more demanding in terms of nutrients and water (Diepenbrock and Pasda, 1995). Although both crops thrive in similar environments, direct comparisons of their response to increasing P availability with respect to P use efficiencies are not available, and since a two-year pot experiment using soil mixture (chapter 3 and 4 in this dissertation) shows the high P requirement and low P UE of safflower compared to sunflower. Therefore, the aim of this study is to directly compare the P use efficiency of safflower as compared to sunflower in nutrient solution under controlled conditions.

## 2. Material and methods

### 2.1 Experimental conditions

An experiment using safflower (*Carthamus tinctorius* L., variety 'Sabina') and sunflower (*Helianthus annuus* L., variety 'Salut R M') was carried out in the period from May to August 2006 in a greenhouse in which the day night temperature was adjusted to 28°C, and 15°C, respectively, with additional lightening (intensity at canopy level equals to 260  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Young plants of safflower (*Carthamus tinctorius* L. variety Sabina) and sunflower (*Helianthus annuus* L. variety Salut R M') were grown in aerated nutrient solution and randomised completely. Five phosphorus levels (in  $\text{KH}_2\text{PO}_4$  form) were used for both species (0.05, 0.1, 0.2, 0.4, 0.8 mM) in 5 L plastic pot in eight replicate for each treatment. Other nutrients added were 5.0, 4.0, 1.0, 0.7, 0.5 mM N, K, Ca, Mg, and Fe respectively in the following chemical forms:  $\text{K}_2\text{SO}_4$ , KCl,  $\text{KNO}_3$ ,  $\text{Ca}(\text{NO}_3)_2 \times 4 \text{H}_2\text{O}$ ,  $\text{NH}_4\text{NO}_3$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , Fe-NaEDTA. Micronutrients were added in adequate amounts ( $\mu\text{M}$ ): 2.97  $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$ , 1.24  $\text{ZnCl}_2$ , 0.66  $\text{CuCl}_2 \times 2 \text{H}_2\text{O}$ , 24.75  $\text{H}_3\text{BO}_3$ , 0.083  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \times 4 \text{H}_2\text{O}$ , and 0.0413  $\text{NiCl}_2$ .

Achenes were germinated between moist paper tissues and the roots of three-days-old, germinated achenes were passed through wholes of Styrofoam plates floating on aerated 0.2 mM  $\text{CaSO}_4$  solution. Two seven-days-old uniform-sized seedlings (roots become around

five centimetres long and the first germination leaves arise) were transferred to each 5 L pot provided with lids, containing half the concentrations of nutrients solution for each P treatment. Nutrient solutions were constantly aerated, and the initial solution pH was 5.8, which was monitored during the first two weeks of the experiment every other day. Abundance of nutrients was checked every other day using nitrate strips (nitrate as indicator); analytical test strips range from 10-500 mg/L  $\text{NO}_3^-$  (Merck KGaA, Darmstadt, Germany) to ensure that the nutrients are not depleted by the growing plants. When the nitrate in the highest P level treatments is less than 10 mg/L (the two cultivars were checked separately), new nutrient solutions substituted the old ones in all pots for the same species. The volume of remaining nutrient solution before changing to new one and after harvest was measured and a sample from each pot was taken to be analysed for P. After 50 percent flowering, the plants received all nutrient without P, and new nutrient solutions (-P) were added when nitrate was depleted after nitrate test for each cultivar independent of the other species. The total P supply (mg P pot<sup>-1</sup>) for each treatment was calculated from the number of times where new nutrient solution was added and the P content (mg) of the added nutrient solution for each pot individually. The remaining P in the nutrient solution after renewing the nutrient solutions was calculated by multiplying the volume of the remained nutrient solution by the P concentration in the sample taken from that solution for each pot individually. Aphids were controlled with regular pesticide applications of Metasystox<sup>®</sup> (S- [2-Ethylsulfinyl] ethyl] O, O-dimethyl phosphorothionate), and infestations with *Perenospora* sp. were controlled by application of Amistar<sup>®</sup> (azoxystrobin) according to manufacturers recommendation.

### 2.2. Harvesting and analytical procedures

Growth parameters were monitored along the growing period. A young mature blade (YMB) of each plant was taken for leaf area measurement using leaf area scanner. Plants were harvested in two growth stages (anthesis and maturity), four replicates of each P treatment for each species were harvested at 50 percent flowering stage (end of June), and the other four replicates were let to mature (end of July). Each pot was harvested individually when it reached the stage of maturity. Plants were separated into capitula, leaves, stems, roots. Leaves and stems were separated to upper and lower parts by cutting the stem into two equal parts in length in both harvest stages, achenes were also separated from the mature plants. All plant parts were dried (except achenes that were dried at room temperature in a well-aerated area) at 70°C until constant weight in a drying oven, grinded to pass a 1.5 mm sieve, of which, after thorough mixing, a sub-sample of 5 g was ball-milled to a fine powder. The

samples were prepared for P analysis using dry ashing method (Dechassa, 2001), in which 50 mg of dried sample was ashed in a crucible at 450°C in a muffle furnace overnight. Then 1 ml of 0.35 M HNO<sub>3</sub> solution was added, and after swirling left for at least 10 minutes. After addition of 9 ml of purified water (18.2 MΩ cm<sup>-1</sup>), the sample is filtered through ashless filter paper (blue band, Whatman®, Schleicher und Schüll, Whatman International Ltd, England) into polypropylene tubes. Total P of the plant material was measured using colorimetric method (Ammonium-Vanadate-Molybdate) according to Gericke and Kurmies, 1952, and in the remaining nutrient solutions using the colorimetric method according to Schüller, 1969.

### 2.3. Statistics and yield component analysis

All statistical analysis was carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means with respect to the influence of P supply were carried out using the GLM procedure considering a fully randomised design. Where appropriate, data were log transformed to maintain homogeneity of variance. The Bonferoni procedure was employed with multiple t-tests in order to maintain an experimentwise  $\alpha$  of 5%.

Response curves were derived from the relationship between each parameter tested (e.g. g DM pot<sup>-1</sup>) on the y-axis and the amount of P accumulated in the plants, P supply, or P concentration in DM (e.g. for P accumulated in plants; mg P total plant<sup>-1</sup> pot<sup>-1</sup>) using the following Michaelis-Menten-type equation

$$\text{e.g.: Yield parameter} = (A_{\text{max}} \times (\text{mg P})) / (c + (\text{mg P}))$$

with 'A<sub>max</sub>' as an estimate of maximum yield, and 'c' as the P accumulation or P concentration in DM required for half maximum yield, corresponding to the K<sub>m</sub> in Michaelis-Menten kinetics. The Michaelis-Menten equation proved superior to the Mitscherlich curve and has been widely used to describe nutrient efficiency (Gourley et al., 1994, Steingrobe and Claassen, 2000). Curve fitting was carried out using the procedure NLIN in SAS, employing the Gauss-Newton algorithm. The Michaelis-Menten equation was applied to compare both species in terms of accumulation efficiency (mg P accumulated pot<sup>-1</sup>), and DM response curves based on accumulated P and P concentration in DM.

Linear regression was used to compare the linear relations between P supply and some parameters (external P requirement, P recovery, P concentration in DM) using the procedure 'mixed' in SAS program. Significant difference was based on the 95% confidence limit for the 'a'; slope and 'b'; the Y-intercept of the linear equations of the two species.

NUE may be broken down into its components and expressed in a multiplicative fashion as: Nutrient use efficiency [ $\text{g DM (g P supply)}^{-1}$ ] = P uptake efficiency ( $(\text{mg P accumulated. (g P supply)}^{-1})$ )  $\times$  P utilization efficiency ( $(\text{g DM produced (mg P accumulated)}^{-1})$ ). In order to quantify the impact of individual NUE components (uptake and utilization efficiencies) multiple regression analysis is biased as the mathematical product, rather than a statistical relationship, of ‘Uptake efficiency’ and ‘Utilization efficiency’ result in NUE. Hence, a component analysis according to Piepho (1995) was employed, allowing the contribution of individual components of NUE to be quantified. This approach assumes that the SD of log-transformed yield is close to the coefficient of variance of the yield, uses the log-transformed component data and interprets values of  $C_i = \text{Cov} [\log (\text{NUE}), \log (\text{component}_i)]$  as an aggregate measure of the  $i^{\text{th}}$  component’s contribution to the variability in yield.

### 3. Results

#### 3.1 Growth parameters

Both crops responded strongly to increasing P supply with respect to growth. Growth and achene yield of safflower increased up to 185.8 mg P per pot (0.2 mM [P])<sup>\*</sup>, and sunflower’s optimal growth (DM production) was achieved at 532.7 mg P pot<sup>-1</sup> (0.2 mM [P]) but the sunflower plants didn’t gave consistent achene yield as a response of increasing P supply because problems of pollination and the presence of very high percent of hollow achenes.

Leaf area and stem diameter were increased as solution [P] increased (Table 5.1). Plant height of both species was affected in deficient solution [P] but was more pronounced in sunflower because safflower is a highly branching plant and the number of branches was highly affected in deficient solution [P]. Deficient solution [P] reduced the number of leaves in both species as a result of reducing new leaf formation in the upper half of the plant while the number of leaves in the lower half was not affected in both species. The secondary branches of safflower were totally inhibited under severe deficit solution [P], and also the number of primary branches was decreased under inadequate solution [P]. The number of capitula per plant in safflower was increased with increasing external solution [P] (Table 5.2).

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\* [P] indicates P concentration in the nutrient solution.

**Chapter 5 Phosphorus use efficiency in nutrient solution**

**Table 5.1:** Effect of P supply on growth parameters of safflower and sunflower<sup>1)</sup>.

P supply (mM)	P supply (mg pot <sup>-1</sup> )	Leaf area (cm <sup>2</sup> )	Number of leaves plant <sup>-1</sup>			Plant height (cm)	Stem diameter (mm)
			upper	lower	total		
Safflower							
0.05	46.5	20.8±3.4 B	26.8±2.6 C	13.0±0.8 A	39.8±2.2 C	73.0±5.0 B	5.5±0.4 C
0.1	92.9	32.3±4.2 A	67.0±7.1 B	13.0±0.8 A	79.5±6.9 B	83.8±3.9 A	7.4±0.2 B
0.2	185.8	38.4±2.0 A	87.5±9.9 A	11.0±1.6 A	98.5±11.2 A	86.0±3.6 A	8.3±0.4 A
0.4	371.6	37.4±4.8 A	78.0±5.9 BA	10.8±1.0 A	88.5±6.8 BA	87.8±2.1 A	8.1±0.2 A
0.8	743.3	36.9±2.3 A	84.8±3.2 A	12.0±1.4 A	96.8±3.9 A	86.0±3.7 A	8.7±0.3 A
Sunflower							
0.05	133.2	110.9±8.1 D	16.0±0.8 B	12.0±0.8 A	27.8±1.3 C	163.8±10.3 C	15.2±0.7 C
0.1	266.3	167.0±13.9 C	17.3±1.0 B	13.0±1.4 A	30.0±1.6 BC	188.8±8.5 B	18.9±1.1 B
0.2	532.7	264.5±5.0 A	19.8±1.0 A	11.5±1.0 A	31.0±0.8 BA	218.8±8.5 A	20.8±0.8 BA
0.4	1065.4	231.5±14.3 B	22.0±0.8 A	11.8±1.2 A	33.8±1.0 BA	212.5±2.9 A	22.4±1.8 A
0.8	2130.7	209.6±5.9 B	20.3±1.3 A	11.8±1.7 A	32.0±1.4 A	208.8±2.5 A	23.1±0.9 A

<sup>1)</sup>Figure within each column followed by the same letter are not significantly different ( $p < 0.05$ ,  $n = 4$ ), values are means ± SD.

**Table 5.2:** Effect of P supply on number of branches and capitula of safflower<sup>1)</sup>.

P supply (mM)	Primary	Secondary	Total	Capitula plant <sup>-1</sup>
0.05	5.7±0.3 C	0.3±0.5 C	5.9±0.3 C	6.8±0.5 C
0.1	6.5±0.4 BC	6.5±1.2 B	13.0±0.9 B	14.8±1.0 B
0.2	7.5±0.6 BA	6.6±1.0 B	14.1±1.1 B	15.0±0.8 BA
0.4	7.5±0.2 BA	6.4±0.9 B	13.9±0.9 B	14.0±0.8 BA
0.8	7.8±0.6 A	9.3±0.7 A	17.1±1.1 A	16.3±0.5 A

<sup>1)</sup>Figure within each column followed by the same letter are not significantly different ( $p < 0.05$ ,  $n = 4$ ), values are means  $\pm$  SD.

Total dry matter of both species in both harvesting times was improved with increasing external solution [P] (Tables 3, 4, and 5). The dry matters of both upper and lower leaves of both plants were improved, taking in consideration that the number of leaves of the upper half in both species (measured at anthesis) was increased with increasing solution [P] which can be the cause for the improved total dry matter of this part of the plants, but this parameter (number of leaves) was not affected by increasing solution [P] in the lower part of both plants. The dry matter of both upper and lower parts of the stems of both species were positively influenced in the same manner, as external solution [P] increased resulting in an increment in the dry matter of stem of both plants. Dry matter of safflower capitula was increased with increasing solution [P] but that of sunflower was not affected. Root dry matter of both species was not affected with increasing P fertilizer in the solution.

## Chapter 5 Phosphorus use efficiency in nutrient solution

**Table 5.3:** Effect of P supply on growth parameters of safflower and sunflower at anthesis<sup>1)</sup>.

P (mM)	Leaf DM (g pot <sup>-1</sup> )			Stem DM (g pot <sup>-1</sup> )			Capitula DM (g pot <sup>-1</sup> )	Root DM (g pot <sup>-1</sup> )	TDM (g pot <sup>-1</sup> )
	upper	lower	total	upper	lower	total			
Safflower									
0.05	1.3±0.2 C	2.8±0.4 C	4.1±0.4 C	2.0±0.3 C	5.3±0.9 B	7.2±1.0 C	6.5±0.5 C	4.4±0.6 C	22.2±1.3 C
0.1	3.0±0.4 B	4.3±0.2 BA	7.4±0.4 B	5.1±0.7 B	9.5±0.5 A	14.6±1.2 B	11.7±1.5 B	6.6±0.8 BA	40.2±3.5 B
0.2	4.3±0.5 A	4.5±0.5 BA	8.8±0.9 BA	6.4±0.2 A	9.2±1.6 A	15.6±1.7 BA	12.7±2.8 B	5.4±0.6 BC	42.4±5. B7
0.4	3.8±0.4 BA	3.9±0.3 B	7.7±0.7 BA	6.3±0.4 A	8.8±1.0 A	15.1±0.8 BA	12.3±0.7 B	5.8±0.4 BC	40.9±1.9 B
0.8	4.1±0.2 A	5.0±0.8 A	9.1±0.9 A	6.7±0.1 A	11.2±1.6 A	17.9±1.7 A	17.1±1.9 A	7.7±0.3 A	51.7±4.6 A
Sunflower									
0.05	18.9±1.4 C	11.7±1.2 C	30.6±2.0 C	16.3±0.8 B	32.0±3.7 B	48.3±3.9 B	12.3±1.6 A	18.7±2.7 A	109.8±8.8 B
0.1	28.4±2.7 B	15.4±1.3 BC	43.7±3.5 B	26.0±3.3 A	50.0±2.5 A	76.0±4.8 A	13.6±1.9 A	16.3±3.5 A	149.7±12.1 A
0.2	31.2±3.0 B	17.1±2.2 B	48.3±4.0 B	22.5±3.2 A	57.5±5.0 A	80.0±6.8 A	11.0±1.2 A	16.3±4.1 A	155.6±13.6 A
0.4	42.3±1.1 A	19.5±2.2 BA	61.7±2.9 A	26.6±4.2 A	61.4±6.4 A	88.0±10.3 A	9.8±2.1 A	20.6±1.7 A	180.1±15.8 A
0.8	39.2±4.5 A	23.3±2.0 A	62.5±5.7 A	24.2±1.8 A	62.4±9.9 A	86.6±11.4 A	12.0±3.6 A	20.0±3.6 A	181.2±20.6 A

<sup>1)</sup> Figure within each column followed by the same letter are not significantly different ( $p < 0.05$ ,  $n = 4$ ), values are means  $\pm$  SD.

## Chapter 5 Phosphorus use efficiency in nutrient solution

**Table 5.4:** Effect of P supply on leaves and stem DM of safflower and sunflower at maturity<sup>1)</sup>.

P (mM)	Leaf DM (g pot <sup>-1</sup> )			Stem DM (g pot <sup>-1</sup> )		
	upper	lower	total	upper	lower	total
Safflower						
0.05	1.1±0.4 C	2.1±0.4 B	3.2±0.7 C	1.9±0.3 C	3.8±0.8 B	5.7±1.1 C
0.1	2.8±0.3 B	3.4±0.4 A	6.1±0.5 B	5.4±1.1 B	6.4±0.9 A	11.7±1.8 B
0.2	3.1±0.6 B	3.8±0.6 A	6.9±1.2 B	6.4±0.4 BA	7.7±0.7 A	14.1±1.0 BA
0.4	3.2±0.3 B	3.4±0.3 A	6.6±0.5 BA	5.4±0.5 B	6.8±0.5 A	12.2±1.0 B
0.8	4.7±0.6 A	4.1±0.7 A	8.8±1.1 A	7.7±0.5 A	7.6±0.7 A	15.3±1.1 A
Sunflower						
0.05	16.5±2.4 C	12.4±1.7 B	28.9±1.3 C	13.5±2.4 B	29.3±1.9 C	42.8±3.8 C
0.1	26.8±3.1 B	17.1±0.4 A	43.9±3.4 B	22.1±2.2 A	51.2±4.4 B	73.3±5.8 B
0.2	40.2±5.1 A	17.4±2.5 A	57.6±3.5 A	27.9±6.1 A	62.5±6.7 BA	90.4±9.5 BA
0.4	38.2±3.7 A	15.9±0.5 BA	54.0±3.9 A	26.3±2.9 A	61.5±3.3 BA	87.7±2.6 BA
0.8	39.9±3.1 A	19.0±2.7 A	58.9±1.9 A	28.3±3.2 A	71.0±9.6 A	99.3±11.5 A

**Table 5.5:** Effect of P supply on the dry matter (g pot<sup>-1</sup>) of Capitula, roots, achenes, and total plant of safflower and sunflower at maturity<sup>1)</sup>.

P (mM)	Capitula DM	Roots DM	Achene yield	TDM
Safflower				
0.05	5.8±0.8 C	3.7±0.7 C	9.9±1.6 C	28.2±4.0 D
0.1	12.7±1.7 B	4.7±0.5 CB	18.7±1.3 B	54.0±3.7 C
0.2	15.1±0.7 BA	5.0±0.4 B	24.5±2.4 A	65.6±4.4 B
0.4	12.8±1.1 B	5.3±0.3 B	23.0±0.5 A	59.9±2.5 BC
0.8	17.2±1.6 A	7.0±0.2 A	25.6±0.9 A	73.7±4.8 A
Sunflower				
0.05	17.2±2.0 B	15.2±1.3 B	28.2±3.4 A	132.3±8.0 C
0.1	23.3±3.9 BA	19.5±2.6 BA	20.9±3.6 A	180.8±6.9 B
0.2	22.2±4.1 BA	24.4±4.3 A	25.2±23.5 A	219.9±17.4 A
0.4	28.6±4.9 A	14.0±2.1 B	40.3±18.5 A	224.7±23.1 A
0.8	21.8±3.3 BA	18.9±2.7 BA	24.1±15.5 A	217.0±17.3 BA

<sup>1)</sup> In both tables (5.4, 5.5); figure within each column followed by the same letter are not significantly different ( $p < 0.05$ ,  $n = 4$ ), values are means ± SD.

### 3.2. Effect of P supply on $C_{\min}$ , P accumulation and P recovery

#### 3.2.1. Difference in $C_{\min}$ between the two species

The minimum concentration of P in the nutrient solution of which net influx of P in roots appears to be zero, and still remaining in the nutrient solution, although the plant is suffering from P deficiency ( $C_{\min}$ ) doesn't differ significantly between both species at two deficient P levels (0.05, and 0.1 mM P), measured at both anthesis and maturity (Table 5.6). Also there was no significant difference in  $C_{\min}$  in the same plant at both mentioned P deficient levels.

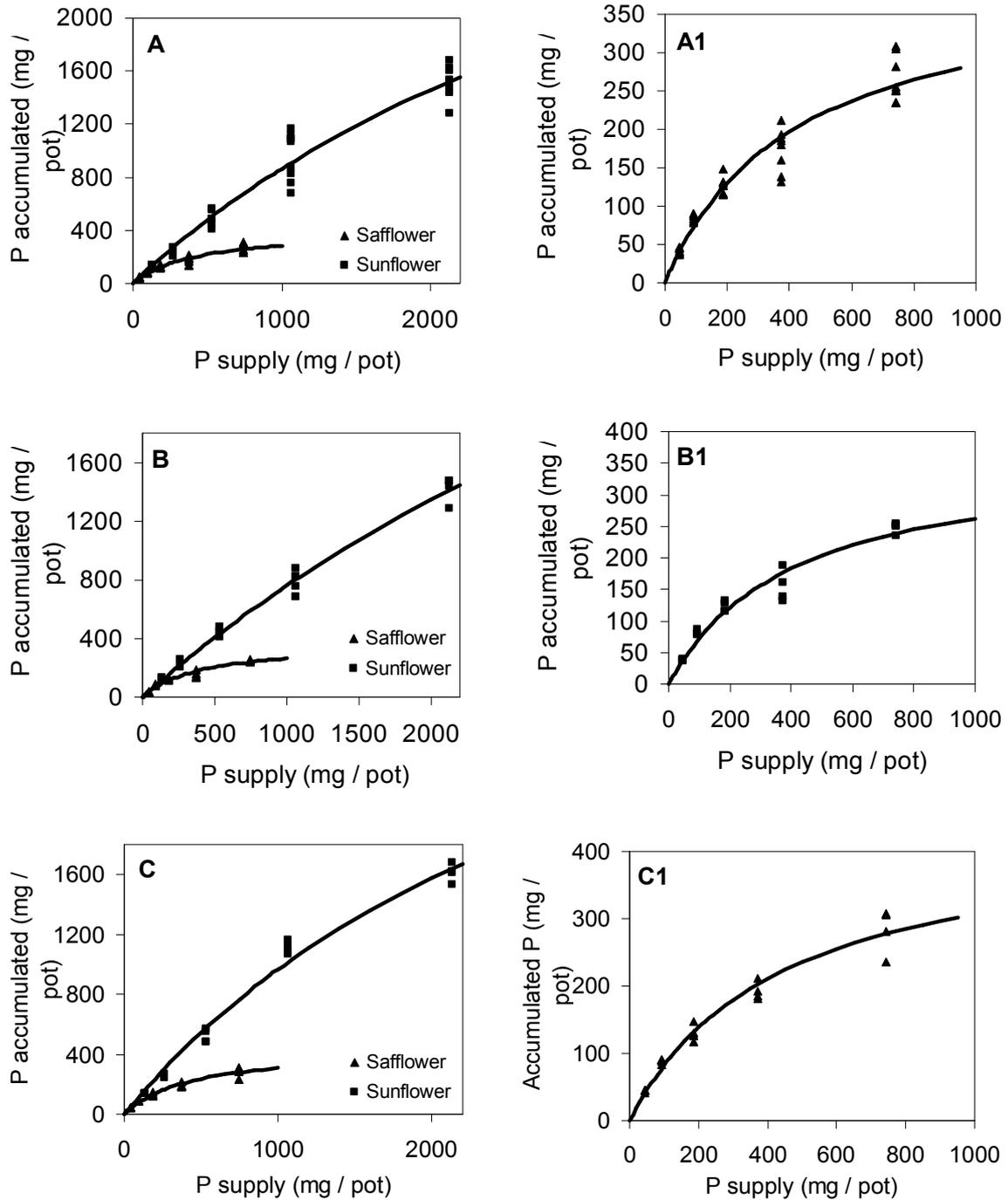
**Table 5.6:** Phosphorus  $C_{\min}$  (mg P L<sup>-1</sup>) values at two P deficient supplies in safflower compared to sunflower at anthesis and maturity<sup>1)</sup>.

P supply	Anthesis		Maturity	
	Safflower	Sunflower	Safflower	Sunflower
0.05	0.24 ± 0.14 A <sup>n.s</sup>	0.23 ± 0.06 A	0.43 ± 0.29 A <sup>n.s</sup>	0.37 ± 0.06 A
0.01	0.41 ± 0.21 A <sup>n.s</sup>	0.61 ± 0.32 A	0.36 ± 0.01 A <sup>n.s</sup>	0.39 ± 0.01 A

<sup>1)</sup> Figure within each column followed by the same letter are not significantly different, n.s represent not significant between two species at the same P level and the same experiment (anthesis or maturity), ( $p < 0.05$ ,  $n = 4$ ), values are means ± SD.

#### 3.2.2. Effect of P supply on P accumulation

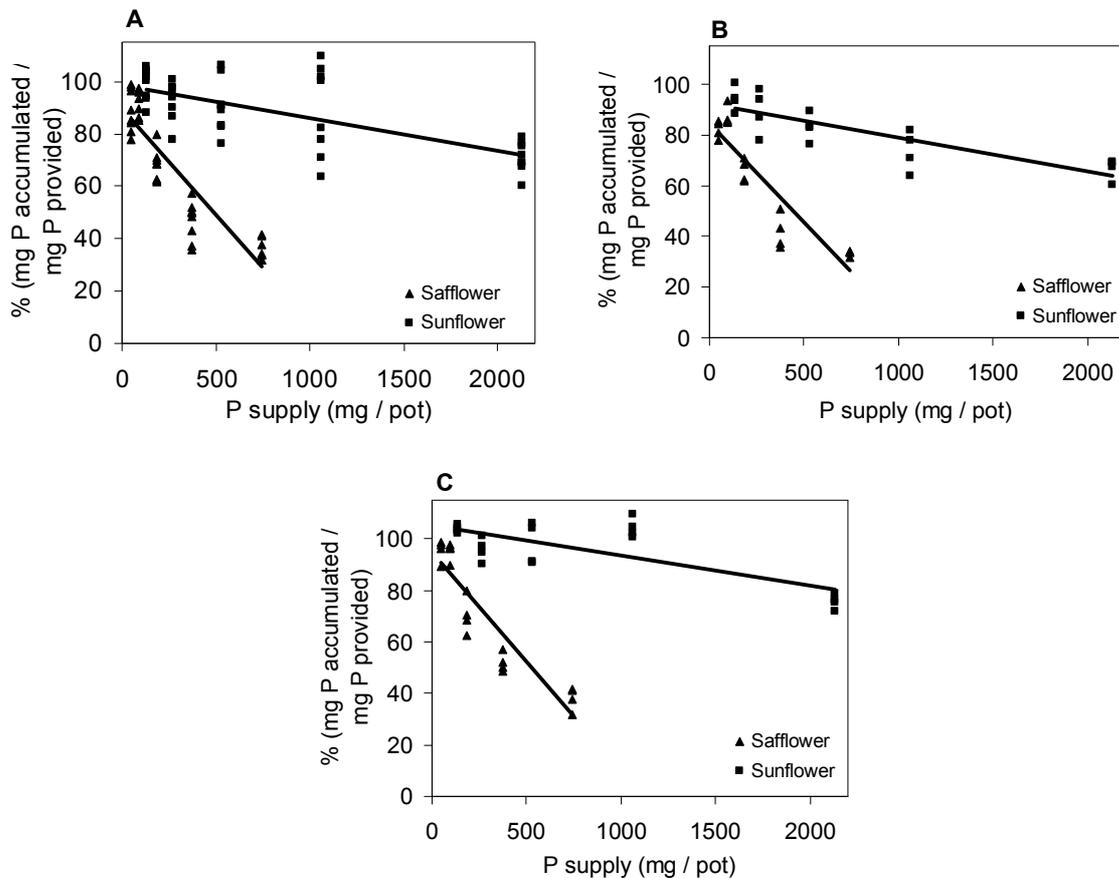
As the total P supplies (mg P pot<sup>-1</sup>) at equivalent [P] (mM P) are not the same for the two crops (Table 5.1), the comparison of means is not helpful. Therefore, the P accumulation of both species related to P supply was best fitted using Michaelis-Menten-type equations (Fig. 5.1). Although this type of equation is applied, less explanation can be given from the differences of the equation constant between the two plants as will be shown in the yield response curves later. Both species accumulated increasing P amounts in their shoots as P supply increased.  $A_{\max}$ , and  $K_m$  were significantly higher in sunflower compared to safflower at anthesis, maturity, and when both data are pooled.



**Figure 5.1:** P accumulation response curves for safflower and sunflower based on the P supply per pot. Michaelis-Menten-type equations are given as: Accumulated P = ( $A_{max} \times (\text{mg P supplied})$ ) / ( $c + (\text{mg P supplied})$ ), with ‘P’ representing the P supplied per pot, ‘c’ the  $K_m$ , ‘A’ the maximum P accumulation potential. [A] Accumulated P<sub>sunflower (anthesis and maturity)</sub> =  $(4638.6^* \times P) / (4359^* + P)$ , [A], [A1] Accumulated P<sub>safflower (anthesis and maturity)</sub> =  $(404.6 \times P) / (421.0 + P)$ , [B] Accumulated P<sub>sunflower (maturity)</sub> =  $(5767.4^* \times P) / (6582.3^* + P)$ , [B], [B1] Accumulated P<sub>safflower (maturity)</sub> =  $(368.3 \times P) / (405.0 + P)$ , [C] Accumulated P<sub>sunflower (anthesis)</sub> =  $(4262.5^* \times P) / (3410^* + P)$ , [C], [C1] Accumulated P<sub>safflower (maturity)</sub> =  $(440.1 \times P) / (434.4 + P)$ . \* indicates significant difference between the two species in the same constant ( $p < 0.05$ )

3.2.3. Effect of P supply on P recovery

The linear response curves relating P recovery in the plants to P supply for both species (Fig. 5.2) at each harvesting stage and when the data pooled showed that the two species are significantly different from each other in terms of the slope according to the 95% confidence limits. The linear curves of sunflower lay over that of safflower. The P recovery decrease with increasing P supply in both species, but the slope of this decrease is significantly less in sunflower than in safflower; which indicates that sunflower has advantage over safflower to recover added P.



**Figure 5.2:** P recovery response curves for safflower and sunflower based on the P supply per pot. Linear regressions are given. **[A]** Recovered P (%) sunflower (anthesis and maturity) =  $-0.0124^* \times P \text{ supply (mg pot}^{-1}) + 98.587^{n.s}$  ( $r^2 = 0.45^{***}$ ), Required P (g pot<sup>-1</sup>) safflower (anthesis and maturity) =  $-0.0811 \times P \text{ supply (mg pot}^{-1}) + 89.412$  ( $r^2 = 0.80^{***}$ ), **[B]** Recovered P (%) sunflower (maturity) =  $-0.0132^* \times P \text{ supply (mg pot}^{-1}) + 92.175^{n.s}$  ( $r^2 = 0.70^{***}$ ), Required P (g pot<sup>-1</sup>) safflower (maturity) =  $-0.0777 \times P \text{ supply (mg pot}^{-1}) + 84.435$  ( $r^2 = 0.82^{***}$ ), **[C]** Recovered P (%) sunflower (anthesis) =  $-0.0115^* \times P \text{ supply (mg pot}^{-1}) + 105^{n.s}$  ( $r^2 = 0.56^{***}$ ), Required P (g pot<sup>-1</sup>) safflower (anthesis) =  $-0.0844 \times P \text{ supply (mg pot}^{-1}) + 94.39$  ( $r^2 = 0.85^{***}$ ). \* in linear equation constants indicates significant difference between the same constants in both species ( $p < 0.05$ ). \*, \*\*, \*\*\* for  $r^2$  indicate significant correlation within each plant at  $p < 0.05, 0.01, \text{ and } 0.001$  respectively, n.s indicates not significant.

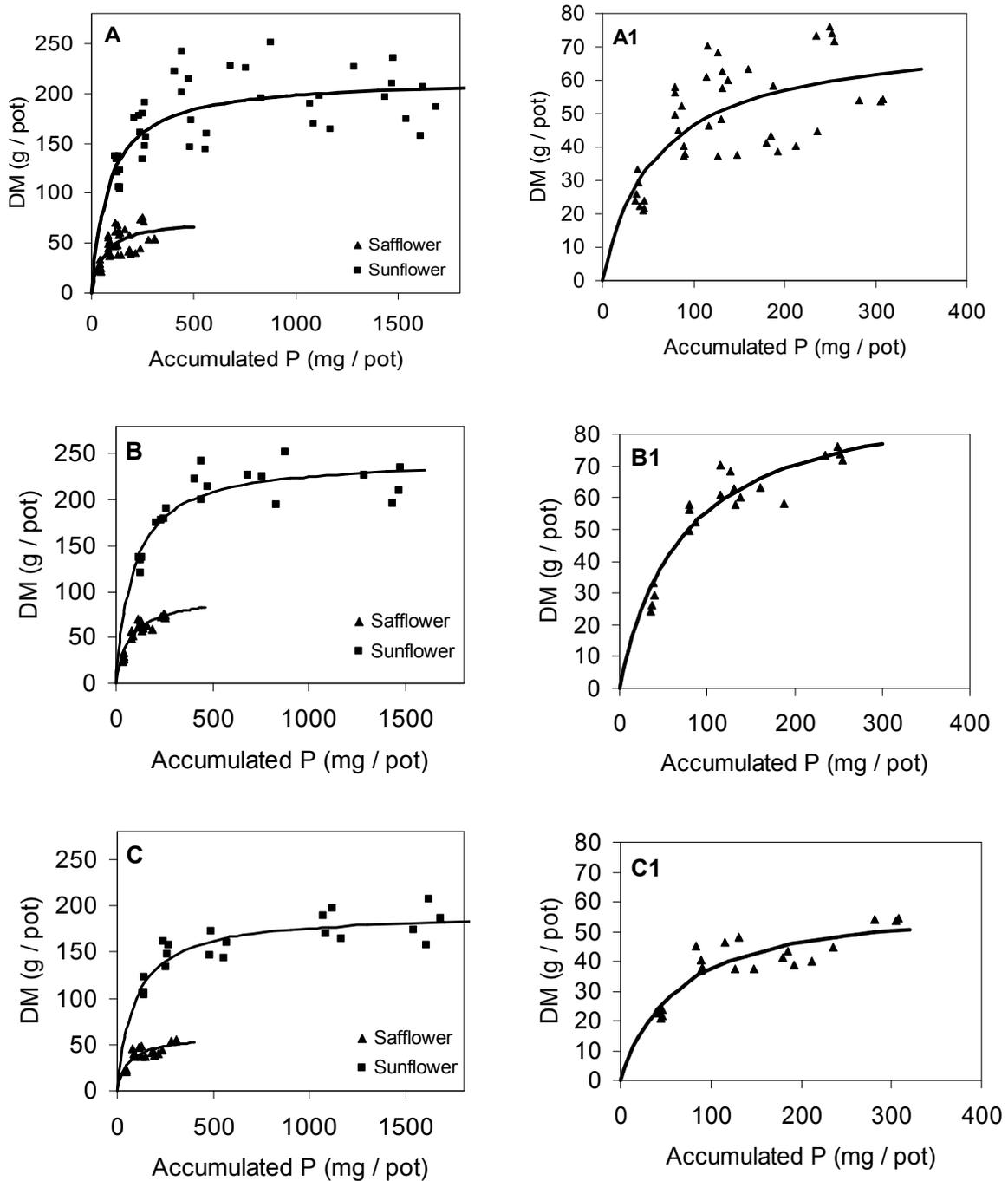
The y-intercept was not significantly different between species and reveals that at the levels of P supply near zero; both species could recover the same percentage of external P supply.

At anthesis, both species removed almost all added external solution P at low external solution [P] and this percentage is sharply decreased in safflower with increasing P level to reach less than 40% at the higher P supply, while sunflower still remove all added P at deficient and optimal P supply then decreased to nearly 80%, at the highest external [P].

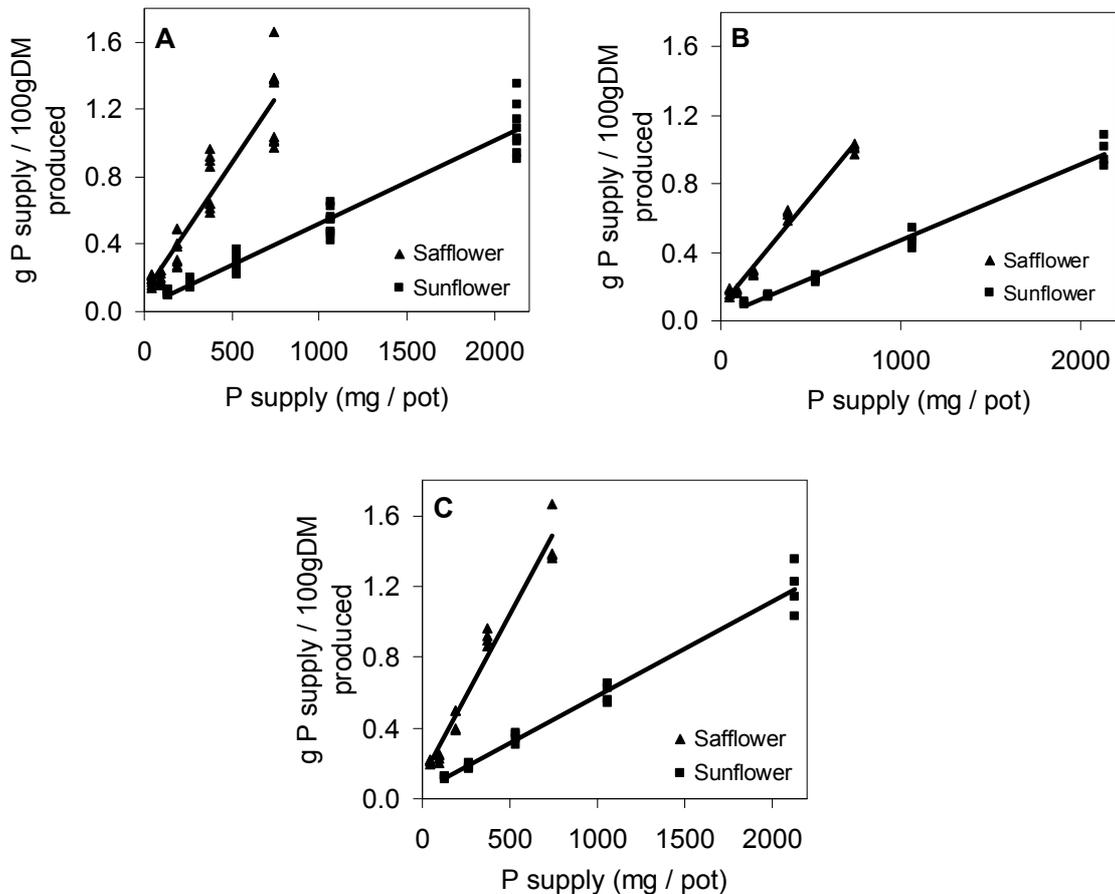
### 3.3. Effect of P supply on P utilization efficiencies

#### 3.3.1. Effect of P supply on yield response curves

The dry matter (DM) response curves relating the accumulated P in DM with the DM produced (Fig. 5.3) are homologous to the efficiency ratios. The functional relationship between nutrient supply and yield parameters may be described in several ways. Polynomial functions are easily applied, but do not allow interpreting their coefficients in a straightforward fashion. The classical Mitscherlich equation has often been used to describe yield responses, but in order to characterize nutrient efficiency the Michaelis-Menten equation has been more frequently employed (e.g. Gourley et al., 1994, Steingrobe and Claassen, 2000). In analogy to enzyme kinetics, the P accumulation required to produce 50 % of the predicted maximum yield (term 'c') corresponds to the  $K_m$  in Michaelis-Menten kinetics and essentially describes the curvature of the graph. It is thus a good indicator of the sensitivity of a crop to reduced nutrient supply, hence its nutrient efficiency. However, this approach requires a well-defined response curve from which the yield maximum can be deduced. The data of DM for both crops was applied from both experiment harvesting stages (anthesis and maturity) and when all data are pooled. Characterizing nutrient efficiency according to this approach reveals that  $K_m$  of both species is not significantly different, which indicates that both species have the same efficiency to use accumulated P for DM production at 50% maximal DM yield, because the term 'c' is always the same for sunflower and safflower (Fig. 5.3).



**Figure 5.3:** Dry matter (DM) response curves for safflower and sunflower based on the total P accumulated in above-ground biomass per pot. Michaelis-Menten-type equations are given as:  $DM = (A_{max} \times (mg\ P)) / (c + (mg\ P))$ , with ‘mg P’ representing the P accumulated in biomass per pot, ‘c’ the  $K_m$ , ‘A’ the maximum yield potential. [A] TDM sunflower (anthesis and maturity) =  $(215^* \times (mg\ P)) / (85.04^{ns} + mg\ P)$ , [A], [A1] TDM safflower (anthesis and maturity) =  $(73.7 \times (mg\ P)) / (58.10 + mg\ P)$ , [B] TDM sunflower (maturity) =  $(246.0^* \times (mg\ P)) / (90.8^{ns} + mg\ P)$ , [B], [B1] TDM safflower (maturity) =  $(95.5 \times (mg\ P)) / (72.2 + mg\ P)$ , [C] TDM sunflower (anthesis) =  $(192.3^* \times (mg\ P)) / (94.0^{ns} + mg\ P)$ , [C], [C1] TDM safflower (anthesis) =  $(60.7 \times (mg\ P)) / (62.8 + mg\ P)$ . \* indicates significant difference between the two species in the same constant ( $p < 0.05$ ).



**Figure 5.4:** P requirement (for production of 100g DM) response curves for safflower and sunflower based on the P supply per pot. Linear regressions are given. [A] Required P (g pot<sup>-1</sup>) sunflower (anthesis and maturity) = 0.0005\* × P supply (mg pot<sup>-1</sup>) + 0.0339<sup>n.s</sup> (r<sup>2</sup> = 0.96\*\*\*), Required P (g pot<sup>-1</sup>) safflower (anthesis and maturity) = 0.0016 × P supply (mg pot<sup>-1</sup>) + 0.1008 (r<sup>2</sup> = 0.89\*\*\*), [B] Required P (g pot<sup>-1</sup>) sunflower (maturity) = 0.0004\* × P supply (mg pot<sup>-1</sup>) + 0.0226\* (r<sup>2</sup> = 0.98\*\*\*), Required P (g pot<sup>-1</sup>) safflower (maturity) = 0.0013 × P supply (mg pot<sup>-1</sup>) + 0.0849 (r<sup>2</sup> = 0.98\*\*\*), [C] Required P (g pot<sup>-1</sup>) sunflower (anthesis) = 0.0005\* × P supply (mg pot<sup>-1</sup>) + 0.0452<sup>n.s</sup> (r<sup>2</sup> = 0.98\*\*\*), Required P (g pot<sup>-1</sup>) safflower (anthesis) = 0.0018 × P supply (mg pot<sup>-1</sup>) + 0.1166 (r<sup>2</sup> = 0.97\*\*\*). \* in linear equation constants indicates significant difference between the same constants in both species (p < 0.05). \*, \*\*, \*\*\* for r<sup>2</sup> indicate significant correlation within each plant at p < 0.05, 0.01, and 0.001 respectively. n.s indicates not significant.

### 3.3.2. Effect of P supply on agronomic P efficiency (external P requirement)

The term ‘external nutrient requirement’ refers to the amount of nutrient in the media required to produce a given percentage of maximum yield (Föhse et al., 1988; Fox, 1981). Accordingly we adopted a calculation that defines the required external P quantity (in g) to produce 100 g of DM. Comparing the linear response curves of both species (Fig. 5.4), obviously shows the higher requirement of safflower for external P than sunflower at both

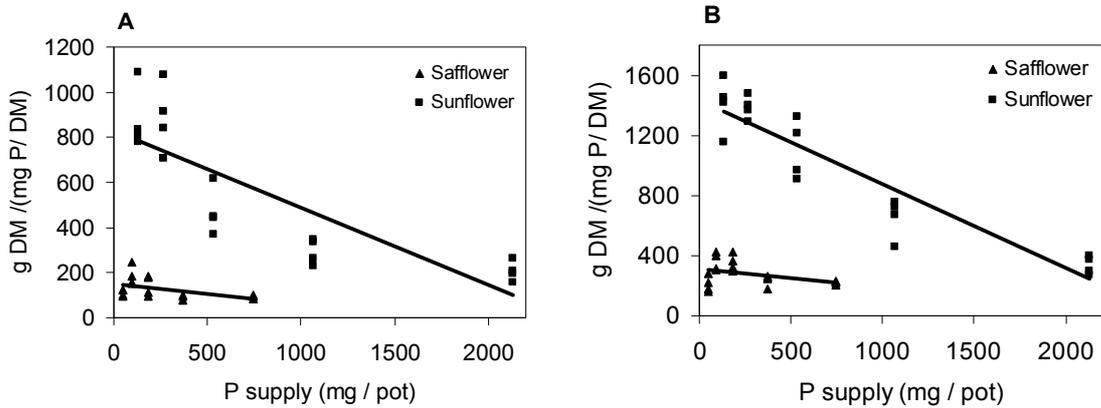
harvesting times (anthesis and maturity) and when data are pooled. This can be proved by the significantly large slope of the linear relationship between P supply and P requirement of safflower compared to that of sunflower. The y-intercept indicates the requirement of external P at near zero P supply, was higher in safflower than sunflower but the difference was significant at maturity only.

### 3.3.3. Effect of P supply on utilization index

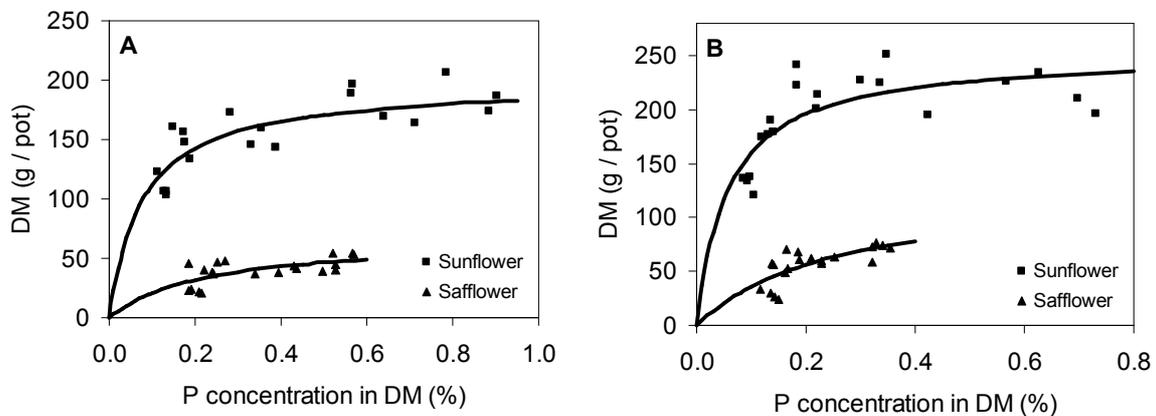
According to the comparison of the linear curves of both species at both harvesting stages (Fig. 5.5); it was observed that the linear curves of both species are significantly differing from each other in both slope and y-intercept at maturity and in y-intercept at anthesis only. The sunflower response curve lay significantly higher than that of safflower, indicating the higher utilization index values in the former compared to the later. At the very low P supplies (y-intercept) sunflower can produce much higher DM per unit of P concentration than safflower. In both species, at the higher P levels, P use efficiency decreased, implying the 'law of diminishing returns' in P use for production of dry matter.

The DM response curve based on the P concentration in DM is homologous to the term utilization index. It was applied to the Michaelis-Menten equation and represents more clear response (Fig. 5.6) than the calculated UI based on the P supply. The response curves showed the high  $A_{max}$  and low  $K_m$  values of sunflower compared to safflower indicating clearly the higher utilization efficiency of the former compared to the later in term of this efficiency indicator. Consequently, sunflower required less P concentration in DM to produce 50% of the maximum yield ( $K_m$ ) than safflower, in addition, the former had a significantly higher DM production potential ( $A_{max}$ ) than the later.

The shoot P concentration at 50% of the maximum yield ( $K_m$ ) of sunflower was less than that of safflower. However sunflower required a low level of external P to produce fixed amount of yield compared to safflower. The use of agronomic use efficiency and to less extent ER and UI as efficiency indicators involve the uptake of the nutrient and its utilization to produce final yield and doesn't indicate the mechanism through which the efficient cultivar interprets its efficiency, this difference between the two species implied that the superior P efficiency of sunflower to that of safflower is associated with P utilization efficiency, and P uptake efficiency (P recovery), but the contribution of both efficiency components to overall NUE still not clear. For this reason, the contribution of uptake efficiency and utilization efficiency to the over all P use efficiency can be evaluated, according to Piepho (1995) (Table 5.7).



**Figure 5.5:** P utilization index (PUI) ( $\text{g DM} / (\text{g P} (\text{g DM})^{-1})$ ) response curves for safflower and sunflower in term of DM production based on the P supply per pot. Linear regressions are given. **[A]**  $\text{PUI}_{\text{sunflower (anthesis)}} = -0.3414^{\text{n.s.}} \times \text{P supply (mg pot}^{-1}) + 828.35^*$  ( $r^2 = 0.68^{***}$ ),  $\text{PUI}_{\text{safflower (anthesis)}} = -0.0848 \times \text{P supply (mg pot}^{-1}) + 148.69$  ( $r^2 = 0.24^{\text{n.s.}}$ ), **[B]**  $\text{PUI}_{\text{sunflower (maturity)}} = -0.5583^* \times \text{P supply (mg pot}^{-1}) + 1436.6^*$  ( $r^2 = 0.86^{***}$ ),  $\text{PUI}_{\text{safflower (maturity)}} = -0.1179 \times \text{P supply (mg pot}^{-1}) + 310.53$  ( $r^2 = 0.15^{\text{n.s.}}$ ). \* in linear equation constants indicates significant difference between the same constants in both species ( $p < 0.05$ ). \*, \*\*, \*\*\* for  $r^2$  indicate significant correlation within each plant at  $p < 0.05$ , 0.01, and 0.001 respectively. n.s indicates not significant.



**Figure 5.6:** Dry matter (DM) response curves for safflower and sunflower based on the P concentration in above-ground biomass per pot. Michaelis-Menten-type equations are given as:  $\text{DM} = (\text{A}_{\text{max}} \times (\text{P concentration})) / (\text{c} + (\text{P concentration}))$ , with ‘Pconc’ representing the P concentration in above-ground biomass per pot, ‘c’ the  $K_m$ , ‘A’ the maximum DM yield potential. **[A]**  $\text{DM}_{\text{sunflower (anthesis)}} = (197.3^* \times (\text{Pconc.})) / (0.0765^* + \text{Pconc.})$ ,  $\text{DM}_{\text{safflower (anthesis)}} = (66.8 \times (\text{Pconc.})) / (0.2196 + \text{Pconc.})$ , **[B]**  $\text{DM}_{\text{sunflower (maturity)}} = (252.7^* \times (\text{Pconc.})) / (0.0581^* + \text{Pconc.})$ ,  $\text{DM}_{\text{safflower (maturity)}} = (128 \times (\text{Pconc.})) / (0.257 + \text{Pconc.})$ . \* indicates significant difference between the two species in the same constant ( $p < 0.05$ ).

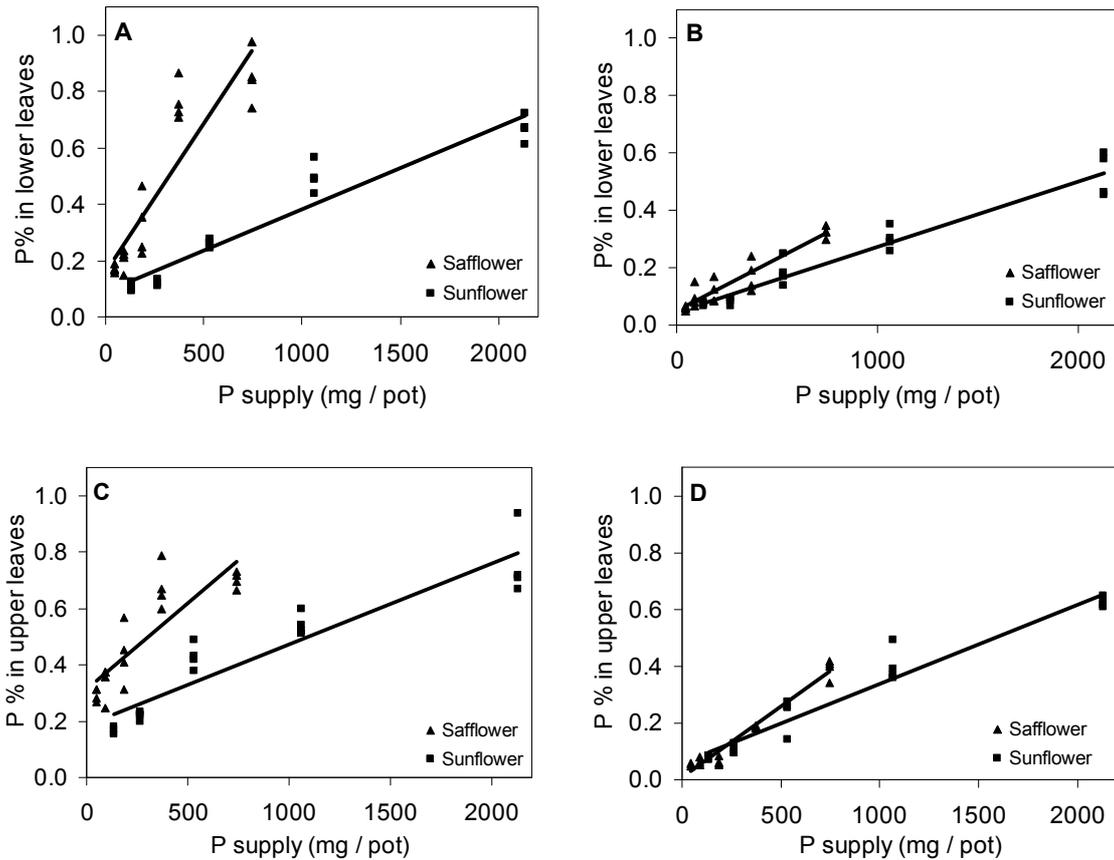
**3.3.4. Contribution of uptake efficiency and utilization efficiency to phosphorus use efficiency of safflower and sunflower**

According to Moll et al., (1982), the nutrient use efficiency is defined as the yield per unit of nutrient available in the soil (supplied), and has two primary components; uptake efficiency (accumulated nutrient / supplied), and utilization efficiency (yield/ accumulated nutrient), in which all parameters are expressed in the same units (e.g., g/plant). The  $c_i$  coefficients, based on the variance of log-transformed uptake and utilization efficiency (components of P UE), were calculated to quantify the contribution of each component to final P UE variability, and the yield component analysis according to Piepho (1995) was adapted for these calculations (Table 5.7). It was found that in both harvest stages, and when data are pooled, both uptake and utilization efficiency are important to the final P UE in safflower, but in sunflower the utilization efficiency is influencing the final P UE much more than the uptake efficiency.

**Table 5.7:** Estimation of variation coefficients (VC) of phosphorus use efficiency (P UE), SD and variation of log-P UE, and  $c_i$  coefficients for phosphorus uptake efficiency, and phosphorus utilization efficiency as components of P UE of safflower and sunflower<sup>1)</sup>.

Species	VC of PUE (%)	SD Log-PUE (× 100)	Variance (× 100)	C <sub>i</sub> for efficiency components	
				Uptake (× 100)	Utilization (× 100)
Maturity					
Safflower	60.42	73.64	54.24	28.68	25.57
Sunflower	69.48	84.09	70.71	10.95	59.75
Anthesis					
Safflower	64.98	77.74	60.44	28.24	32.20
Sunflower	72.73	84.15	70.82	6.88	63.93
Pooled					
Safflower	63.94	76.73	58.88	26.59	32.29
Sunflower	71.10	83.82	70.25	7.74	62.51

<sup>1)</sup>Calculations according to Piepho (1995). (n = 20 for each harvesting stage, n = 40 when pooled).

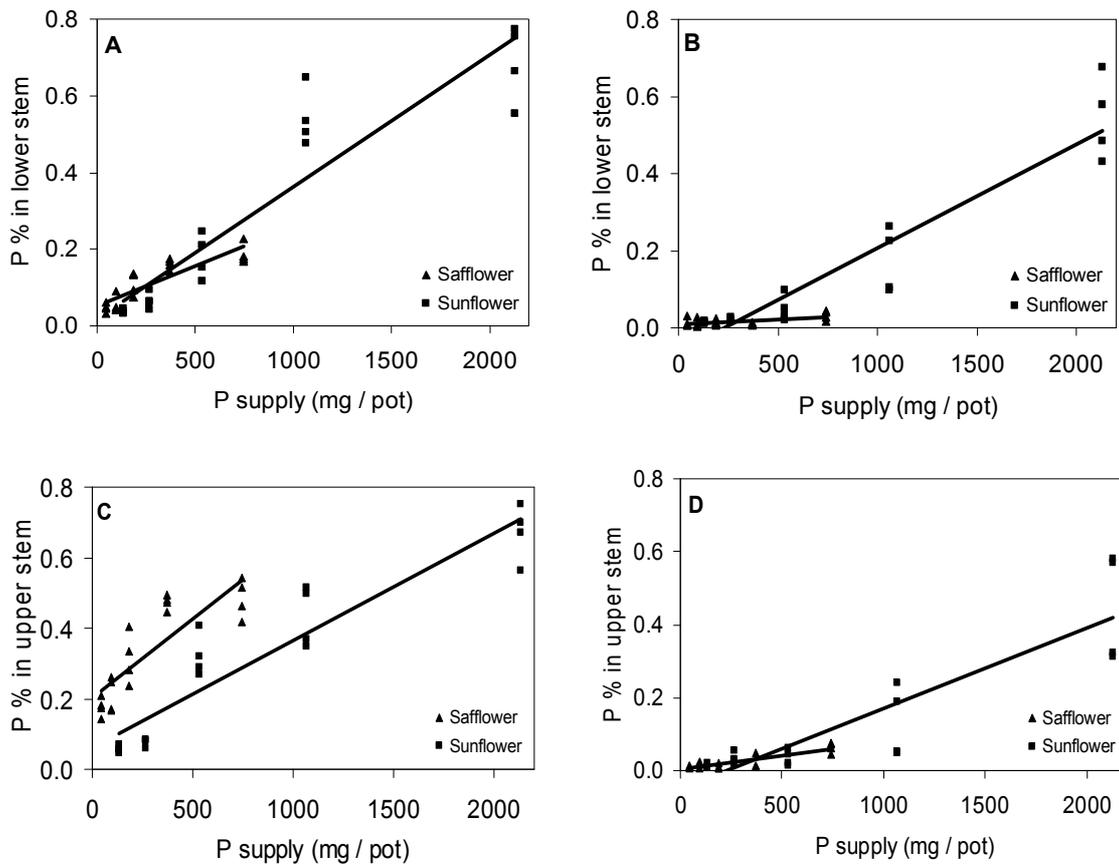


**Figure 5.7:** P concentration response curves for safflower and sunflower leaves based on the P supply per pot. Linear regressions are given. [A] P concentration (%) sunflower lower leaves (anthesis) =  $0.000294^* \times \text{P supply (mg pot}^{-1}) + 0.0882^{\text{n.s}}$  ( $r^2 = 0.93^{***}$ ), P concentration (%) safflower lower leaves (anthesis) =  $0.0011 \times \text{P supply (mg pot}^{-1}) + 0.1589$  ( $r^2 = 0.82^{***}$ ), [B] P concentration (%) sunflower lower leaves (maturity) =  $0.00023^* \times \text{P supply (mg pot}^{-1}) + 0.0447^{\text{n.s}}$  ( $r^2 = 0.94^{***}$ ), P concentration (%) safflower lower leaves (maturity) =  $0.000365 \times \text{P supply (mg pot}^{-1}) + 0.0486$  ( $r^2 = 0.89^{***}$ ), [C] P concentration (%) sunflower upper leaves (anthesis) =  $0.000287^* \times \text{P supply (mg pot}^{-1}) + 0.1851^*$  ( $r^2 = 0.87^{***}$ ), P concentration (%) safflower upper leaves (anthesis) =  $0.00061 \times \text{P supply (mg pot}^{-1}) + 0.3142$  ( $r^2 = 0.73^{***}$ ), [D] P concentration (%) sunflower upper leaves (maturity) =  $0.00028^* \times \text{P supply (mg pot}^{-1}) + 0.0606^*$  ( $r^2 = 0.95^{***}$ ), P concentration (%) safflower upper leaves (maturity) =  $0.00051 \times \text{P supply (mg pot}^{-1}) + 0.0055$  ( $r^2 = 0.96^{***}$ ). \* in linear equation constants indicates significant difference between the same constants in both species ( $p < 0.05$ ). \*, \*\*, \*\*\* for  $r^2$  indicate significant correlation within each plant at  $p < 0.05$ , 0.01, and 0.001 respectively. n.s indicates not significant.

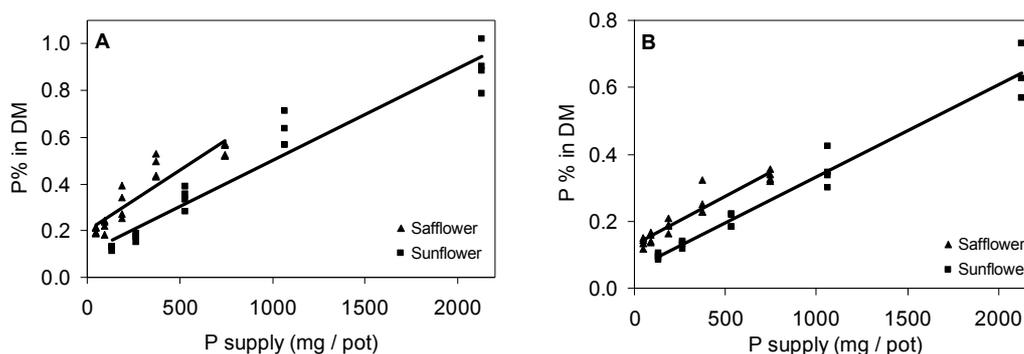
### 3.3.5. Phosphorus translocation

Because achene yield in sunflower was not consistent along the P supply, the real translocation efficiency was not possible to be calculated for this specie. The ability of a cultivar to reduce the nutrient concentration of its lower parts or the supporting plant part as stem can indicate its efficiency in translocation (Baligar et al., 2001). Accordingly, the concentration of P in lower leaves of safflower was significantly higher than that of sunflower at anthesis and maturity (Fig. 5.6). Also safflower's higher leaves still containing higher concentration

of P compared to that of sunflower, but sunflower maintain the same slope of the curve in both upper and lower leaves when compared at anthesis and maturity separately, while the slope of the curve in safflower's lower leaves is much higher than that of its upper leaves. On the other hand the P concentration in both species are statistically not different in lower and upper stem parts, and safflower contained less P concentration in both lower and upper stem parts compared to those of sunflower at maturity (Fig. 5.7). Comparing the two species in terms of P concentration of total DM reveals that safflower contains significantly higher values than sunflower at anthesis, but the difference was not significant at maturity.



**Figure 5.8:** P concentration response curves for safflower and sunflower stems based on the P supply per pot. Linear regressions are given. [A] P concentration (%) sunflower lower stem (anthesis) =  $0.0003^{n.s} \times P \text{ supply (mg pot}^{-1}) + 0.018^{n.s}$  ( $r^2 = 0.87^{***}$ ), P concentration (%) safflower lower stem (anthesis) =  $0.00021 \times P \text{ supply (mg pot}^{-1}) + 0.0533$  ( $r^2 = 0.77^{**}$ ), [B] P concentration (%) sunflower lower stem (maturity) =  $0.00027^* \times P \text{ supply (mg pot}^{-1}) - 0.0634^*$  ( $r^2 = 0.90^{***}$ ), P concentration (%) safflower lower stem (maturity) =  $3E-05 \times P \text{ supply (mg pot}^{-1}) + 0.0087$  ( $r^2 = 0.35^{n.s}$ ), [C] P concentration (%) sunflower upper stem (anthesis) =  $0.0003^{n.s} \times P \text{ supply (mg pot}^{-1}) + 0.0599^*$  ( $r^2 = 0.88^{***}$ ), P concentration (%) safflower upper stem (anthesis) =  $0.00045 \times P \text{ supply (mg pot}^{-1}) + 0.2023$  ( $r^2 = 0.71^{***}$ ), [D] P concentration (%) sunflower upper stem (maturity) =  $0.00022^* \times P \text{ supply (mg pot}^{-1}) - 0.049^{n.s}$  ( $r^2 = 0.81^{***}$ ), P concentration (%) safflower upper stem (maturity) =  $7E-05 \times P \text{ supply (mg pot}^{-1}) + 0.0031$  ( $r^2 = 0.71^{n.s}$ ). \* in linear equation constants indicates significant difference between the same constants in both species ( $p < 0.05$ ). \*, \*\*, \*\*\* for  $r^2$  indicate significant correlation within each plant at  $p < 0.05$ , 0.01, and 0.001 respectively. n.s indicates not significant.



**Figure 5.9:** P concentration response curves for safflower and sunflower DM based on the P supply per pot. Linear regressions are given. **[A]**  $P \text{ concentration } (\%)_{\text{safflower (anthesis)}} = 0.000393^* \times P \text{ supply } (\text{mg pot}^{-1}) + 0.1061^* (r^2 = 0.94^{***})$ ,  $P \text{ concentration } (\%)_{\text{sunflower (anthesis)}} = 0.000512 \times P \text{ supply } (\text{mg pot}^{-1}) + 0.2032 (r^2 = 0.84^{***})$ , **[B]**  $P \text{ concentration } (\%)_{\text{sunflower (maturity)}} = 0.000281^{\text{n.s}} \times P \text{ supply } (\text{mg pot}^{-1}) + 0.05498^* (r^2 = 0.96^{***})$ ,  $P \text{ concentration } (\%)_{\text{safflower (maturity)}} = 0.00029 \times P \text{ supply } (\text{mg pot}^{-1}) + 0.1304 (r^2 = 0.91^{***})$ . \* in linear equation constants indicates significant difference between the same constants in both species ( $p < 0.05$ ). \*, \*\*, \*\*\* for  $r^2$  indicate significant correlation within each plant at  $p < 0.05$ , 0.01, and 0.001 respectively. n.s indicates not significant.

## 4. Discussion

### 4.1. Growth and morphology

This nutrient solution experiment was conducted according to the results obtained from the two-year pot experiment using soil regarding P supply in this dissertation (chapter 3, 4). In agreement with results of the soil experiment, and this nutrient solution experiment, it was reported that P deficiency limits shoot growth of safflower (Zaman, 1988; Padmavathi et al., 2003), and sunflower (Cannor and Sadras, 1992; Grove and Sumner, 1982). In this investigation, the reduction in leaf area could be the cause for reduced dry matter of the lower leaves of both species as a result of deficient P supply (Freedman et al., 1989). In the upper part of leaves, the reduced number of leaves (Lynch et al., 1991) as well as the reduced leaf area contributes to reduce the dry matter of leaves of the plants and finally the total dry matter in both species under P deficiency. The contribution of the stem in reducing dry matter as affected by sub-optimal external solution [P] may be caused by the reduced stem diameter and reduced height of the plants as a response of P deficiency. As found in both experiments concerning P supply (soil and nutrient solution experiments), the effect of P supply on increasing the number of branches per plant was reported in safflower (El-Nakhlawy, 1991; Ahmed et al., 1985; Singh et al., 1995). Interestingly, the secondary branches are extremely reduced with decreasing P supply, and they were totally inhibited under extreme deficient P supply.

### 4. 2. Phosphorus use efficiency

The evaluation of nutrient use efficiency (NUE) is useful to differentiate plant species for their ability to absorb and utilize nutrients for maximum yields and can be evaluated through many definitions (Baligar et al., 2001). The definition of NE as plant yield per unit of nutrient supply results in its dependence on two interrelated groups of plant factors, namely a) plant properties related to uptake efficiency, which is nutrient uptake relative to its supply and b) factors related to utilization efficiency representing plant yield relative to nutrient uptake (Sattelmacher et al., 1994). Unfortunately, the characterization of nutrient supply under field conditions has to face several uncertainties related to the loss of nutrients and dependence of their availability on soils and climatic conditions as well as on water supply. However, screening for NE requires a simpler rooting substrate, solution cultures are less adequate due to the lack of physical root-soil interactions as well as of nutrient mobilization from less and organic fractions (Sauerbeck and Helal, 1990).

#### 4.2.1 $C_{\min}$ , P accumulation and P recovery

As uptake efficiency depends on soil parameters and root physiology parameters (Williams and Yanai, 1996); the soil parameters are not able to be studied in nutrient solution in which the nutrient is all the time available to the root system. Under ample supply conditions, the capacity of the uptake mechanism ( $A_{\max}$ ) rather than its affinity ( $K_m$ ) will be of primary significance in the case of mobile nutrients such as nitrate, but in the less mobile nutrients such as P, under low nutrient solution concentration, the affinity of the uptake mechanism  $K_m$  and  $C_{\min}$  are of significance (Sauerbeck and Helal, 1990).

Response curves rather than comparing means at different P supplies were applied to differentiate the response of both species because the total P supply (mg P pot<sup>-1</sup>) differed between the species at equivalent external [P] (mM). This difference in the total P supply received at equivalent [P] due to the difference in N demand of each species because the nutrient solutions were renewed for each species individually after nitrate test in the highest [P] was depleted. This gives the opportunity to make the plants not deficient for other nutrients rather than the nutrient under study (P). Response curves for each germplasm were derived either using Michaelis-Menten equations or linear regressions and both regression models were tested for invariance to determine whether the two response curves were significantly different ( $p < 0.05$ ).

The measures related to the P uptake used in this study to assess differences between the two species were:  $C_{\min}$  (solution P concentration at which net uptake is zero), P accumulation

(mg P accumulated  $\text{pot}^{-1}$ ) and P recovery (% (mg P accumulated / mg P provided)) (Blair and Cordero, 1978; Elliot and Läuchli, 1985). Unfortunately, the small-volume nutrient solution culture techniques are of limited effectiveness in screening for root morphological factors critical in the acquisition of P from the soil. One reason is that in solution culture, nutrients are continually brought to root surfaces by agitation. Also, during most of the growth period the P concentration in the nutrient solution is much higher than in the soil solution. As a result, several adaptive features induced by low P, such as root hair initiation and growth, may not be detected (Gerloff, 1987).

### 4.2.1.1 Phosphorous minimal concentration in solution ( $C_{\min}$ )

The minimum P concentration in the nutrient solution where the P influx in the roots is zero ( $C_{\min}$ ) is an uptake mechanism factor obtained in this nutrient solution experiment and related to genetic difference between sunflower and safflower, was found to not differ between the two species under investigation (Table 5.6). This indicates that the two species doesn't differ between each other in the genetic affinity in term of  $C_{\min}$ .

### 4.2.1.2. P accumulation

In this investigation, the higher  $A_{\max}$  in sunflower compared to safflower in terms of P accumulation based on P supply (Fig. 5.1) indicates the higher accumulation potential of the former compared to the later. But because sunflower produces more biomass and accumulates more nutrients (including P and N), it also received more P, thus,  $A_{\max}$  may can't be interpreted as high accumulation efficiency. The  $K_m$  in this case can be misleading which indicates the P supply at which half maximum P accumulation could be reached, but it reveals in this case that sunflower has the affinity to accumulate much external P and its P supply for half maximum accumulation is higher than safflower.

### 4.2.1.3. P Recovery

The difference in P recovery between the two species (Fig. 5.2) is possibly according to the higher absorption affinity of available P by roots of sunflower compared to safflower; ranking sunflower is more efficient than safflower in this absorption affinity.

Generally, the difference in P uptake efficiency between plants indicates mechanisms differentiating the two species in terms of P uptake efficiency including soil factors and plant factors (Baligar et al., 2001). It was reported that the most important parameters controlling nutrient uptake are the average dissolved nutrient concentration (soil parameter) and the

maximal rate of nutrient uptake (root physiological parameter), and the next most important parameter is the effective diffusion coefficient (soil parameter) (Williams and Yanai, 1996). Availability of nutrients at root surfaces in soil is controlled by movement in the soil solution and by contacts generated through root growth and extension. The importance of root growth and morphology in nutrient access cannot be adequately evaluated in agitated solution cultures (Gerloff et al., 1983), but genetic aspects related to P influx and efflux, rate of P transport in roots and shoots, affinity to uptake ( $K_m$ ), threshold concentration  $C_{min}$ , could be evaluated using this kind of nutrient solution cultures which control the overall P recovery.

### 4.2.2. Utilization efficiency

Nutrient utilization efficiency can be interpreted in many ways to characterize different genera, species or genotypes in terms of efficient and inefficient in utilization. Efficiency ratio (ER) or amount of biomass produced per unit of nutrient present in the tissues (Coltman et al., 1985; Woodend, 1986). The utilization index (Siddiqi and Glass, 1981; Woodend, 1986), which is defined as biomass per unit of tissue nutrient concentration was proposed by Siddiqi and Glass (1981) as an improved measure that, unlike the efficiency ratio, takes differences in the amount of produced biomass into consideration. Agronomic efficiency denotes the biomass, or harvestable product, produced per unit of nutrient applied (Blair and Cordero, 1978; Moll et al., 1982).

Measures used in this study to assess differences between the two species were in terms of P utilization efficiency: shoot dry mass ( $\text{g DM pot}^{-1}$ ) response curve (Blair and Cordero, 1978; Gourley et al 1993) based on P accumulation which is homologous to P efficiency ratio ( $\text{g DM (mg P}^{-1}\text{)}$ ) based on P supply (Baligar et al., 1990; Godwin and Blair, 1991), external P required to achieve certain percentage of yield (Föhse et al., 1988) based on P supply, P utilization index ( $\text{g DM}^{-2} (\text{mg P}^{-1})$ ) (Siddiqi and Glass, 1981); and shoot dry mass ( $\text{g DM pot}^{-1}$ ) response curve based on the P concentration in DM which is similar to P utilization index, and finally P translocation by comparing both species in terms of P concentration in lower and upper stems.

#### 4.2.2.1. Growth response curve based on P accumulated in DM

The response of safflower and sunflower in terms of DM production based on P accumulation (Fig. 5.3) interpreted according to M-M equation revealed that both species have the same  $K_m$  values which indicates that both crops have the same efficiency in utilizing internal P to produce 50% DM (low P supply). These results show that safflower is at least not more

efficient than sunflower in utilizing absorbed P and hence not to be considered a low input cultivar compared to sunflower, as it needs the same P amount as sunflower to produce half maximum DM yield.  $A_{\max}$  was higher in sunflower compared to safflower which indicates the maximum DM production potential and is not related to utilization efficiency. Growth response curve didn't fit for our data in the previous experiment in this work conducted in soil; accordingly ER was calculated and based to P supply, which is homologous to the growth response curve applied in this nutrient solution experiment. The ER values in the pot experiment reveals that sunflower was more efficient in utilizing absorbed P than safflower at optimal and moderate P deficiency supplies for the production of all yield parameters (DM, achenes, oil).

### 4.2.2.2 External P requirement

The higher external P requirement for safflower to produce fixed amount of DM was higher compared to sunflower (Fig. 5.4). These data obtained from the external P requirements from the nutrient solution experiment supports the results obtained from the previous soil experiment (see paragraph 3.2.1 in chapter 4) indicating that sunflower is more efficient in utilizing external P than safflower at suboptimal and optimal P supplies to express higher DM and achene yield.

### 4.2.2.3 Utilization Index

The Utilization index (UI) according to Siddiqi and Glass (1981), is an efficiency indicator which takes the difference in biomass produced into consideration, not just the amount of P accumulated. It showed the superiority of sunflower over safflower in the efficiency of utilizing internal P (similar to DM response curve base on P concentration) and supports the findings of the previous experiment in this work conducted in soil (Fig. 5.5, 5.6).

The results obtained from this nutrient solution experiment and the previous experiment in this work conducted in soil indicate that difference between some efficiency indicators (Efficiency Ratio and Utilization Index) supports the conclusions of other authors that ranking species for nutrient efficiency can vary according to the definition used (Blair, 1993; Gourley et al., 1994). However, in our study the difference between ER and UI was not conflicting. But the interpretation of utilization efficiency in terms of UI was clearer than in terms of ER. UI interpreted as DM production curve based on P concentration in DM revealed the lower  $K_m$  value in sunflower compared to safflower (Fig. 5.6) supporting the conclusion that safflower is less P efficient in utilizing internal P, while the ER show no

statistical difference between the two species in term of  $K_m$  values which also proves at least that safflower is not a low input crop compared to sunflower.

#### 4.2.2.4. Translocation/remobilisation within the plant

As a result of the inconsistency of the achene yield in sunflower as affected by P supply, the real translocation efficiency was not possible to be calculated for this species. But the ability of a cultivar to reduce the nutrient concentration of its lower parts or the supporting plant part as stem can indicate its efficiency in translocation (Baligar et al., 2001). Concerning our results, the ability of sunflower at anthesis to have the same P concentration in lower and upper leaves may indicate more translocation efficiency of P from those parts compared to safflower. The P concentration in lower and upper plant parts, along with the P concentration in total DM at both anthesis and maturity were not clear to conclude a difference in the efficiency in remobilisation between the two plants. With, the less P concentration in the lower leaves of sunflower is interpreted as efficiency of translocation, or as less P requirement is not clear from these data.

#### 4.2.2.5. Mechanisms related to utilization efficiency

The main factors contributing to the higher efficiency of efficient cultivar are (i) efficient remobilisation of P from vegetative plant organs to grains, and (ii) lower P requirement for yield formation (Sattelmacher et al., 1994), (iii) P storage in vacuoles, (iv) lower element concentration in supporting parts, particularly the stem (Baligar et al., 2001). Substantial genetic variation, however, has been reported in P utilization efficiency whether these variations relate to differences in P assimilation and / or compartmentation on the cellular level is not known. However the prospects of increasing nutrient utilization efficiency in P through better utilization on the cellular level are considered to be rather poor but can be improved through screening methods and breeding programs between P deficiency tolerant traits (Sattelmacher et al., 1994).

### 5. Conclusion

Although only limited information on the nutrient requirement under field conditions may be obtained from pot experiments, it is evident that – in terms of DM and achene yield formation – safflower is performing inferior to sunflower under P-limited conditions and cannot be regarded a low input species in terms of its P requirement. Safflower is less efficient than

sunflower in utilizing absorbed P at low P availability and at their respective optimal P supplies. The results obtained using different efficiency indicators illustrates that the ranking may depend on the definitions used. The calculation of utilization index includes however, both yield and plant nutrient concentration, and is likely to be complicating the identification of potential mechanisms associated with enhanced nutrient efficiency. The use of agronomic use efficiency as an efficiency indicator involves the uptake of the nutrient and its utilization to produce final yield and doesn't indicate the mechanism through which the efficient cultivar interprets its efficiency. Indeed, little is known on the physiological mechanisms responsible for different utilization efficiency, and further research efforts should aim at identifying the mechanisms responsible for differences in P utilization efficiency of sunflower (P efficient) and safflower (P inefficient). Similarly, no information is available concerning the uptake efficiency under field conditions, and thus there is need for further research to prove the low P uptake efficiency of safflower and to identify the responsible mechanisms.

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## Chapter 6

### Effects of Potassium Supply on Growth and Yield of Safflower (*Carthamus tinctorius* L.) as compared to Sunflower (*Helianthus annuus* L.)

#### Summary

Safflower may have a certain production potential under German conditions, particularly in organic farming where the putatively low nutrient requirement is highly welcomed. However, current knowledge regarding the nutrient requirements of safflower as compared to similar oil crops is limited. It was thus the aim of this study to determine the growth and yield response of safflower (*Carthamus tinctorius* L.) as compared to sunflower (*Helianthus annuus* L.) with respect to K supply. Three safflower and two sunflower plants were cultivated in 6 L Mitscherlich pots. Both species responded strongly to increasing K supply with respect to plant growth and yield. Growth and yield of safflower increased up to 1 g K per pot, while the optimum for sunflower was 3.0 g K per pot. Safflower out-yielded sunflower at low K supply, while at high K level the opposite was observed. Supply of K affected virtually all yield components in both species, though to different degree. The number of capitula in safflower was only slightly affected and the number of achenes per capitulum was only reduced under severe K deficiency, while single achene mass increased with increasing K supply. To the contrary, in sunflower, the number of achenes per capitulum strongly responded to the K supply, as did the single achene mass. Oil yield in safflower was affected by K deficiency mainly due to reduced achene yield, not oil concentration. To the contrary, oil yield in sunflower was severely affected by low K supply due to both reduced achene yield and lower oil concentration. Multiple regression analysis indicate that in sunflower, the stem DM and the total amount of K accumulated in the above-ground biomass was most important, while in safflower the total amount of K and N accumulated had the highest impact. It may be concluded that sunflower is more sensitive to inadequate K supply than safflower.

#### 1. Introduction

The oil derived from safflower and sunflower represents a share of 0.07 and 7.3 % of the global production, respectively, while corresponding figures for Europe are 0 and 74.9% of safflower and sunflower achenes produced globally (FAO, 2007a,b). As possibilities to expand planted acreage are limited on a global scale, appropriate production intensities are

required. Particularly in developing countries, where the proportion of less fertile soils is particularly high, it may be difficult to fulfil the nutritional requirements of high-yielding crops. Fertilizer application represents an important measure to correct nutrient deficiencies and to replace elements removed in the products harvested (Dambroth and El Bassam, 1990; Sattelmacher et al., 1994).

Next to nitrogen, potassium (K) is the second most abundant mineral macronutrient in plants (Marschner, 1995). It fulfils important functions as inorganic osmolyte (turgor, stomatal regulation), counter ion in trans-membrane transport processes (photosynthesis, respiration, photosynthate translocation), and influences the activity of more than 60 enzymes (Marschner, 1995). Large amounts of K are required by high-yielding crops, and although total K contents of most soils are rather high, availability is usually low due to enclosure of K in silicates and strong adsorption by K-specific binding sites (Marschner, 1995; Russel, 1988). Applying K fertilisers thus represents an efficient measure to ensure adequate K supply. Particularly under conditions of low-input agriculture or resource limitations, K availability in the soil may be severely restricted, to which genotypes currently selected may not be well adapted (Glass et al., 1981). More efficient varieties of a given crop may be considered (Marschner, 1995; Gerloff and Gabelman, 1983), but large differences with respect to K demand between closely related, alternative crops may also be worth exploiting. Safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.), both belonging to the *Asteraceae*, are important oil crops in tropical and subtropical areas. Safflower is a highly branched, herbaceous, thistle-like annual, 30-150 cm tall with globular flower heads (capitula), characterised by a strong taproot, which enables it to thrive in dry climates and can access and utilize nutrients from below the root zone of cereal crops (Li and Mündel, 1997). The oil crop sunflower, however, is much taller, usually un-branched, lacks a tap root, and is usually considered being more demanding in terms of nutrients and water (Diepenbrock and Pasda, 1995). Indeed, considering yields the cost benefit ratio in safflower is about 40% of that obtained in sunflower due to the comparatively lower cost of its cultivation (Kumar, 2000).

Several studies suggest that high-yielding varieties are more sensitive to reduced nutrient availability input than the varieties with lower yield potential (Fischbeck, 1988; Dambroth and El Bassam, 1990). A similar response may be hypothesised for safflower vs. sunflower, which is known for its comparatively high K demand (Grove and Sumner, 1982). While the impact of N (Diepenbrock and Pasda, 1995; Steer and Hocking, 1984; Connor and Sadras, 1992) and P supply (Singh et al, 1973; Zubriski and Zimmerman, 1974) for yield formation

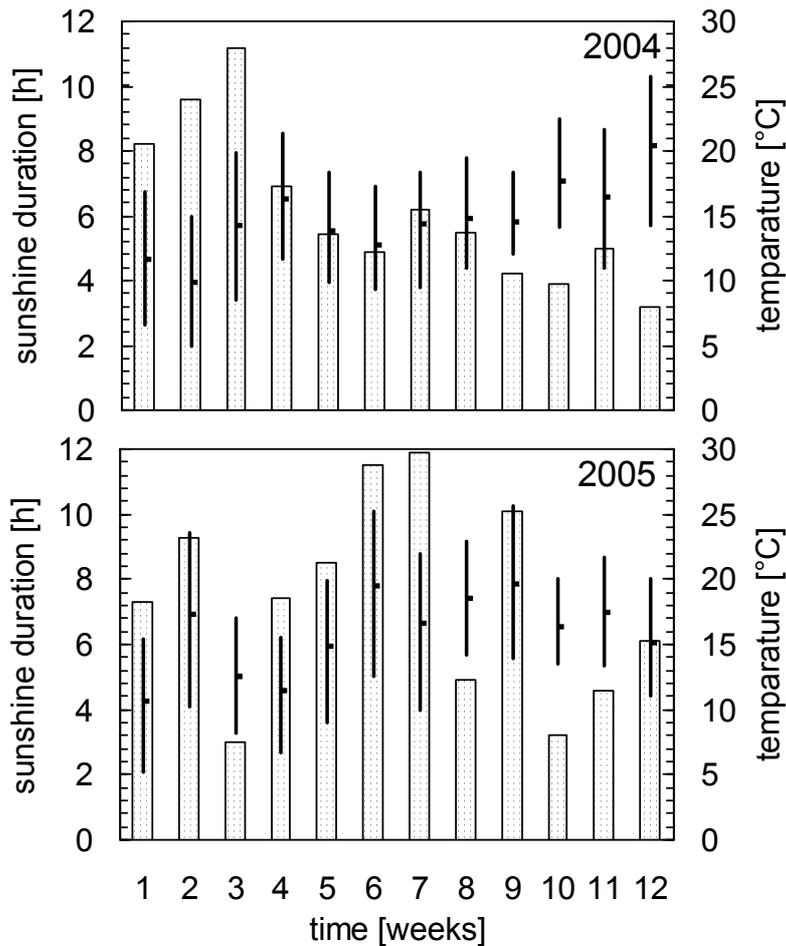
of sunflower has been extensively characterised, the influence of K supply for safflower yield formation has found little attention. Although both crops thrive in similar environments, direct comparisons of their response to increasing K availability with respect to yield and quality are not available; in particular the putatively lower K requirement of safflower is not sustained. Therefore, the aim of this study is to directly compare the impact of K supply on growth, yield and quality of safflower and sunflower under semi-controlled conditions. Special emphasis was given to the response of yield components.

## 2. Materials and Methods

### 2.1. Experimental Conditions

Pot experiments using safflower (*Carthamus tinctorius* L., variety 'Sabina') and sunflower (*Helianthus annuus* L., variety 'Salut R M') were conducted in the period from May to October 2004 and 2005 under semi-controlled conditions. Five K levels (0.0, 0.25, 0.5, 1.0, and 2.0 g per pot) were used in four replicates for both species in 2004. Other nutrients added were 0.72, 2.0, and 1.0 g per pot of P, N, and Mg respectively. In 2005, based on the results obtained in 2004, K levels were adapted to 0.25, 0.5, 1.0, 2.0, and 3.0 g per pot for safflower and to 0.5, 1.0, 2.0, 3.0, and 4.0 g per pot for sunflower. N supply was the same as in 2004 for safflower and increased to 4.0 g per pot for sunflower, and P supply was increased to 2.0 g P per pot for both species in 2005. Micronutrients were added in adequate amounts for both species in both years. Three safflower and two sunflower plants per Mitscherlich pot (6 l), cultivated in a mixture of equal volumes of sand, limed nutrient-poor soil, and perlite, were cultivated under outside conditions until the end of flowering (middle of August), and maintained under greenhouse conditions (day night temperature was adjusted to 28 °C, and 15 °C respectively) with additional lightening (intensity of light supplementation at canopy level was 260  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Before being used in the experiment, soil (limed nutrient-poor Ferrasol containing 9.6 mg  $\text{g}^{-1}$  organic matter, pH[CaCl<sub>2</sub>] 5.9), sand (grain size 0.4-0.6 mm) and perlite (Perligran<sup>®</sup>; Germany, pH[CaCl<sub>2</sub>] 7.0, 95% pores volume) were analysed using the calcium acetate lactate (CAL) method according to Schüller (1969) and found to contain 28, 0, and 0 mg P, and 56, 8, and 0 mg K  $\text{kg}^{-1}$ , respectively. Total N in soil, sand and perlite was analysed using an elemental analyser 1108 (Carlo-Erba, Milan, Italy) coupled to an isotope ratio mass spectrometer (Finnigan MAT Delta C) and contained 1.0, 0.0, and 0.0 mg  $\text{g}^{-1}$ , respectively. The substrate mixture thus contained 18 mg available P, 17 mg available K, and 56 mg total N  $\text{kg}^{-1}$ . Irrigation water

was analysed for the contents of K and found to contain 7.6 and 8.9 mg L<sup>-1</sup> in 2004 and 2005 respectively. Aphids were controlled with regular pesticide applications of Metasystox<sup>®</sup> (S- [2-Ethylsulfinyl) ethyl] O, O-dimethyl phosphorothionate), and infestations with *Peronospora* sp. were controlled by application of Amistar<sup>®</sup> (azoxystrobin) according to manufacturers recommendation. Daily air temperature and sunshine duration were calculated as weekly average (Fig. 6.1).



**Figure 6.1:** Weekly means of daily sunshine duration (bars) and average, lowest and highest daily air temperatures during the experimental period of both years (15th May – 15th August).

### 2.2. Harvesting and analytical procedures

Growth and yield parameters were monitored along the growing period. Plant parts (achenes, capitula, leaves, stems) were separated at physiological maturity, dried (except achenes that were dried at room temperature in a well-aerated area) at 70°C until constant weight in a drying oven, grinded to pass a 1.5 mm sieve, of which, after thorough mixing, a sub-sample

of 5 g was ball-milled to a fine powder. Oil was extracted from crushed achenes using Soxhlet extraction method (SOX THERM 2000, S 206 AK/S2006A, Gerhardt, Bonn, Germany). Extracted oil samples were light-protected and stored directly after extraction in non-transparent polypropylene tubes at  $-18^{\circ}\text{C}$ . Iodine values were determined according to Matissek et al. (1992). For K determination samples were prepared by dry ashing (Dechassa, 2001). 50 mg of dried samples were ashed in a crucible at  $450^{\circ}\text{C}$  in a muffle furnace overnight. Then 1 ml of 0.35 M  $\text{HNO}_3$  solution was added, and after swirling left for at least 10 minutes. After addition of 9 ml of purified water ( $18.2 \text{ M}\Omega \text{ cm}^{-1}$ ), the sample is filtered through ashless filter paper (Whatman® #42, Whatman International Ltd, UK) into polypropylene tubes. Total K of the plant material was measured using inductively-coupled plasma optical emission spectrometry (Spectro Analytical Instruments GmbH, Kleve, Germany).

### 2.3. Statistics and yield component analysis

All statistical analysis was carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means with respect to the influence of K supply were carried out using the GLM procedure considering a fully randomised block design, treating years (2004 and 2005) separately. Where appropriate, data were log transformed to maintain homogeneity of variance. The Bonferoni procedure was employed with multiple t-tests in order to maintain an experimentwise  $\alpha$  of 5%.

Achene yield may be broken down into its components and expressed in a multiplicative fashion as: Achene yield [ $\text{g plant}^{-1}$ ] = (capitula  $\text{plant}^{-1}$ )  $\times$  (achenes capitulum $^{-1}$ )  $\times$  (mass achene $^{-1}$  [g])

Initially Pearson correlations were calculated to test the relation between individual yield components and achene yield. The NOMISS option was used in order to obtain results consistent with subsequent multiple regression calculations. In order to quantify the impact of individual yield components multiple regression analysis is biased as the mathematical product (or the sum in case log-transformed data are used), rather than a statistical relationship, of all individual yield components represents the achene yield. Hence, a yield component analysis according to Piepho (1995) was employed, allowing the contribution of individual components to be quantified. This approach assumes that the SD of log-transformed yield is close to the coefficient of variance of the yield. The approach uses the log-transformed component data and interprets values of  $C_i = \text{Cov}[\log(\text{yield}), \log(\text{component}_i)]$  as an aggregate measure of the  $i^{\text{th}}$  component's contribution to the variability in yield. Finally, the association of selected individual traits with respect to achene yield

was evaluated. Due to the high multi-collinearity of many traits and the limited degrees of freedom stemming from the limited number of observations, selection of traits was done on the basis of hypotheses concerning the importance of various traits for achene yield. In addition to Pearson correlations standardised partial regression coefficients were calculated to evaluate the contribution of each trait to the yield variation upon varied K supply, representing direct effects as used in path coefficient analysis (Dewey and Lu, 1959).

### 3. Results and discussion

#### 3.1. Effect of K on growth and morphology

Both crops responded strongly to increasing K supply with respect to growth and morphology (Table 6.1). Growth and yield of safflower increased up to 1 g K per pot in both years. Increasing K supply in safflower affect particularly stem dry matter, capitula dry matter, and number of branches (Table 6.2) resulting in increased total dry matter. However plant height and leaf DM were affected at the lowest K supply only (Table 6.1). Sunflower growth and yield parameters were continuously increased by increasing K supply in the first experimental year and the optimum K supply could not be deduced. In 2005, K supply covered a wider range and growth and achene yield was optimal at 3 g K per pot. In sunflower, stem dry matter and capitula dry matter were strongly reduced in response to K deficiency resulting in reduced total dry matter, as plant height and leaf DM were also strongly retarded at low K supply. In agreement with our findings, K nutrition was found to have a positive influence on dry-matter production in rapeseed (Khan et al., 2004) and cotton (Sawan et al., 2006). Cakmak et al. (1994) stated that K nutrition had pronounced effects on carbohydrate partitioning by affecting either phloem export of photosynthates (sucrose) or growth rate of sink and / or source organs, retarding plant growth and development.

Deficient K supply was accompanied with leaf burns, plant wilting and lodging, and these symptoms were more pronounced in K-deficient sunflower than in safflower (data not shown). As K is mobile within the plant it is well documented that K is retranslocated to places of new growth (buds, flowers), and under severe deficiency the source organs become chlorotic and finally necrotic (Marschner and Cakmak, 1989). In addition to its significance for water homeostasis (Marschner, 1995), lignification of vascular bundles is also impaired (Pissarek, 1973), representing an important factor contributing to the higher susceptibility of K deficient plants to lodging.

## Chapter 6 Effect of K on growth and yield

**Table 6.1:** Effect of K supply on plant height and plant dry matter (DM) of safflower and sunflower in 2004 and 2005 <sup>1)</sup>.

K supply (g pot <sup>-1</sup> )	Plant height (cm <sup>2</sup> )	Leaf DM (g pot <sup>-1</sup> )	Stem DM (g pot <sup>-1</sup> )	Capitula DM (g pot <sup>-1</sup> )	TDM (g pot <sup>-1</sup> )
Safflower (2004)					
0.00	83.4±2.3 C	8.1±0.3 C	11.4±0.9 C	11.1±3.0 D	29.6±2.1 D
0.25	87.8±3.8 B	13.5±1.5 A	16.8±2.4 B	17.4±1.5 C	63.6±6.6 C
0.50	96.0±2.1 A	11.7±0.6 AB	19.2±1.5 B	22.8±1.8 B	77.3±6.9 BC
1.00	99.7±4.0 A	12.9±1.5 AB	25.2±3.9 A	28.8±2.7 A	100.0±9.9 A
2.00	95.7±5.2 AB	9.9±1.5 B	21.6±2.8 AB	23.4±2.7 AB	83.7±7.5 AB
Safflower (2005)					
0.25	85.1±6.5 A	13.6±1.5 A	16.6±2.6 B	18.0±0.5 B	66.4±3.7 B
0.50	84.8±6.6 A	14.6±1.5 A	19.6±3.2 BA	23.8±0.6 A	84.7±8.2 A
1.00	81.8±6.3 A	12.5±1.1 A	18.6±1.4 BA	26.5±2.6 A	87.0±5.6 A
2.00	86.4±1.6 A	13.0±1.7 A	22.2±2.3 A	26.5±2.3 A	93.9±5.6 A
3.00	90.6±3.5 A	12.3±1.7 A	23.1±0.9 A	26.5±3.2 A	95.6±84 A
Sunflower (2004)					
0.00	58.6±8.1 D	8.0±1.4 C	3.0±1.8 E	2.6±0.6 D	12.7± 1.6 E
0.25	106.8±4.7 C	26.0±0.2 B	16.4±2.2 D	11.0±1.2 C	55.8±0.6 D
0.50	112.8±8.2 BC	25.2±2.4 B	22.6±3.6 C	16.6±0.6 B	70.7±5.8 C
1.00	122.3±6.0 AB	27.0±1.8 AB	33.2±2.8 B	26.4±1.4 A	105.7±3.2 B
2.00	131.4±6.7 A	30.2±1.8 A	44.8±4.2 A	31.4±3.8 A	141.9±3.6 A
Sunflower (2005)					
0.50	117.9±4.3 B	48.7±4.0 BA	32.4±1.1 C	18.7±1.0 D	107.8±2.7 D
1.00	127.6±4.6 AB	51.1±2.2 A	41.1±0.7 B	26.0±1.0 C	128.3±3.5 C
2.00	132.1±3.6 A	53.3±2.0 A	52.0±1.2 A	32.4±1.6 B	193.5±3.4 B
3.00	132.6±5.1 A	44.5±4.3 BA	53.3±2.2 A	39.3±2.2 A	218.3±7.0 A
4.00	128.8±1.2 A	39.4±7.0 B	51.6±1.5 A	32.9±1.1 B	202.8±11.7 B

<sup>1)</sup> for a given species and a given year means within each column followed by the same letter are not significantly different,  $p < 0.05$ ,  $n = 4$ .

### 3.2. Effect of K supply on yield components

Supply of K affected virtually all yield components in both species, though to different degree (Table 6.2). In agreement with El-Nacklawy (1991) the number of capitula in safflower was only slightly affected, although corresponding yield components were shown to be rather responsive to K supply in cotton (Sawan et al., 2006) and oilseed rape (Khan et al., 2004; Mahadkar et al., 1996). As a non-branched sunflower variety was used the number of capitula was not affected in this crop.

The number of achenes per capitulum in safflower was only reduced under conditions of severe K deficiency ( $0 \text{ g K pot}^{-1}$ ), while it remained fairly constant in all other treatments (El-Nacklawy, 1991). Interestingly, number of achenes per capitulum was much lower in the second year, while the reverse holds true for the number of capitula per plant. It is speculated that this stems from the higher P and particularly N supply used in the second year that is known to increase the number of achenes per capitulum (El-Nacklawy, 1991; Abdul Sadiq et al., 2000). To the contrary, in sunflower the number of achenes per capitulum strongly responded to the K supply. The positive effect of K supply on the number of achenes per capitulum agrees well with earlier studies in sunflower (Chaudhry and Mushtaq, 1999; Abdul Sadiq et al., 2000; Lewis et al., 1991), and with the increasing number of achenes per pod in rapeseed (Khan et al., 2004; Mahadkar et al., 1996).

Single achene mass (SAM) in safflower is higher as in sunflower because the former has harder shell than the latter. Single achene mass increased with increasing K supply in both years for both species dramatically, even when most extreme deficiency conditions are not considered. Possible explanation for increasing SAM due to the application of K may be due in part to its favourable effects on photosynthesis and phloem loading (Marschner, 1995). The improved assimilate redistribution with increasing K supply may be particularly important for crops where the period between anthesis and maturity is short. Under these conditions plants with improved nutrient status are at greater advantage than those grown under deficiency conditions (Scott et al., 1973). The improved provision of photosynthates results in increasing capitula DM (Table 6.1) and achene yield (Table 6.2). Pearson correlations were calculated in order to provide further insight into the relationship between individual yield components (Table 6.3). Due to the largely different K supply in the two years and complete failure of the zero K treatment, the lowest and highest K treatments of both crops were excluded from the calculations. Indeed, SAM and achene yield are closely interrelated in both crops. In agreement, positive effects of K supply on achene yield being accompanied by increased SAM have been described for sunflower (Chaudhry and Mushtaq; 1999, Abdul

Sadiq et al., 2000, Lewis et al., 1991), cotton (Sawan et al., 2006), and rapeseed (Khan et al., 2004). In sunflower the number of yield components is rather limited as an un-branched variety was used, and virtually all yield components are highly correlated. In safflower, significant negative associations were observed between the number of achenes per capitulum and the number of capitula per plant, while moderately positive relationships ( $r^2 = 0.4$ ) were identified between the number of capitula per plant and either the SAM and the achene yield. In agreement with Tunctürk et al. (2005) the number of achenes per capitulum exhibited no association with achene yield. Pearson correlations are obviously misleading, as they do not allow elucidating the quantitative contribution of the variation of a given yield component to final yield. Therefore  $c_i$  coefficients, based on the covariance of the log-transformed yield components with the log-yields, were calculated to quantify the contribution of each component to the yield variability (Piepho, 1995). The major effect of increasing achene yield in safflower in response to increasing K supply was due to a higher SAM, followed by the number of capitula per plant (Table 6.4). Contribution of the number of achenes per capitulum to overall yield variation was insignificant. To the contrary, in sunflower this yield component proved most significant.

In safflower oil yield was affected by K deficiency, but except for the most severe deficiency conditions in 2004, this was mainly due to reduced achene yield, while oil concentration showed little variation (Table 6.2). Much to the contrary, oil yield in sunflower was severely affected by low K supply not only because of reduced achene yield, but also due to lower oil concentration, which agrees with previous investigations in sunflower (Chaudhry and Mushtaq, 1999, Khan et al., 1999), cotton (Sawan et al., 2006), oilseed rape (Khan et al., 2004), and soybean (Yin and Vyn, 2003). The reduction of oil concentration in response to K deficiency may be related to its significance in photosynthesis and phloem translocation of carbohydrates (Marschner, 1995). Indeed, for soybean it was shown that the resulting reduction in photosynthate transport to the seed decreases oil concentrations (Gaydou and Arrivets, 1983). The oil concentration in sunflower also reached much higher values as in safflower, which further adds to its significance for oil yield.

## Chapter 6 Effect of K on growth and yield

**Table 6.2:** Effect of K supply on achene yield and yield components of safflower and sunflower in 2004 and 2005<sup>1)</sup>.

K supply	Capitula pot <sup>-1</sup>	Achenes capitulum <sup>-1</sup>	SAM <sup>2)</sup> [mg achene <sup>-1</sup> ]	Achene yield [g pot <sup>-1</sup> ]	Oil conc. [g (100 g <sup>-1</sup> )]	Oil yield [g pot <sup>-1</sup> ]
Safflower (2004)						
0.00	6.7±1.19 B	3.36±2.64 B	13.0±6.3 D	0.7±0.2 D	0.9±0.1 C	0.01±0.00 C
0.25	8.0±1.0 AB	22.60±2.59 A	23.1±1.6 C	12.5±1.5 C	13.6±4.6 B	1.75±0.78 B
0.50	8.0±0.7 AB	28.71±3.95 A	34.5±1.9 B	23.7±3.6 B	21.6±4.1 A	5.22±1.58 A
1.00	11.2±1.4 A	22.86±2.89 A	43.7±5.4 A	33.0±2.3 A	22.8±2.0 A	7.53±0.97 A
2.00	8.7±2.2 AB	25.20±5.56 A	47.6±4.1 A	30.0±4.1 AB	22.5±1.5 A	6.80±1.32 A
Safflower (2005)						
0.25	13.1±1.7 A	14.72±2.37 A	32.1±3.1 B	18.2±2.0 C	20.6±2.2 A	3.79±0.78 B
0.5	15.8±1.3 A	14.43±1.32 A	39.1±2.1 B	26.8±3.4 B	22.9±1.1 A	6.14±1.03 A
1.0	14.3±3.1 A	14.85±4.30 A	48.5±3.1 A	29.4±2.0 BA	20.6±0.8 A	6.05±0.46 A
2.0	15.8±0.4 A	13.48±0.48 A	50.3±2.4 A	32.2±1.5 BA	20.4±0.5 A	6.54±0.27 A
3.0	15.2±2.2 A	14.96±0.62 A	49.9±5.3 A	33.7±4.3 A	20.9±1.8 A	7.08±1.43 A
Sunflower (2004)						
0.00	1	0.0±0.0 D	0.0±0.0 E	0.0±0.0 D	0.0±0.0 D	0.0±0.0 C
0.25	1	35±7 D	10.8±1.3 D	0.7±0.2 D	15.5±0.0 C	0.1±0.0 C
0.50	1	263±42 C	13.1±0.6 C	6.9±1.2 C	14.7±2.4 C	1.0±0.3 C
1.00	1	553±70 B	17.3±1.3 B	19.0±2.1 B	26.9±2.7 B	5.1±0.8 B
2.00	1	765±121 A	26.3±1.0 A	40.5±7.6 A	39.8±1.0 A	17.7±0.9 A
Sunflower (2005)						
0.5	1	247±113 C	12.5±1.2 C	6.0±2.1 C	0.0±0.0 D	0.0±0.0 C
1.0	1	446±47 C	11.2±1.2 C	10.1±1.8 C	6.4±2.8 C	0.7±0.4 C
2.0	1	1032±32 B	27.1±0.7 B	55.8±1.2 B	27.8±1.1 B	15.5±0.8 B
3.0	1	1242±17 A	32.7±1.4 A	81.2±2.5 A	34.0±2.4 A	27.6±2.4 A
4.0	1	1224±144 BA	33.0±2.4 A	80.4±4.7 A	37.4±3.8 A	29.9±2.1 A

<sup>1)</sup> for a given species and year means within each column followed by the same letter are not significantly different,  $p < 0.05$ ,  $n = 4$ .

<sup>2)</sup> Single achene mass.

**Table 6.3:** Pearson coefficients between yield and yield components of safflower (above the diagonal) and sunflower (below the diagonal) (pooled over both years)<sup>1)</sup>.

Variables	Achene yield plant <sup>-1</sup>	Capitula plant <sup>-1</sup>	Achenes capitulum <sup>-1</sup>	Mass achene <sup>-1</sup>
Achene yield plant <sup>-1</sup>	-	0.401*	-0.096	0.914***
Capitula plant <sup>-1</sup>	-	-	-0.899***	0.411*
Achenes capitulum <sup>-1</sup>	0.982***	-	-	-0.267
Mass achene <sup>-1</sup>	0.874***	-	0.767***	-

<sup>1)</sup> Variables were log-transformed. Due to largely different K supply in the two years the lowest and highest K treatments of both crops were excluded from the calculations. Significance indicated as \* for  $p < 0.05$ , \*\* for  $p < 0.01$ , and \*\*\* for  $p < 0.001$ ,  $n = 32$  for each crop.

**Table 6.4:** Estimates of variation coefficients (VC) of achene yield, SD and variance of log-yields, and  $c_i$  coefficients for yield components of safflower and sunflower<sup>1)</sup>.

Crop and year	VC Achene yield (%)	SD log-yield (×100)	Variance log-yield (×100)	$C_i$ for yield component		
				Capitula plant <sup>-1</sup> (×100)	Achenes capitulum <sup>-1</sup> (×100)	Mass achene <sup>-1</sup> (×100)
Safflower						
2004	34.42	40.61	16.49	4.34	0.90	11.25
2005	21.66	24.03	5.77	1.19	0.33	4.26
Pooled	28.10	33.34	11.12	4.18	-1.02	7.96
Sunflower						
2004	95.77	156.63	245.33	-	195.48	49.93
2005	85.36	117.00	136.89	-	83.16	53.75
Pooled	100.17	146.22	213.79	-	159.16	54.67

<sup>1)</sup> Calculations according to Piepho (1995). Due to largely different K supply in the two years the lowest and highest K treatments of both crops were excluded from the calculations ( $n = 16$  for each year,  $n = 32$  when pooled).

### 3.2.1. Important traits determining achene yield

The contribution of various traits to the variation of achene yield as affected by variable K supply were evaluated by Pearson correlations, followed by standardized partial regression coefficients, known as direct effects in path coefficient analysis (Dewey and Lu, 1959).

Traits considered were selected to address several hypothesis derived from relationships established in previous investigations. For example, the produced biomass has frequently been discussed in relation to the yield performance of sunflower (Steer and Hocking, 1984; Hocking and Steer, 1989), for which the shoot DM (without achene DM) was taken as a proxy. The leaf area (Connor and Sadras, 1992) or the number of leaves per plant (Steer and Hocking, 1983; Hocking and Steer, 1989) has also frequently been mentioned due to their importance for providing assimilates, for which the leaf DM was used as indicator. The stem DM was included to consider its importance as transient reservoir for assimilates, K, and N (Steer and Harrigan, 1986). The total N accumulated was taken as indicator for overall yield capacity, due to its pivotal importance for yield formation (Connor and Sadras, 1992), while the amount of K available for plant growth was assessed by the total amount of K accumulated in the plants. Finally, the achene K concentration was taken as indicator of K utilisation efficiency with respect to achene yield.

Achene yield was tightly correlated with the size of the vegetative plant, indicated by the straw and stem DM (Table 6.5). Although area and number of leaves has been frequently discussed in relation to achene yield, the total mass of leaves produced was only weakly associated with it, particularly in safflower, which compares well with the moderate influence of K on this trait (Table 6.1). In agreement the correlation between leaf DM and indicators of the size of the vegetative plant (straw and stem DM) was not particularly close (Table 6.5). Interestingly, a tight negative relationship was observed between achene yield and the achene K concentration in sunflower, while in safflower these two traits were not associated. In both crops yield was significantly correlated to the total amount of K and N accumulated in the above-ground biomass, although with respect to N this relationship was much closer for safflower ( $r^2=0.83$ ) than for sunflower ( $r^2=0.55$ ). Interestingly, in sunflower the total K accumulated was tightly correlated to the DM of the stem and straw ( $r^2\approx 0.95$ ), while this relationship was much weaker in safflower ( $r^2\approx 0.59$ ), which may be discussed in the light of the well-known function of the stem as a transient reservoir for K and other nutrients in sunflower (Connor and Sadras, 1992, Hocking and Steer, 1983). It seems that K is more crucial for sunflower, while in yield formation of safflower the N status is more important.

**Table 6.5:** Pearson correlations between achene yield and selected traits of safflower (above the diagonal) and sunflower (below the diagonal). For selection criteria see text. Significance indicated by \* ( $p < 0.05$ ), \*\* ( $p < 0.01$ ) and \*\*\* ( $p < 0.001$ )<sup>1)</sup>.

Variable	Achene yield	Stem DM	Leaf DM	Straw DM	Achene K conc.	Total K accumul.	Total N accumul.
Achene yield		0.726***	0.064	0.731***	-0.042	0.885***	0.825***
Stem DM	0.900***		0.335	0.939***	0.265	0.597***	0.632***
Leaf DM	0.392*	0.673***		0.478**	-0.240	-0.059	0.386*
Straw DM	0.824***	0.975***	0.813***		0.152	0.592***	0.740***
Achene K concentration	-0.897***	-0.811***	-0.326	-0.735***		-0.126	-0.084
Total K accumulated	0.911***	0.949***	0.675***	0.947***	-0.819***		0.727***
Total N accumulated	0.553***	0.721***	0.921***	0.835***	-0.480**	0.799***	

<sup>1)</sup> All values LOG-transformed. Due to largely different K supply in the two years the lowest and highest K treatments of both crops were excluded from the calculations ( $n = 32$ ).

However, Pearson correlations may be misleading, as several traits that show a high correlation with achene yield are highly correlated to each other, resulting in substantial multicollinearity. For example, the amount of K accumulated in the above-ground biomass was associated with the DM of stems and straw (Table 6.5). A highly significant multiple regression model considering selected traits as specified above exhibits an adjusted  $r^2$  of 0.84 and 0.94 for safflower and sunflower, respectively (Table 6.6). Standardised partial regression coefficients, allowing assessment of the direct effect of a certain trait on achene yield, reveal that the leaf DM is in fact negatively associated with yield, and that the stem DM is the most important trait in sunflower, while in safflower the DM of both the stems and the straw are equally important. The importance of the stem DM in sunflower is also seen in the strong response of this trait to different K supply (Table 6.1). The achene K concentration is of marginal importance, indicating that adequate K supply is essential for building photosynthetic capacity, but does not necessarily mean that it accumulates in achenes as the increased provision of assimilates dilutes their K concentration (Table 6.6). While in safflower both N and K accumulated in the shoot are equally important, the impact of K accumulated in

sunflower is rather overwhelming, which agrees well with previous reports stressing the importance of K for yield formation in sunflower (Grove and Sumner, 1982, Lewis et al., 1991, Chaudhry and Mushtaq, 1999). Further aspects of K utilisation efficiency are not considered here as they are covered in a forthcoming publication.

**Table 6.6:** Standardised partial regression coefficients for selected traits and achene yield and statistics for the multiple linear regression model in safflower and sunflower. For selection criteria see text<sup>1)</sup>.

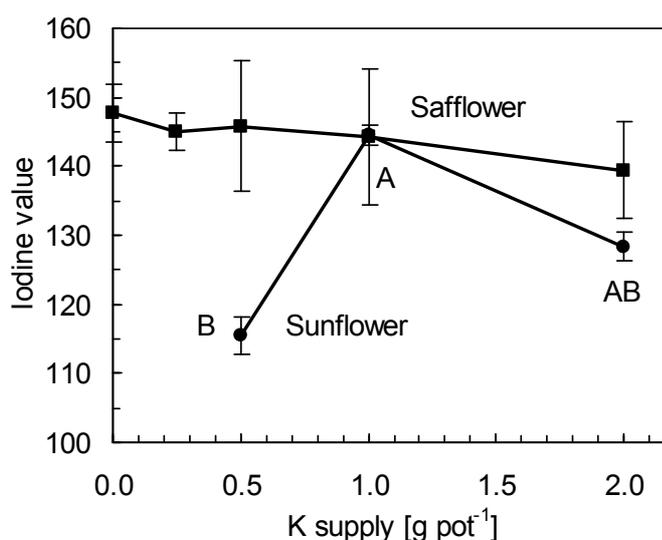
Variable	Safflower	Sunflower
Stem DM	0.206	0.514
Leaf DM	-0.295	-0.501
Straw DM	0.229	0.020
Achene K concentration	-0.116	-0.150
Total K accumulated	0.329	0.485
Total N accumulated	0.351	0.167
Regression model		
Adjusted $r^2$	0.837	0.941
Significance	$p < 0.0001$	$p < 0.0001$

<sup>1)</sup> All values LOG-transformed. Due to largely different K supply in the two years the lowest and highest K treatments of both crops were excluded from the calculations (n = 32).

### 3.3. Effect of K supply on oil quality

Safflower is considered to be a source of the highest quality vegetable oils (Kaffka et al., 2001). As compared to sunflower and olive oil, it is particularly rich in unsaturated fatty acids (Li and Mündel, 1997) and has a higher hypocholesterolemic activity as compared to sunflower oil and other vegetable oils (Kumar, 2000). In agreement, iodine numbers indicating the relative abundance of polyunsaturated fatty acids in the oil were usually higher in safflower than in sunflower and for both species fell in the range of reported values (Kumar, 2000; Roth and Kormann, 2005). In safflower there was only an insignificant trend towards lower iodine values as K supply was increased, while in sunflower K deficiency resulted in a significant reduction of this oil quality parameter (Fig 6.2). In sunflower the beneficial effect of K supply on the accumulation of polyunsaturated fatty acids has been frequently demonstrated (Mekki et al., 1999; Forment et al., 2000), which is seen in the present study only in the severe K deficiency range. In agreement, a similar response was observed for cotton seed

oil (Sawan et al., 2006). To the contrary, influence of K supply on iodine values of safflower oil was insignificant, which has not been reported before. Safflower oil quality is thus not adversely affected by low K status. Apparently, the oil quality is merely influenced by genotype, capitulum position (primary, secondary and tertiary branches), location and stage of maturity as found in safflower (Nagaraj and Reddy, 1997). In addition to genotype, the temperature during oil formation and water stress can affect oil quality as found in sunflower (Connors and Sadras, 1992).



**Figure 6.2:** Effect of K supply on iodine value of safflower and sunflower in 2004. Means assigned the same letter are not significantly different at  $p < 0.05$  ( $n = 4$ ).

#### 4. Conclusion

Both crops responded strongly to varied K supply, resulting in reduced vegetative growth and yield under conditions of limited K supply, and the response of sunflower was particularly strong. As a single-headed sunflower variety was used the variation in achene yield was merely associated with variations in the number of achenes per capitulum, while in safflower the mass per achene was the most responding yield component, followed by the number of capitula per plant. Several traits, which were selected on the basis of previous hypothesis concerning their association with yield formation, were evaluated concerning their importance. In sunflower, the stem DM and the total amount of K accumulated in the above-ground biomass was found to be most important, while in safflower the total amount of K and N accumulated showed the highest standardised partial regression coefficients. Based on this analysis and in view of the relative yield in response to altered K supply it may be concluded that sunflower is more sensitive to inadequate K supply than safflower. The differen-

tial efficiency of safflower and sunflower with respect to K utilisation will be the focus of a forthcoming publication.

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## Chapter 7

### Potassium Efficiency of Safflower (*Carthamus tinctorius* L.) and Sunflower (*Helianthus annuus* L.)

#### Summary

The oil crop safflower may have a certain production potential under low-input conditions (organic farming, developing countries), where the putatively low nutrient requirement is highly welcomed. However, current knowledge regarding the nutrient use efficiency of safflower as compared to similar oil crops is limited. It was thus the aim of this study to determine the potassium use efficiency of safflower (*Carthamus tinctorius* L.) as compared to sunflower (*Helianthus annuus* L.). Safflower and sunflower were cultivated with increasing K supply in a mixture of equal volumes of sand, nutrient-poor limed soil, and perlite in 6 L Mitscherlich pots.

Both species responded strongly to increasing K supply with respect to growth and yield. Safflower out-yielded sunflower at low K supply, while at high K level the opposite was observed. Both species accumulated similar amounts of total K in whole shoots per pot at low, equal K supply, except at extremely low K levels where safflower out-yielded sunflower. However, achene yield of sunflower exceeded that of safflower at optimal and high K supply. K concentration in safflower tissues was significantly lower than in sunflower at optimal and suboptimal K levels. Safflower utilizes absorbed K more efficiently than sunflower to produce achene yield at suboptimal and optimal K supply in terms of efficiency ratio, but only at suboptimal K availability when considering the utilization index. The efficiency of a crop to use accumulated K for dry matter and achene production may be interpreted in terms of Michaelis-Menten (MM) kinetics, specifically addressing the shape of the nutrient response curve. Indeed the efficiency of safflower to use K for growth and yield, analogues to a low  $K_m$  in enzyme kinetics, was higher than in sunflower, while the K accumulation required to initiate yield formation in safflower was significantly lower. Similarly, safflower required much less external K to produce a given amount of achenes than sunflower at low and optimal K supplies. It can be concluded that in terms of K availability safflower represents a low input crop and outperforms sunflower on soils low in available K. The data analysis also reveals that using just one efficiency indicator is usually not sufficient to adequately describe the K efficiency of the crop under consideration.

### 1. Introduction

Next to nitrogen, potassium (K) is the second most abundant mineral macronutrient in plants (Marschner, 1995), and application of K fertilizer has been shown to be particularly effective with respect to yield formation in safflower (El-Nakhlawy, 1988) and sunflower (Grove and Sumner, 1982). Large amounts of K are required by high-yielding crops, and although total K contents of most soils are rather high, availability is usually low due to enclosure of K in silicates and strong adsorption by K-specific binding sites. Thus, applying K fertiliser represents an effective measure to ensure adequate K supply. In addition, in developing countries, where the proportion of less fertile soils is particularly high, it may be difficult to fulfil the nutritional requirements of high-yielding crops (Sauerbeck and Helal, 1990; Marschner, 1995). More efficient varieties of a given crop may be considered (Marschner, 1995; Gerloff and Gabelman, 1983), but large differences with respect to K demand between closely related, alternative crops may also be worth exploiting. In view of resource limitations it is thus desirable to aim for efficient use of K. Therefore, the identification of crops species or cultivars with greater tolerance to suboptimal nutrient availability offers considerable promise for increasing the production potential on marginal land (Baligar et al., 1990; Gourley et al., 1994).

The term 'nutrient efficiency' has been used widely as a measure of the capacity of a plant to acquire and utilize nutrients (Gourley et al., 1994), and may be broken down mechanistically into the ability to acquire nutrient from the soil (uptake efficiency) and the ability to utilize accumulated nutrients for biomass production or yield formation (use efficiency) (Sattelmacher et al., 1994). Definitions of nutrient efficiency vary greatly (Sattelmacher et al., 1994), and in some cases may even be misleading in the quest for identifying mechanisms for enhanced nutrient acquisition and utilization (Gourley et al., 1994). They generally can be divided into those emphasizing productivity and those emphasizing the internal nutrient requirement of the plant. With regard to yield parameters, nutrient efficiency has been defined as the ability to produce a relatively high yield on a substrate that would otherwise limit the production of a standard line (Buso and Bliss, 1988; Graham, 1984). Other definitions of nutrient efficiency, also referred to as 'agronomic efficiency' include production of dry matter (DM) or harvestable products, per unit of nutrient applied (Blair and Cordero, 1978; Sauerbeck and Helal, 1990). The 'external nutrient requirement' refers to the amount of nutrient in the media required to achieve a given percentage of maximum yield (Föhse et al., 1988; Fox, 1981). Yield response per unit of added nutrient has also been used as a measure of nutrient efficiency (Blair, 1983; Baligar et al., 1990).

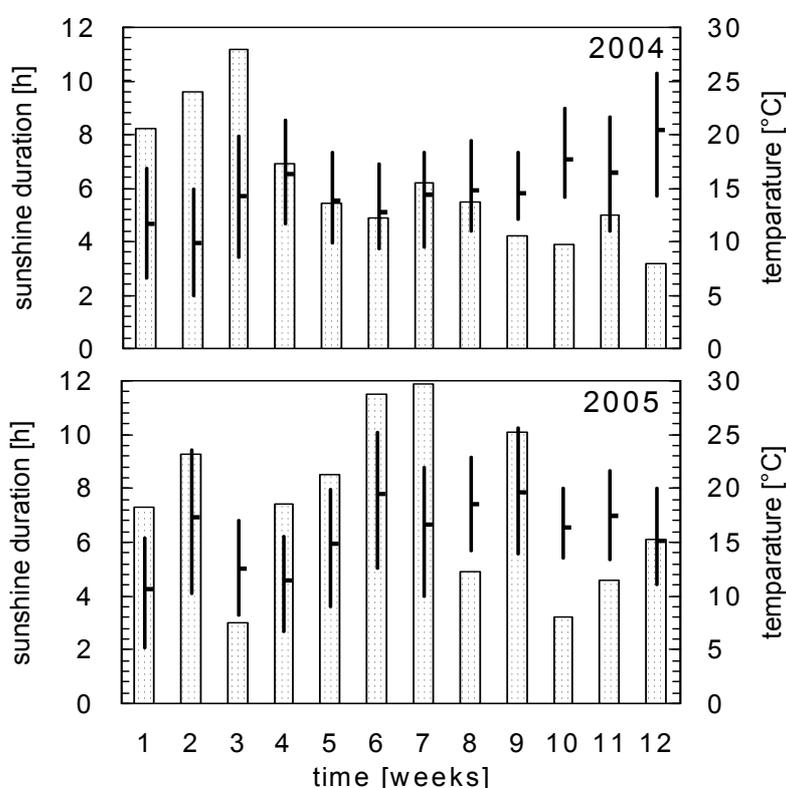
Safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annuus* L.), both belonging to the *Asteraceae*, are important oil crops in tropical areas. Safflower is a highly branched, herbaceous, thistle-like annual, 30-150 cm tall with globular flower heads (capitula), characterised by a strong taproot, which enable it to thrive in dry climates and can access and utilize nutrients from below the root zone of cereal crops (Li and Mündel, 1997). The oil crop sunflower, however, is much taller, usually un-branched, lacks a taproot, and is considered more demanding in terms of nutrients and water (Diepenbrock and Pasda, 1995). Although both crops thrive in similar environments, direct comparisons of their response to increasing K availability with respect to K use efficiencies have not been carried out, and the putatively higher K use efficiency of safflower is not sustained. Therefore, the aim of this study is to directly compare the K use efficiency of safflower as compared to sunflower under semi-controlled conditions.

## 2. Materials and Methods

### 2.1. Experimental Conditions

Experiments using safflower (*Carthamus tinctorius* L., variety 'Sabina') and sunflower (*Helianthus annuus* L., variety 'Salut R M') were conducted in the period from May to October 2004 and 2005 under semi-controlled conditions. In 2004 five K levels (0.0, 0.25, 0.5, 1.0, and 2.0 g per pot) were used in four replicates for both species. Other nutrients added were 0.72, 2.0, and 1.0 g per pot of P, N, and Mg respectively. In 2005, based on the results obtained in 2004, K levels were adapted to 0.25, 0.5, 1.0, 2.0, and 3.0 g per pot for safflower and to 0.5, 1.0, 2.0, 3.0, and 4.0 g per pot for sunflower. N supply was the same as in 2004 for safflower and increased to 4.0 g per pot for sunflower. P supply was increased to 2.0 g P per pot for both species in 2005, and micronutrients were always added in adequate amounts. Three safflower and two sunflower plants were cultivated individually in Mitscherlich pot (6 L), in a mixture of equal volumes of limed nutrient-poor soil (limed nutrient-poor Ferrasol containing 9.6 mg g<sup>-1</sup> organic matter, pH[CaCl<sub>2</sub>] 5.9), sand (grain size 0.4-0.6 mm), and perlite (Perligran<sup>®</sup>, Germany, pH[CaCl<sub>2</sub>] 7.0, 95% pores volume) under outside conditions until the end of flowering (middle of August), and maintained under greenhouse conditions (day night temperature was adjusted to 28 °C, and 15 °C respectively) with additional lightening (intensity of light supplementation at canopy level was 260 μmol m<sup>-2</sup> s<sup>-1</sup>). Before being used in the experiment, soil, sand and perlite were analysed using the calcium acetate lactate (CAL) method according to Schüller (1969) and found to contain 28, 0, and 0 mg P, and 56, 8, and 0 mg K kg<sup>-1</sup>, respectively. Total N in soil, sand and perlite was analysed using

an elemental analyser 1108 (Carlo-Erba, Milan, Italy) coupled to an isotope ratio mass spectrometer (Finnigan MAT Delta C) and contained 1.0, 0.0, and 0.0 mg g<sup>-1</sup>, respectively. The substrate mixture thus contained 18 mg available P, 17 mg available K, and 56 mg total N kg<sup>-1</sup>. Irrigation water was analysed for the contents of K and found to contain 7.6 and 8.9 mg L<sup>-1</sup> in 2004 and 2005 respectively. Aphids were controlled with regular pesticide applications of Metasystox<sup>®</sup> (S- [2-Ethylsulfinyl) ethyl] O, O-dimethyl phosphorothionate), and infestations with *Perenospora* sp. were controlled by application of Amistar<sup>®</sup> (azoxystrobin) according to manufacturers recommendation. Daily air temperature and sunshine duration were calculated as weekly average (Fig. 7.1).



**Figure 7.1:** Weekly means of daily sunshine duration (bars) and average, lowest and highest daily air temperatures during the experimental period of both years (15<sup>th</sup> May – 15<sup>th</sup> August).

## 2.2. Harvesting and analytical procedures

Plant parts (achenes, capitula, leaves, stems) were separated at physiological maturity, dried (except achenes that were dried at room temperature in a well-aerated area) at 70°C until constant weight in a drying oven, grinded to pass a 1.5 mm sieve, of which, after thorough mixing, a sub-sample of 5 g was ball-milled to a fine powder. Oil was extracted from crushed achenes using Soxhlet extraction (SOX THERM 2000, S 206 AK/S2006A, Gerhardt, Bonn, Germany). For K determination samples were prepared by dry ashing (*Dechassa*,

2001). 50 mg of dried samples were ashed in a crucible at 450°C in a muffle furnace overnight. Then 1 ml of 0.35 M HNO<sub>3</sub> solution was added, and after swirling left for at least 10 minutes. After addition of 9 ml of purified water (18.2 MΩ cm<sup>-1</sup>), the sample was filtered through ashless filter paper (Whatman® #42, Whatman International Ltd, UK) into polypropylene tubes. Total K of the plant material was measured using inductively-coupled plasma optical emission spectrometry (Spectro Analytical Instruments GmbH, Kleve, Germany).

### 2.3. Statistical analysis

All statistical analysis was carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means with respect to the influence of K supply were carried out using the GLM procedure considering a fully randomised design, treating years separately. Where appropriate, data were log transformed to maintain homogeneity of variance. With multiple t-test the Bonferoni procedure was employed in order to maintain an experimentwise  $\alpha$  of 5%.

Response curves for DM and achene yield production were derived from the relationship between each yield parameter (g pot<sup>-1</sup>) and the amount of K accumulated in the plant's shoots (mg K pot<sup>-1</sup>) using the following Michaelis-Menten-type equation

$$\text{Yield} = (A_{\text{max}} \times (\text{mg K} - b) / (c + (\text{mg K} - b)))$$

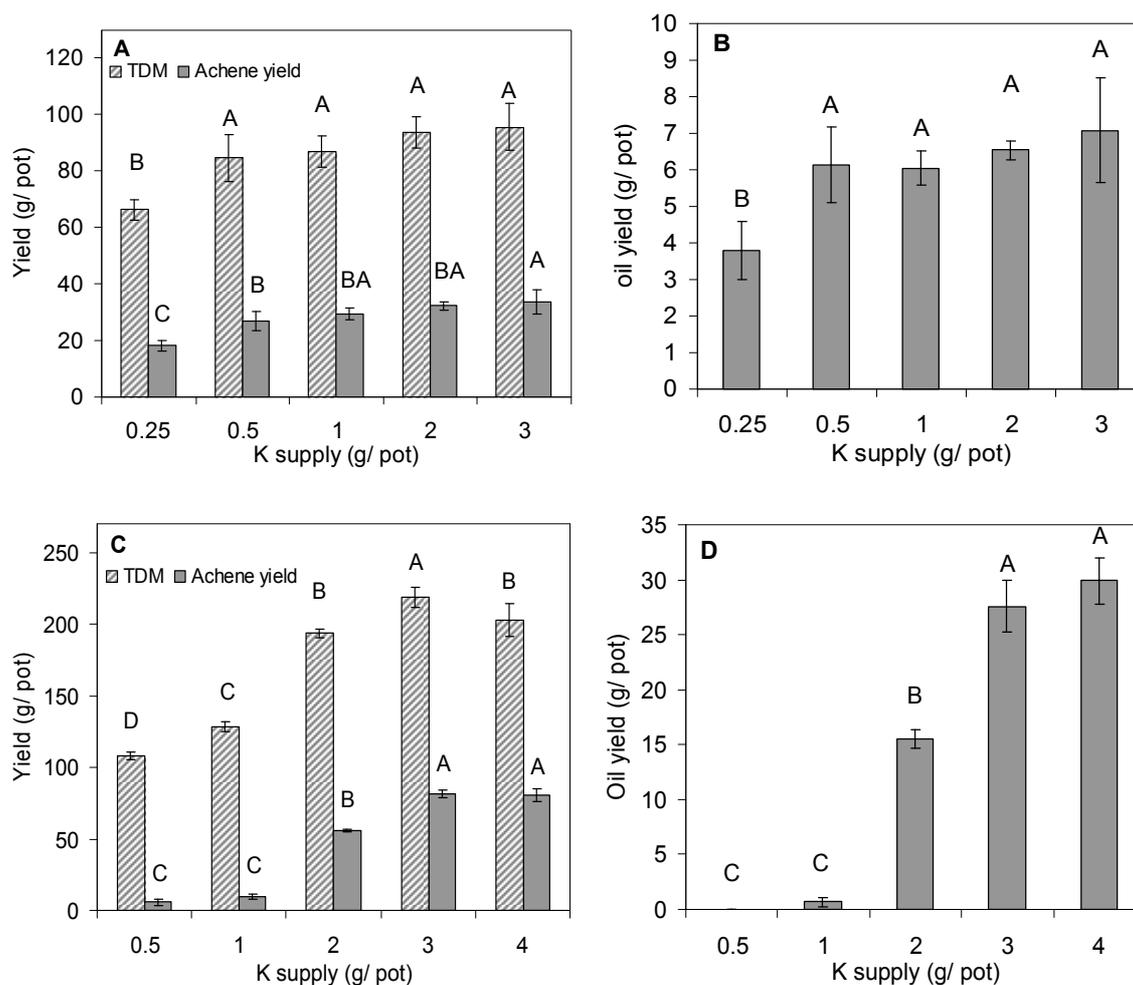
with 'A<sub>max</sub>' as an estimate of maximum yield, 'b' as the minimum K accumulation required for yield formation (achenes) corresponding to the c<sub>min</sub> in enzyme kinetics, and 'c' as the K accumulation required for half maximum yield, corresponding to the K<sub>m</sub>. The term 'b' was omitted in the case of DM, as there is no K-free biomass. The Michaelis-Menten equation proved superior to the Mitscherlich curve and has been widely used to describe nutrient efficiency (e.g. *Gourley et al.*, 1994, *Steingrobe and Claassen*, 2000). Curve fitting was carried out using the procedure NLIN in SAS, employing the Gauss-Newton algorithm. The internal K requirement of the two species is defined as 'c', the amount of K accumulation (in mg per pot) required to produce 50 % of predicted maximum yield (*Föhse et al.*, 1988).

## 3. Results and discussion

### 3.1. Effect of K supply on K accumulation and uptake efficiency

Both crops responded strongly to increasing K supply with respect to growth and yield (Fig. 7.2). Growth and yield of safflower increased up to 0.5 g K per pot, while sunflower's optimal growth and yield was achieved at 3 g K pot<sup>-1</sup> in the experiment of 2005. Data for 2004 are not shown as the optimal K supply was not reached in sunflower. Both species

accumulated increasing and similar K amounts in their shoots at equal K levels in 2004 except at the very low level (0 g K pot<sup>-1</sup>) at which safflower accumulated twice the amount of K than sunflower (Table 7.1). In 2005, where K supplies are largely extended, sunflower accumulated higher amounts of K than safflower. At their respective optimal K supply (0.5 g K, and 3 g K pot<sup>-1</sup> for safflower and sunflower, respectively) sunflower accumulated more than 4-times as much K as safflower.



**Figure 7.2:** TDM and achene yield (A, C) and oil yield (B, D) of safflower (A, B) and sunflower (C, D) over a range of K supply (means  $\pm$  SD,  $n = 4$ ). Bars assigned the same letter are not significantly different ( $p < 0.05$ ).

## Chapter 7 Potassium use efficiency

**Table 7.1:** Effect of K supply on total accumulated K, K concentration, and plant K uptake efficiency of safflower and sunflower <sup>1)</sup>.

K supply (g pot <sup>-1</sup> )	Safflower		Sunflower	
	total K accumulated (mg K pot <sup>-1</sup> )			
K	2004	2005	2004	2005
0.00	154.0 ± 12.7 E*	-	82.1 ± 11.2 E	-
0.25	296.3 ± 48.0 D <sup>n.s</sup>	457.6 ± 14.1 C <sup>n.s</sup>	352.1 ± 21.4 D	-
0.50	529.0 ± 32.9 C <sup>n.s</sup>	671.9 ± 41.1 C*	528.4 ± 23.1 C	988.3 ± 40.5 D
1.00	952.7 ± 63.6 B <sup>n.s</sup>	1116.5 ± 141.6 B <sup>n.s</sup>	912.6 ± 35.2 B	1161.0 ± 57.7 D
2.00	1302.8 ± 94.9 A <sup>n.s</sup>	1736.2 ± 186.0 A*	1386.0 ± 30.5 A	2116.5 ± 197.9 C
3.0	-	1965.4 ± 81.2 A*	-	3006.6 ± 73.4 B
4.0	-	-	-	3378.7 ± 209.6 A
	K concentration (mg K (g DM) <sup>-1</sup> )			
0.00	5.0 ± 0.3 D*	-	6.4 ± 0.4 C	-
0.25	5.0 ± 0.5 D*	6.9 ± 0.2 C <sup>n.s</sup>	6.9 ± 0.2 C	-
0.50	6.9 ± 0.7 C <sup>n.s</sup>	8.0 ± 0.5 C <sup>n.s</sup>	7.5 ± 0.1 B	9.3 ± 0.6 C
1.00	9.6 ± 0.5 B*	12.9 ± 1.8 B*	8.7 ± 0.1 A	9.0 ± 0.3 C
2.00	15.6 ± 0.4 A*	18.5 ± 1.1 A*	8.9 ± 0.3 A	11.0 ± 1.1 C
3.0	-	20.6 ± 1.1 A*	-	13.8 ± 0.6 B
4.0	-	-	-	16.7 ± 1.8 A
K	K uptake efficiency (mg K accumulated (mg K provided) <sup>-1</sup> )			
0.00	/±/	-	/±/	-
0.25	1.19 ± 0.19 A*	1.87 ± 0.06 A <sup>n.s</sup>	1.44 ± 0.04 A	-
0.50	1.06 ± 0.07 A <sup>n.s</sup>	1.34 ± 0.08 B*	1.06 ± 0.05 B	1.98 ± 0.08 A
1.00	0.95 ± 0.06 A <sup>n.s</sup>	1.12 ± 0.14 B <sup>n.s</sup>	0.91 ± 0.03 C	1.16 ± 0.06 B
2.00	0.67 ± 0.03 B <sup>n.s</sup>	0.87 ± 0.09 C <sup>n.s</sup>	0.71 ± 0.03 D	1.06 ± 0.1 B
3.0	-	0.66 ± 0.03 D*	-	1.00 ± 0.03 B
4.0	-	-	-	0.85 ± 0.05 C

<sup>1)</sup> Means ± SD not assigned the same letter within each column indicate significant difference ( $p < 0.05$ ,  $n = 4$ ). \* and n.s indicate significance and insignificant difference between species in a given year, respectively ( $p < 0.05$ ).

Our results agree with *Spear et al.*, (1978b), who found that sunflower is a high K demanding crop, whose K uptake rate exceeds the mean of 10 cassava cultivars. Increasing provi-

sion of K caused a dramatic increase of the K concentration ( $\text{mg K (g DM)}^{-1}$ ) in both species and both years. The same response was documented in sunflower plants (*Spear et al.*, 1978a, b, c; *Grove and Sumnar*, 1982) grown on increasing K supplies. The K concentration in safflower tissues was lower than that in sunflower at low levels of K supply (0 and  $0.25 \text{ g K pot}^{-1}$ ) according to the strong reduction of DM production in sunflower, but concerning the respective optimal K supply rates, sunflower exceeded safflower with respect to concentrating K in their tissues, or, on the other hand, sunflower requires a higher tissue K concentration to perform optimally in terms of yield production.

Potassium uptake efficiency (*Moll et al.*, 1982) was the same in both species at most equivalent levels of both years, but sunflower was slightly more efficient than safflower at 0.25, 0.50, and  $3.0 \text{ g K pot}^{-1}$ . At their respective optimal supply levels, safflower was more efficient in K uptake than sunflower. In both species and both years, the uptake efficiency increased with decreasing K supply (Table 7.1). Several examples show that some plants grow satisfactory under variable conditions of soil nutrient supply by inducing nutrient uptake through modifications of uptake mechanisms as general response to nutrient stress (*Marschner*, 1995). The size of the root system, the physiology of uptake and the ability of plants to increase K solubility in the rhizosphere are considered as mechanisms of uptake efficiency (*Steingrobe and Claassen*, 2000), but these mechanisms are not clearly documented and understood (*El Dessougi et al.*, 2002).

### 3.2. Effect of K supply on K utilization efficiencies

To characterize different genera, species or genotypes for nutrient use efficiency (NUE), researchers include many criteria such as; presence or absence of deficiency symptoms (*Diers and Fehr*, 1989; *Jensen et al.*, 1986), absolute growth at a limiting nutrient level (*Chisholm and Blair*, 1988; *Schettini et al.*, 1987), relative growth obtained by comparing growth at limiting and adequate nutrient levels (*Coltman et al.*, 1985; *Gerloff*, 1987), efficiency ratio (ER) or amount of biomass produced per unit of nutrient present in the tissues (*Coltman et al.*, 1985; *Woodend*, 1986), and utilization coefficient as the inverse of the whole plant nutrient concentration, the latter being expressed on a dry matter basis (*Steenbjerg and Jakobsen*, 1963; *Loneragan and Asher*, 1967). The utilization index (*Siddiqi and Glass*, 1981; *Woodend*, 1986), which is defined as biomass per unit of tissue nutrient concentration, was proposed by *Siddiqi and Glass* (1981) as an improved measure that, unlike the efficiency ratio, takes differences in the amount of produced biomass into consideration. Agronomic efficiency denotes the biomass, or harvestable product, produced per

unit of nutrient applied (*Blair and Cordero, 1978; Moll et al., 1982*). While such measures are relatively easily obtained, they may not reflect, or be correlated with agronomic NUE. Despite the considerable differences in the utilization of absorbed nutrients, there is as yet little understanding of the underlying cause of these differences (*Gourley et al., 1994; Svečnjak and Rengel, 2006*).

### 3.2.1. Effect of K supply on agronomic K efficiency

The term 'external nutrient requirement' refers to the amount of nutrient in the media required to produce a given percentage of maximum yield (*Föhse et al., 1988; Fox, 1981*). Accordingly we adopted a calculation that defines the required external K quantity (g) to produce 1 kg of achenes. It is obvious that safflower's requirement for external K decrease significantly when the K supply is reduced (Table 7.2). To the contrary, sunflower's agronomic K requirement increased with decreasing K supply and was much higher than that of safflower at their respective optimal K supplies, considering that 0.5 g K, and 3.0 g K per pot were the optimal K supplies for safflower and sunflower, respectively. As a genotype that is able to realize a yield above average under conditions of suboptimal nutrient supply has been named nutrient efficient (*Graham, 1984; Sattelmacher et al., 1994*), safflower, unlike sunflower, can be considered a low input oil crop adapted to grow and produce under K-limited conditions.

The harvest index (HI), which indicates the partitioning of assimilates to economic sinks (*Sattelmacher et al., 1994*), was higher in safflower than in sunflower at suboptimal K supply in 2005, and in 2004 throughout. However, sunflower had a higher HI than safflower at their respective optimal K supplies (Table 7.2). The HI increased by increasing K supply in both species and was strongly reduced at low K supply, particularly in sunflower.

## Chapter 7 Potassium use efficiency

**Table 7.2:** Effect of K supply on the external K requirement, K utilization index according to *Siddiqi and Glass (1981)*, and harvest index of safflower and sunflower in 2004 and 2005<sup>1)</sup>.

K supply (g pot <sup>-1</sup> )	Safflower		Sunflower	
K	2004	2005	2004	2005
External K requirement for achene production (g N applied (kg achenes) <sup>-1</sup> )				
0.00	/±/	-	/±/	-
0.25	20.0 ± 2.0 C *	14.0 ± 2.0 D	352.0 ± 94.0 A	-
0.50	22.0 ± 4.0 C *	19.0 ± 3.0 D*	75.0 ± 15.0 B	103.0 ± 24.0 A
1.00	31.0 ± 2.0 B *	34.0 ± 2.0 C*	53.0 ± 6.0 C	92.0 ± 9.0 A
2.00	68.0 ± 9.0 A *	62.0 ± 3.0 B*	45.0 ± 2.0 C	36.0 ± 1.0 C
3.0	-	90.0 ± 12.0 A*	-	37.0 ± 1.0 C
4.0	-	-	-	50.0 ± 3.0 B
K utilization index for achene production (g achene / (g K (g DM) <sup>-1</sup> ))				
K	2004	2005	2004	2005
0.00	1.5 ± 0.54 C*	-	0.0 ± 0.0 E	-
0.25	24.9 ± 2.2 B *	26.5 ± 3.2 AB	1.1 ± 0.3 D	-
0.50	35.2 ± 7.4 A*	33.9 ± 5.9 A*	9.2 ± 1.4 C	6.3 ± 2.0 C
1.00	34.6 ± 4.0 A*	23.1 ± 3.4 B*	22.0 ± 1.5 B	11.1 ± 1.9 B
2.00	18.8 ± 3.5 B*	17.5 ± 1.5 C*	41.9 ± 8.3 A	51.4 ± 5.8 A
3.0	-	16.5 ± 2.9 C*	-	59.0 ± 3.3 A
4.0	-	-	-	48.7 ± 7.6 A
Harvest index (g achenes g DM <sup>-1</sup> )				
K	2004	2005	2004	2005
0.00	2.3 ± 0.9 D*	-	0.0 ± 0.0 E	-
0.25	21.6 ± 3.4 C*	26.0 ± 1.6 B <sup>n.s</sup>	1.4 ± 0.2 D	-
0.50	30.5 ± 2.2 B*	31.5 ± 1.2 A*	10.9 ± 1.2 C	5.7 ± 2.1 C
1.00	33.0 ± 1.3 AB*	33.8 ± 1.5 A*	18.1 ± 2.3 B	7.8 ± 1.3 C
2.00	35.9 ± 2.4 A*	34.3 ± 2.0 A*	29.6 ± 1.6 A	28.8 ± 0.6 B
3.0	-	35.2 ± 1.5 A <sup>n.s</sup>	-	37.2 ± 1.3 A
4.0	-	-	-	39.7 ± 1.6 A

<sup>1)</sup> Means ± SD not assigned the same letter within each column indicate significant difference ( $p < 0.05$ ,  $n = 4$ ). \* and n.s indicate significance and insignificant difference between species in a given year, respectively ( $p < 0.05$ ).

### 3.2.2. Effect of K supply on K utilization efficiency

In terms of DM production, safflower showed higher potassium ER values than sunflower at low K supply in both years, but decreased to values lower than those of sunflower at the

highest K supply (Table 7.3). Safflower was also much more efficient in utilising K to produce achenes and oil than sunflower at similar levels in both years except the highest K supply, where sunflower had significantly higher potassium ER than safflower. At their respective optimal K supplies, safflower was found to be more efficient than sunflower in utilizing K for DM and achene production, and both species had the same K utilization efficiency in terms of oil production. These results are in agreement with the agronomic K efficiency discussed earlier. The low K utilization efficiency of sunflower agrees with the assessment by *Spear et al.* (1978b) who found that at maximum yield, sunflower is less efficient in utilizing K for DM production than 10 cultivars of cassava, and had half the efficiency coefficient compared to maize when the three species (sunflower, cassava, and maize) were grown with increasing K concentrations in solution culture. The ability of safflower to utilize potassium more efficiently in DM production, together with the capacity to maintain continued growth at low potassium supply (*Spear et al.*, 1978a) may be interpreted as an adaptation of safflower to soils of poor K status, as discussed previously with respect to different genotypes of wheat (*Zhang et al.*, 1999) and snap bean (*Shea et al.*, 1968).

Both species showed increasing KER to produce DM with decreasing K supply (*Spear et al.*, 1978b, *Asher and Loneragan*, 1967), in order to adapt to nutrient-poor environments simply by enhancing their nutrient use-efficiency (NUE), and for more economical use of each unit of nutrient acquired for DM production (*Hocking and Steer*, 1989). In safflower, both achene - and oil-based KER increased with decreasing K supply, but not at very low K levels. To the contrary, sunflower achenes and oil KER decreased with decreasing K supply. Commonly, NUE is measured in relation to vegetative growth without consideration of economic productivity of crops. Vegetative measures can be useful, particularly in forage crops, but their validity in quantification of utilization efficiency in grain crops is questionable (*Woodend and Glass*, 1993). It appears that if genotypes differ considerably for harvest index and if yield is not positively correlated with total biomass, vegetative measures for KUE are unreliable as indicator of agronomic KUE (*Woodend and Glass*, 1993). Our findings indicate that safflower and sunflower differ for harvest index (Table 7.2), therefore vegetative measures of KUE should not be used as selection criteria for improving agronomic KUE.

**Table 7.3:** Effect of increasing K supply on potassium efficiency ratios (KER) for the production of dry matter, achenes, and oil (g DM, achenes, oil g<sup>-1</sup> K).

K supply (g/pot)	KER dry matter		KER achenes		KER oil	
	Safflower	Sunflower	Safflower	Sunflower	Safflower	Sunflower
2004						
0.00	200±11.3 A*	155±9.0 A	5±1.7 D*	0±0.0 D	0.1±0.0 D	0.0±0.0E
0.25	200±20.2 A*	147±15.7 AB	43±4.6 A*	2±0.5 C	5.8±2.0BC*	0.3±0.1D
0.50	147±14.6 B	134±7.9 B	45±6.9 A*	13±2.7 B	11.4±1.3 A*	2.0±0.6C
1.00	105±5.6 C	116±5.2 C	35±1.4 B*	21±1.7 A	7.9±0.6 B*	5.6±0.8B
2.00	64±1.5 D*	102±3.1 D	23±1.7 C	28±5.6 A	5.2±0.7 C*	11.0±2.3A
2005						
0.25	145±4.3 A	-	40±4.8 A	-	8.3±1.8 A	-
0.50	126±8.1 B*	107±6.8 A	40±3.4 A*	6±2.0 B	9.1±1.2 A*	0±0 C
1.00	79±10.8 C*	111±3.6 A	27±2.7 B*	9±1.3 B	5.5±0.8 B*	0.6±0. C3
2.00	54±3.1 D*	92±8.8 B	19±2.1 C*	27±2.9 A	3.8±0.5 B*	7.4±1.0 B
3.0	49±2.6 D*	73±3.2 C	17±1.6 C*	27±0.9 A	3.6±0.6 B*	9.2±0.7 A
4.0	-	60±6.1 C	-	24±2.8 A	-	8.9±0.9 BA

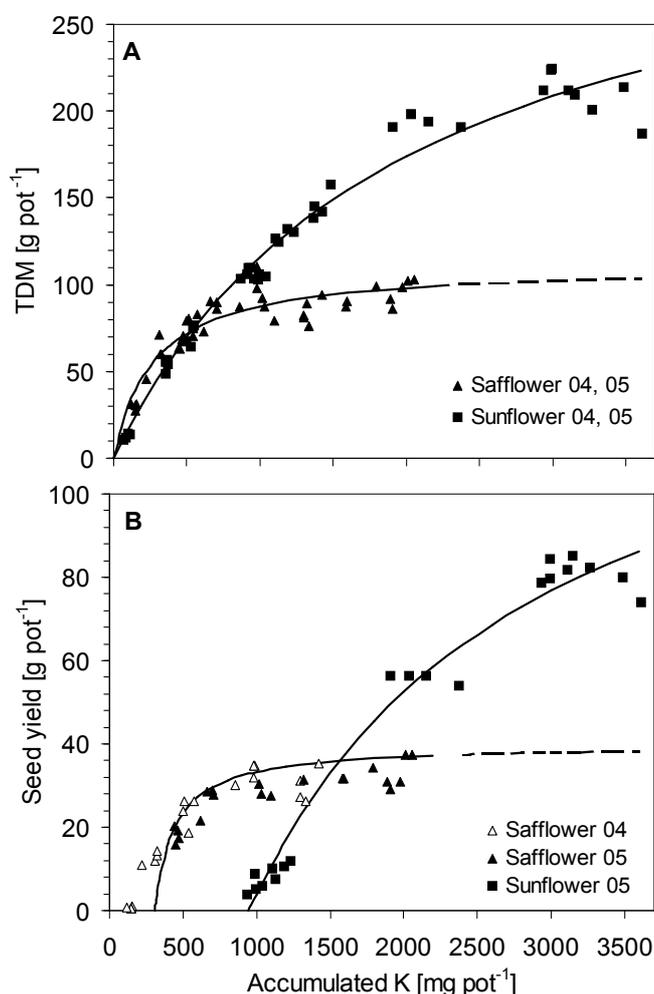
<sup>1)</sup> Means ± SD not assigned the same letter within each column indicate significant difference ( $p < 0.05$ ,  $n = 4$ ). \* indicates significant differences between species in a given year ( $p < 0.05$ ).

To avoid the interpretation of the dilution effect under low K supply as utilization efficiency, utilization index was calculated (*Siddiqi and Glass, 1981*), which takes the difference in biomass produced into consideration, not just the amount of K accumulated. It is clearly observed that safflower has a higher K utilization index in terms of achene production at suboptimal K supplies compared to sunflower, but the latter exceeded the former significantly at optimal and high K supplies (Table 7.3). These findings are in the same line with those based on evaluation of the efficiency ratio, comparing both crops at suboptimal K supply, while the two utilization efficiency indicators (ER, UI) show conflicting results when both species are compared at their respective optimal K supply. This makes the use of just one utilization indicator sometimes misleading (*Chisholm and Blair, 1988, Siddiqi and Glass, 1981, Gourley et al., 1994*). The K utilization index increased with decreasing K supply in safflower in both years but not at the lowest K level (0.0 K per pot), while it increases in sunflower with increasing K supply. This finding proves again that safflower can be considered a low input crop with respect to K availability.

### 3.3. Effect of K supply on yield response curves

The functional relationship between nutrient supply and yield parameters may be described in several ways. Polynomial functions are easily applied, but do not allow interpreting their coefficients in a straightforward fashion. The classical Mitscherlich equation has often been used to describe yield responses, but in order to characterize nutrient efficiency the Michaelis-Menten equation has been more frequently employed (e.g. *Föhse et al.*, 1991, *Gourley et al.*, 1994, *Steingrobe and Claassen*, 2000). As the data analysis requires variation in both dimensions, it is best applied to parameters describing efficiency ratios (Fig. 7.3). In analogy to enzyme kinetics, the K accumulation required to produce 50 % of the predicted maximum yield (term 'c') corresponds to the  $K_m$  in Michaelis-Menten kinetics and essentially describes the curvature of the graph. It is thus a good indicator of the sensitivity of a crop to reduced nutrient supply, hence its nutrient efficiency. However, this approach requires a well-defined response curve from which the yield maximum can be deduced. The data of TDM for both crops was taken from both years but in the case of achene yield response curve, the data of both years were pooled in safflower because the response could be adequately described using a single curve, allowing coverage of a wider range of data, while for sunflower the data of 2004 were not used as the optimal growth and yield were not reached. Characterizing nutrient efficiency according to this approach indicates that safflower is less sensitive to inadequate K supply and thus more K efficient than sunflower with respect to both DM production and achene yield, because the term 'c' is always lower for safflower (Fig. 7.3). As expected, the response curve concerning TDM does not require an additional term to account for unproductive K accumulation, as K-free biomass is not to be considered (Fig. 7.3A). The minimal accumulated K per pot to trigger achene yield formation (term 'b'), corresponding to the  $c_{min}$  in enzyme kinetics, was lower in safflower than in sunflower indicating the capability of the former to realize achene yield at marginal K accumulation as compared to sunflower (Fig. 7.3B). These striking differences in the threshold value 'b' and the curvature 'c' resulted in the two response curves intersecting, and represents a typical example of the difficulties arising from different definitions of nutrient efficiency as discussed by *Sattelmacher et al.* (1994). Based on data by *Thiraporn et al.* (1987) they argued that two genotypes, of which one outperforms the other at low nutrient availability, while the opposite holds true at high nutrient supply, could both be considered nutrient efficient, as the more responding genotype converts applied nutrients more efficiently into yield. As we follow the definition that a more efficient genotype is able to realize a yield above average under conditions of suboptimal nutrient supply (*Graham*, 1984, *Sattelmacher*

et al., 1994), safflower can be considered a more efficient crop in terms of the ability to utilize internal K to produce agronomic yield under suboptimal K supply.



**Figure 7.3:** Functional relationship between yield (TDM, achenes) and K accumulated in the above-ground biomass per pot. Michaelis-Menten-type equations are given as: Yield parameter =  $(A_{\max} \times (\text{mg K accumulated} - b)) / (c + (\text{mg K accumulated} - b))$ , with 'mg K' representing the K accumulated in the above-ground biomass per pot, 'c' the  $K_m$ , ' $A_{\max}$ ' the maximal yield potential and 'b' the minimal N accumulation required for yield formation. [A]  $TDM_{\text{sunflower}} = (347.3 \times \text{mg K}) / (1994.8 + \text{mg K})$ ,  $TDM_{\text{safflower}} = (111.5^* \times \text{mg K}) / (273.5^* + \text{mg K})$ , [B]  $\text{Seed yield}_{\text{sunflower}} = (150.6 \times (\text{mg K} - 946)) / (1976 + (\text{mg K} - 946))$ ,  $\text{Seed yield}_{\text{safflower}} = (39.8^* \times (\text{mg K} - 310.2^*)) / (133.7 + (\text{mg K} - 310.2^*))$ . \* indicates that coefficients for safflower are significantly different from those for sunflower based on the 95% confidence limits.

With respect to the physiological mechanisms responsible for genotypic differences of K utilisation efficiency, confined here to the capacity for normal growth and metabolism at relatively low tissue concentration, only a few studies identified the underlying mechanisms. Generally speaking, these may be related to the root to shoot translocation of nutrients, nutrient accumulation and remobilization within the shoot, compartmentalization within the cell, elemental substitution of Na for K function, and at the biochemical level the critical K

concentrations required for enzymatic reactions (e.g. the biochemical sensitivity of pyruvate kinase to the K concentration) (*Baligar et al., 2001; Gerloff and Gabelman, 1983*).

### 4. Conclusion

Although only limited information on the nutrient requirement under field conditions may be obtained from pot experiments, it is evident that – in terms of achene yield formation – safflower is performing superior to sunflower under K-limited conditions and can be regarded a low input species in terms of its K requirement. Safflower is more efficient than sunflower in utilizing absorbed K at low K availability and at their respective optimal K supplies. In terms of uptake efficiency, sunflower overrides safflower at low and optimal K supply. The results obtained using different efficiency indicators illustrates that the ranking may depend on the definitions used. The calculation of utilization index includes however, both yield and plant nutrient concentration, and is likely to be complicating the identification of potential mechanisms associated with enhanced nutrient efficiency. The use of agronomic use efficiency as an efficiency indicator involves the uptake of the nutrient and its utilization to produce final yield and doesn't indicate the mechanism through which the efficient cultivar interprets its efficiency. Indeed, little is known on the physiological mechanisms responsible for different utilization efficiency, and further research efforts should aim at identifying the mechanisms responsible for differences in K utilization efficiency of safflower (K efficient) and sunflower (K inefficient). Similarly, no information is available concerning the uptake efficiency under field conditions, and thus there is need for further research to prove the high K uptake efficiency of safflower and to identify the responsible mechanisms.

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## Chapter 8

### Suitability of Safflower (*Carthamus tinctorius* L.) cultivation in the northern part of Germany under organic and low input farming conditions

#### Abstract

Safflower represents an important oil crop internationally and may have a certain production potential under German conditions, particularly in organic farming where the putatively low nutrient requirement is highly welcomed. It has a wide adaptation from 45°S to 60°N and can be cultivated also in regions with a temperate climate, therefore was cultivated as a potential oil crop in Germany before the Second World War, and competed well with other oil crops developed at that time. To assess the suitability of safflower cultivation as an oil crop under the climatic conditions of the northern part of Germany under low input organic farming, two field experiments were conducted from April to October 2004 and 2005 in Lindhöft-Kiel (Latitude; 54.45, Longitude, 9.97, Altitude; 15m) in a clay/sand soil. Ten safflower cultivars ((1) Sabina, (2) PI-572475/Saffire, (3) PI-209286, (4) PI-253518, (5) PI-253555, (6) BS-62915, (7) BS-62924, (8) CART-19/89, (9) DO-13/03 and (10) DO-15/03) were cultivated in a Randomised Complete Block Design (RCBD).

The highest oil yielding cultivars were cultivar 3, and cultivar 8, while the lowest yielding cultivars were cultivars 2, 9, and 10. Cultivars 1, 4, 6 and 7 didn't differ from the highest yielding cultivars significantly while cultivar 5 was closed to the lowest yielding ones. Pearson correlation reveals that oil yields of the ten cultivars (pooled) exhibited tight positive correlations with plant height, plant dry matter, number of capitula plant<sup>-1</sup>, number of achenes capitulum<sup>-1</sup> and thousand achene mass (TAM), but were not correlated with days to full flowering or oil concentration. Interestingly, the vegetative growth (DM) was highly and significantly correlated with the number of capitula plant<sup>-1</sup> in all cultivars individually, and in most of them (7 cultivars) in term of thousand achene mass, and half of them (4 cultivars) in term of achene plant<sup>-1</sup>, and negatively correlated with oil concentration in most of the cultivars under study (7 cultivars) indicating the competition of the oil concentration with other yield components when vegetative growth is improved. Yield component analysis reveals that the number of achenes capitulum<sup>-1</sup> had the major influence on the variation of the oil yield of the ten cultivars under study (pooled), followed by the number of capitula plant<sup>-1</sup> and the TAM, while the oil concentration had slightly negative influence, and the plant density was negatively associated with oil yield.

Although two years field experiment are not enough to analyse the performance of safflower adaptability to certain region, it can be concluded that the most adaptive cultivars which produce significantly higher yield are PI-209286 originated from Romania, and cultivar CART-19/89 originated from Poland, while the less adaptive cultivars were DO-13/03 and DO-15/03 originated with German origin in addition to the Canadian cultivar PI-572475/Saffire. The most striking yield component that may contribute to the high yield production according to the comparison of means, Pearson correlation with oil yield and yield component analysis is the number of achene per capitulum, which can be used as a base for selecting cultivars adaptive to the humid cold area of the northern part of Germany. The growth period (between full flowering and harvest) which influence this yield component must be given attention (to avoid low temperature and rainfall by tuning the sowing date) to improve yield at these conditions. This yield component is correlated with TAM, but the negative correlation between TAM and oil concentration must be balanced by tuning the date of sowing and the growing practice or through breeding of adaptive cultivars.

### 1. Introduction

Safflower represents an important oil crop internationally and may have a certain production potential under German conditions, particularly in organic farming where the putatively low nutrient requirement is highly welcomed. Safflower (*Carthamus tinctorius L*) is a herbaceous annual plant and a member of the Composite family. It is highly branched, thistle-like; whose achenes contain 35-45% oil (Weiss, 2000). It is native to parts of Asia, the Middle East, and Africa (Armah-Agyeman et al., 2002), and one of the humanity's oldest crops (Li and Mündel, 1997). In the past, safflower was grown for its flowers, which were used in making dyes for clothing and food, but today, it is grown mainly for its oil (Weiss, 2000). Safflower's oil has been produced commercially and for export for more than 50 years in around 60 countries, but over half is produced in India and the production in the U.S.A., Mexico, Ethiopia, Argentina and Australia comprises most of the remainder (Li and Mündel, 1997). The oil derived from safflower represent a share of 0.07% of the global production of oil achenes, while the share of Europe in safflower achenes produced globally is marginal (FAO, 2007 a, b).

Cultivation of safflower has a wide adaptation from 45°S to 60°N and can be cultivated also in regions with a temperate climate (Mündel, 2001). However large-scale commercial cropping is practiced between 30° and 45° N and in fairly low-altitude areas (below 1000 meters) (Esendal, 1997, Weiss, 2000). Safflower grows on a wide range of soils and pH (5-8), but

for commercial production highest yields are obtained on fairly deep, well-drained, somewhat sandy loam of neutral reaction (Weiss, 2000). However, safflower production under excessive moisture and high humidity conditions is seriously stifled by diseases (Mündel, 2001). Therefore, the success of safflower introduction into new areas will largely depend on the disease resistance (Esendal, 2001) and the extent of improvement in yield and oil content (Malleshappa et al., 2003).

There is only small evidence of the suitability to cultivate safflower in Central Europe (Reinbrecht et al., 2005). Before the Second World War, safflower was cultivated as a potential oil crop in Germany (Scheibe and Yekta, 1934, Scheibe, 1938, Sessous, 1940), and was held worthy to produce edible oil. Accordingly, specific safflower genotypes originating mainly from European botanical gardens were selected and proved to compete well with other oil crops developed at that time. Unfortunately, all knowledge about which genotypes detected by Scheibe is lost, also in gene banks (Reinbrecht et al 2005). Winter oil seed rape is nowadays the major oil crop in Germany, and safflower grown conventionally cannot compete well with this crop (Reinbrecht et al 2005). Since there is some evidence that organically grown oilseed rape is highly susceptible to a wide range of insects and other pests (Reinbrecht et al., 2004), and since a Californian study has shown that safflower yielded similar in conventional as well as in organic systems (Clark et al., 1999), it is hypothesized that safflower can be grown successfully according to organic farming practice in Germany. Therefore the objective of our study is to find suitable cultivars by evaluating the yield of ten safflower gene bank accessions, varieties, and breeding lines under low input organic farming conditions in the northern part of Germany.

## **2. Materials and Methods:**

### **2.1. Experimental conditions**

Two field experiments were conducted on 22 April 2004 and 13 April 2005 in Lindhöft (Latitude; 54.45, Longitude, 9.97, Altitude; 15m) in the northern part of Germany (Schleswig-Holstein) in a clay/sand soil which was analysed 14 days after sowing (d.a.s.) for available P, K, and Mg (CAL method; Schüller, 1969), and for total N using an elemental analyser 1108 (Carlo-Erba, Milan, Italy) coupled to an isotope ratio mass spectrometer (Finnigan MAT Delta C) and found to contain 114, 3.5, 7.6, and 6 mg N, P, K, Mg per 100 g soil respectively in 2004, and 152, 4.4, 10.4, and 8 mg N, P, K, Mg per 100 g soil respectively in 2005. Ten safflower cultivars (Sabina, PI-572475/Saffire, PI-209286, PI-253518, PI-253555, BS62915, BS-62924, CART-19/89, DO-13/03, DO-15/03) were cultivated under

organic farming conditions. The cultivars used were selected out of 65 safflower accessions according to the yield performance under organic farming conditions in Germany and Switzerland (Elfadl et al., 2005). The ten cultivars were cultivated in a Randomised Complete Block Design (RCBD), in which four blocks were used as replicates, each block consisted of one replicate of each cultivar as plot, and the ten plots were completely randomised inside each block. Each plot consisted of 12 rows, 10 m long with 25 cm spacing between rows in a sowing density of 50 germinating achenes per m<sup>2</sup>. To achieve the sought plant density for all cultivars, the quantity of achenes (g) to be used in the sowing machine for each cultivar was calculate from the TAM and the germination percentage. Plant densities (plant per m<sup>2</sup>) in the plots were calculated at the germination and full flowering stages by counting plants in two meter length three times per each plot. Weeds between rows were removed (when plants were less than 15 cm height) by blowing machine and the remaining was removed manually when they appear and continued to the beginning of branching stage. To determine nutrient status of the plants, fifteen leaves of the young mature blade (YMB) were collected from fifteen different plants in two meter length of two rows of each plot in the branching vegetative stage (V3-c, i.e.: the appearance of the tertiary branches, >10 mm in length) according to Uslu (1997). The flowering percentage (as the percentage of the buds that started flowering from the total buds) was determined by counting the number of flowers in 100 adjacent buds in the same row two times per each plot every three days from 5% to 95% flowering. The height of the plants was measured in the full flowering stage as the height of the plant from the soil surface to the top of the growing tip, using a meter stick ( $\pm 0.5$  mm) and calculated as the mean of 5 measurements in different locations in each plot. To determine total accumulated nutrients in the plants along with plant dry matter, the whole plants in two meter length of the second right row of each plot were cut from the surface of soil in the physiological ripening stage (after seed filling and before the beginning of leaves senescing), then the real number of the harvested plants is counted. The number of capitula per plant was calculated by counting the number of capitula and the number of plants in two meter length two times per each plot in the full flowering stage and divided by the real number of the plants.

**Table 8.1:** The ten safflower cultivars used in 2004 and 2005 and their country of origin.

Cultivar No.	Cultivar name	Origin	Cultivar No.	Cultivar name	Origin
1	Sabina	Czech	6	BS62915	Germany
2	PI-572475/Saffire	Canada	7	BS-62924	Germany
3	PI-209286	Romania	8	CART-19/89	Poland
4	PI-253518	Austria	9	DO-13/03	Germany
5	PI-253555	Portugal	10	DO-15/03	Germany

## 2.2. Harvesting and analytical procedures

Yield was obtained by harvesting 8 m length and 1.7 m width of each plot using a harvesting machine on 09.10.04 and 21.09.05. Achenes were collected, dried on 28 °C in air forced oven, cleaned from hay, weighed and yield was calculated as kg per ha. Thousand achene mass was measured by counting one thousand achene by a machine then the counted achenes were weighed. The oil concentration in the achenes was determined using Near Infrared (NIR) Spectroscopy (Carl Zeiss Jena GmbH, Jena, Germany, with the software Nircal 4.21, Büchi Labortechnik AG, Flawil, Switzerland); in which 394 samples were used as reference values for NIRS calibration. Oil was extracted from the reference samples using accelerated solvent method (ASE) and shows high correlation with the results obtained from the NIR method ( $r^2 > 0.9$ , Q-value >75%). Plant samples for analysis were dried at 65°C to constant weight, grinded to pass 1.5 mm diameter, from which, 5 g samples were grinded by a ball machine to obtain a very fine homogenous powder. For mineral nutrient determination; samples were prepared by using dry ashing method (Dechassa, 2001); in which 50 mg of the 50 °C dried fine sample were weighed in porcelain crucible then ashed in a muffle furnace at 450 °C overnight (more than 10 hours). The ash was cooled at room temperature, 1 ml of 0.35 N HNO<sub>3</sub> was added, shaken smoothly and left 10 minutes before adding 9 ml water (purified to 18.2 MΩ cm<sup>-1</sup> using a Milli-Q water purifier from Millipore Co.). The digest was filtered through ashless filter papers (blue band, Whatman International Ltd, England) into polypropylene tubes. P, K, and Mg were measured using the inductively coupled plasma (ICP, Spectro Analytical Instruments GmbH, Kleve, Germany). Total nitrogen was analysed by weighing 2-3 mg of the 50 °C dried fine sample and run as for soil samples. The climatic parameters for the area of study in both years are obtained from (Deutscher Wetterdienst) and expressed as weekly average (Table 8.2) for the growing season and whole year (Table 8.3).

### 2.3. Statistics

All statistical analysis was carried out using SAS (SAS Institute Inc., Cary, USA, Release 8.02, 2001). The results of both years were pooled and used as one data set with eight replicates instead of two years and four replicates in each one. Comparisons of means were carried out using the procedure 'mixed' considering the two years (2004 and 2005) as one trial (pooled) and estimating two trials from multi ones randomly. The Tuckey test was employed with multiple t-tests in order to maintain an experimentwise  $\alpha$  of 5%.

Oil yield may be broken down into its components and expressed in a multiplicative fashion as:

$$\text{Oil yield [Kg oil ha}^{-1}\text{]} = (\text{plant per m}^2) \times (\text{capitula plant}^{-1}) \times (\text{achenes capitulum}^{-1}) \times (\text{mass 1000 achene}^{-1}\text{[g]}) \times (\text{oil concentration [g g}^{-1}\text{]})$$

Initially Pearson correlations were calculated to test the relation between individual traits (morphological parameter or yield components) and oil yield. In order to quantify the impact of individual yield components multiple regression analysis is biased as the mathematical product (or the sum in case log-transformed data are used), rather than a statistical relationship, of all individual yield components represents the oil yield. Hence, a yield component analysis according to Piepho (1995) was employed, allowing the contribution of individual components to be quantified. This approach assumes that the SD of log-transformed yield is close to the coefficient of variance of the yield, uses the log-transformed component data and interprets values of  $C_i = \text{Cov}[\log(\text{yield}), \log(\text{component}_i)]$  as an aggregate measure of the  $i^{\text{th}}$  component's contribution to the variability in yield.

## Chapter 8 Screening of safflower cultivars

**Table 8.2:** Weekly means of daily temperature, sun shine duration (h), rainfall (mm), and relative humidity (%) within the experimental period from 22.4.04 to 9.10.04, and from 13.04.05 to 21.09.05 (Deutscher Wetterdienst; Wetterstation Kiel-Holtenau).

Week No.	2004									2005								
	Temperature (°C)			Sun shine (h)		Day (h)	Rainfall (mm)		RH (%)	Temperature (°C)			Sun shine (h)		Day (h)	Rainfall (mm)		RH (%)
	Av	Max.	Min.	Av	Sum	Av	Av	Sum	Av	Av	Max.	Min.	Av	Sum	Av	Av	Sum	Av
1	10.5	15.6	6.5	5.7	40.2	12.7	0.8	4.6	76	9	13.3	6	5.5	38.2	12.3	0.2	1.4	74.1
2	11.5	16.7	7.4	4.2	29.3	13.3	1.8	12.9	72.6	6.3	10.8	2.1	11.1	77.6	12.7	0	0	65
3	11.9	16.4	8.7	2.9	20.5	13.7	0.8	5.5	81.3	12.5	17.1	8.9	3.8	26.5	13.3	3.1	21.9	75.9
4	11.6	16.4	6	6.4	45.1	14.3	0	0.2	70.4	7.9	12.4	3.3	5.8	40.8	13.7	4.8	33.3	78.3
5	9.5	14.6	4.7	10	70	14.8	1.4	9.5	64.2	9.4	14.1	2.9	8.6	60.1	14.3	0.3	1.9	70
6	13.6	19.2	7.6	12	84.1	15.3	0	0.2	63.9	13	18.3	7.1	7.3	51	14.8	1.4	9.5	64.3
7	15.1	20.3	10.6	7	49	16.0	3.2	22.2	70	16.3	21.9	10.4	8.5	59.2	15.3	1.2	8.1	65.3
8	15.2	19.6	10.7	6.5	45.4	16.3	0.4	2.5	70.3	12.2	16.5	7.9	3.8	26.7	16.0	3	20.7	70.9
9	12.8	18.2	8.6	5.9	41.6	16.7	5.5	38.4	73.4	11.8	16.5	6.8	8.2	57.2	16.3	1.7	12	64.9
10	13.9	17.4	9.8	4.3	25.5	17.0	3	20.7	76.6	18.1	24	10.7	10.1	71	16.7	0.8	5.5	64.9
11	14.2	18.8	10	5.9	41.5	17.3	6.9	34.7	73.6	17.4	22.9	10.9	12.3	85.8	17.0	1.3	8.8	65.3
12	14.6	18.6	12	2.9	20.5	17.7	1.9	13.6	74.7	18.4	23.3	13.1	7.8	54.1	17.3	3.1	21.9	65.9
13	17	21.3	13.9	5.2	36.7	17.3	0.8	5.7	73.9	19.3	24.1	14	9.1	63.8	17.7	0.2	1.7	67.3
14	16.1	21.2	11.5	5.3	37	17.0	3.2	22.1	74.4	18.1	23.2	13.7	4.9	34.6	17.3	1.6	11.1	70.6
15	19.4	25	12.7	3.4	24	16.8	0.6	3.9	68.3	15.3	18.9	12.3	3.6	25.3	17.0	8.9	62.2	79.4
16	22.2	27.8	16.1	3.1	21.3	16.7	0	0	64	17.7	22.5	13.6	4.6	32.5	16.8	3.3	23.3	78.3
17	18.7	22.3	14.7	3.2	22.6	16.3	5.2	36.1	76.3	14.3	19.1	10.1	6.6	46.2	16.7	3.4	24.1	76.3
18	16.2	21.2	11.8	2.9	20.3	15.7	3.1	21.5	70	14.9	18.2	11.7	2.9	20.3	16.3	0.3	2.3	77
19	14.9	19	11.3	4.7	32.8	15.0	6	42.2	78.4	18.7	24.6	13.6	8.2	57.3	15.7	0	0	73.1
20	17.2	23.5	10.9	4	27.9	14.7	0	0	68.7	15.7	20.1	11.2	7.5	52.5	15.0	2.2	15.5	72.1
21	15.2	20.2	10.8	3.9	27.4	14.3	1.9	13.8	67.9	17.6	24	11.5	10.1	70.7	14.7	0.12	0.9	66.1
22	13.1	17.2	9.9	4.8	33.5	13.7	5.7	40.1	71.6	17.2	21.5	12.4	6	42.1	14.3	0.8	5.4	72.8
23	12.3	15.3	9.4	2.8	19.9	13.0	3.7	26.2	83.7	13.1	18	7.4	5.2	41.9	13.7	0.3	2.4	71.1
24	11.6	16.1	8.3	4.9	49.8	12.7	0.1	0.9	77.2	-	-	-	-	-	13.0	-	-	-

**Table 8.3:** Climatic parameters in whole year and within the experimental periods in 2004 and 2005

Parameter	2004	2005
	Whole year	
Average temperature (°C)	9.3	9.4
Maximum temperature (°C)	13	13.2
Minimum temperature (°C)	5.6	5.4
Sum of Average temperature (degree)	3398.6	3413.2
Relative Humidity (%)	76.3	75.6
Rainfall daily average (mm)	2.1	1.8
Rainfall sum (mm)	772.9	649.2
Sunshine daily average (h)	3.9	5
Sunshine sum (h)	1409.4	1811
	Experimental period	
Average temperature (°C)	14.5	14.5
Maximum temperature (°C)	19.2	19.4
Minimum temperature (°C)	10.2	9.6
Sum of Average temperature (degree)	2482	2354
Relative Humidity (%)	72.6	70.8
Rainfall daily average (mm)	2.3	1.8
Rainfall sum (mm)	377.5	293.9
Sunshine daily average (h)	5.1	7
Sunshine sum (h)	865.9	1135.4
Hot days > 20 °C	10	17
15-20 °C	57	67
10-15 °C	94	50
< 10 °C	10	28
Maximum T ° reached (weekly average)	27.8 °C	24.6 °C
Minimum T ° reached (weekly average)	4.7 °C	2.1 °C

### 3. Results

#### 3.1. Difference between cultivars in growth and morphology

The plant densities at germination and flowering stages were found to be not significantly different for all cultivars, it ranged from 43 (cultivar 7) to 58 (cultivar 9) germinated plant

per m<sup>2</sup> and from 31 (cultivar 10) to 41 (cultivar 9) at flowering. All cultivars didn't differ significantly in plant height and biomass production (g DM plant<sup>-1</sup>). Plant height ranged from 88.1 cm (cultivar 5) to 102.0 cm (cultivar 9), and plant biomass ranged from 19.3 g DM plant<sup>-1</sup> (cultivar 2) to 26.2 g DM plant<sup>-1</sup> (cultivar 10). The earliest 95% flowering was observed in cultivars number 2 and 5 and the latest cultivar was number 9 in one week difference. The cultivars 1, 6, 7, 8, and 10 didn't differ significantly from the latest one, while cultivars 3 and 4 were not different significantly from the earliest one.

**Table 8.4:** morphological parameters of safflower cultivars (cultivar names see Table 8.1).

Cultivar	Plant height (cm)	Days to 95% flowering	Biomass (g DM plant <sup>-1</sup> )
1	95.3 A	127.0 AB	21.4 A
2	90.9 A	120.5 D	19.3 A
3	92.4 A	123.5 BCD	21.6 A
4	88.4 A	122.3 CD	26.1 A
5	88.1 A	121.3 D	22.6 A
6	95.6 A	125.4 ABC	24.6 A
7	93.9 A	124.1 BCD	26.0 A
8	95.0 A	125.9 ABC	23.3 A
9	102.0 A	128 A	20.3 A
10	93.1 A	125.3 ABC	26.2 A

Means ± SD not assigned the same letter within each column indicate significant difference ( $p < 0.05$ ,  $n = 8$ ).

### 3.2. Difference between cultivars in yield and yield components

The significantly highest achene yield production was achieved by cultivars 3 and 8 (Table 8.6), while cultivars 2, 9, and 10 produced the lowest. Cultivars 1, 4, 6, and 7 produced less achene yield than the highest yielding ones but the difference was not significant, while cultivar 5 yielded better than the least yielding cultivars but didn't differ from them significantly. Oil concentration was very low and the same in all cultivars (in the narrow range between 11.8 and 13.4%). Number of capitulum per plant was the highest in cultivar 4 (13.4 capitula plant<sup>-1</sup>), while cultivars 1, 2, 3, 5, and 6 had less number of capitula plant<sup>-1</sup>. Cultivars 7, 8, 9, and 10 had no significant difference between cultivars having the highest and the lowest number of capitula plant<sup>-1</sup>. These data shows that the high achene yielding cultivars (3 and 8) didn't differ significantly from the low achene yielding cultivars (9 and 10) in terms of number of capitula plant<sup>-1</sup>. The highest number of achenes per capitulum was

found in the highest yielding cultivars (3 and 8), while the lowest value of this yield component was found in the lowest achene yielding cultivars (9 and 10). The highest TAM was found in cultivars 1, 3 and 5 while the lowest value was found in cultivar 2, but the high yielding cultivar 8 didn't differ from the low yielding cultivars (9, 10) in terms of this yield component.

**Table 8.5:** Yield and yield components in safflower cultivars (C.V names see Table 8.1).

Cv.	Capitula plant <sup>-1</sup>	Achene capitulum <sup>-1</sup>	TAM <sup>1)</sup>	Achene yield (kg ha <sup>-1</sup> )	Oil content (%)	Oil yield (kg ha <sup>-1</sup> )
1	9.3 B	9.8 AB	25.3 A	753.9 AB	12.1 A	90.1 ABC
2	9.2 B	6.1 BC	20.5 B	406.0 C	13.4 A	54.9 DC
3	9.5 B	12.2 A	25.1 A	928.6 A	12.4 A	114.9 A
4	13.1 A	9.5 AB	22.5 AB	881.8 AB	12.9 A	109.9 AB
5	9.4 B	7.9 ABC	24.6 AB	610.7 BC	11.8 A	70.4 CD
6	9.5 B	9.3 AB	25.5 A	799.6 AB	12.8 A	100.0 BA
7	11.0 AB	8.6 ABC	24.7 AB	819.7 AB	12.3 A	96.6 ABC
8	10.3 AB	11.9 A	23.9 AB	968.3 A	12.6 A	120.6 A
9	10.7 AB	4.1 C	21.2 AB	385.7 C	12.0 A	43.5 D
10	9.8 AB	5.8 C	23.5 AB	367.9 C	13.2 A	47.6 D

Means ± SD not assigned the same letter within each column indicate significant difference ( $p < 0.05$ ,  $n = 8$ ). <sup>1)</sup> Thousand achene mass

### 3.3. Difference between cultivars in YMB nutrient status, nutrient accumulation

The ten studied safflower cultivars had the same values of N, P, and K status in young mature blades (YMB), but cultivar number 1 had the highest Mg concentration, while cultivar number 10 had a significantly lower Mg concentration. The high and low yielding cultivars didn't differ in the N, P, K, and Mg status of their YMB (Table 8.6). In terms of total accumulated N, K, and Mg in whole plants harvested at the physiological stage, the ten cultivars also didn't differ significantly (Table 8.6), but in terms of total accumulated P, cultivar 7 had significantly higher values than cultivar 2, and the other cultivars were the same and didn't differ from the highest and lowest P accumulating cultivars.

**Table 8.6:** N, P, K, and Mg concentration ( $\text{mg g}^{-1}$ ) in YMB and their total accumulated amounts per plant at physiological repining stage in safflower cultivars (for cultivar names see Table 8.1).

Cv	Nutrient concentration ( $\text{mg g}^{-1}$ ) in YMB				Accumulated nutrient ( $\text{mg plant}^{-1}$ )			
	N	P	K	Mg	N	P	K	Mg
1	24.9 A	3.8 A	38.8 A	3.1 A	323.4 A	87.2 AB	435.6 A	36.4 A
2	22.2 A	3.4 A	39.2 A	2.8 AB	290.1 A	68.5 B	372.3 A	30.4 A
3	23.6 A	3.6 A	42.5 A	2.9 AB	350.3 A	93.7 AB	459.2 A	36.3 A
4	23.1 A	3.2 A	40.5 A	2.5 AB	405.7 A	103.8 AB	498.3 A	42.0 A
5	22.3 A	3.1 A	38.0 A	2.8 AB	356.3 A	87.8 AB	423.0 A	44.0 A
6	23.6 A	3.1 A	38.8 A	2.6 AB	385.6 A	96.8 AB	495.3 A	40.8 A
7	23.7 A	3.3 A	39.8 A	2.9 AB	420.3 A	116.8 A	523.6 A	52.5 A
8	23.1 A	3.4 A	40.5 A	2.9 AB	397.6 A	96.1 AB	451.1 A	43.6 A
9	22.3 A	3.0 A	40.1 A	2.6 AB	318.8 A	87.5 AB	435.6 A	36.3 A
10	21.7 A	2.9 A	40.4 A	2.4 B	392.0 A	101.7 AB	500.8 A	44.3 A

Means  $\pm$  SD not assigned the same letter within each column indicate significant difference ( $p < 0.05$ ,  $n = 8$ ).

### 3.4. Difference between cultivars in nutrient utilization

Nutrient efficiency ratio (amount of yield produced per unit of nutrient present in tissues) in term of achene yield production (Table 8.7) was different between cultivars under investigation. Cultivars 1, 3, 4, and 8 were found to be more efficient than cultivars 9, and 10 in terms of N and P efficiency ratios. The other cultivars didn't differ from each other and the efficient and non-efficient ones. In terms of K ER, cultivar 3, 4, and 8 were more efficient than cultivars 9, and 10. Cultivar 1 was close to the efficient cultivars, but the others didn't differ from efficient and non-efficient ones. Mg ER was significantly higher in cultivar 1, 3, and 4 than in cultivars 9, and 10. The high yielding cultivar number 8 was close to the efficient ones, while the other cultivars showed no significant difference in terms of Mg ER when compared to cultivars having higher and lower efficiency. Interpreting utilization efficiency as utilization index (yield produced per unit of tissue nutrient concentration) (Table 8.7), cultivars 4 and 8 had the highest efficiency, while cultivar 9 had the lowest value. Cultivars 3 and 6 were close to the efficient ones and cultivars 2, 5, and 10 are close to the non-efficient cultivar. Cultivars 1 and 7 didn't differ significantly from the efficient and less-efficient cultivars.

**Table 8.7:** Difference of nutrient efficiency ratio (ER), and utilization index (UI) of N, P, K, and Mg in term of achene production in safflower cultivars (cultivar names in Table 8.1).

Cv.	Efficiency Ratio (g achene (g nutrient in DM) <sup>-1</sup> )				Utilization Index (g achene / g nutrient (g DM <sup>-1</sup> ))			
	N	P	K	Mg	N	P	K	Mg
1	8.5 A	29.4 A	5.9 AB	74.2 A	1.70 AB	6.15 AB	1.25 AB	14.91 ABCD
2	5.2 AB	20.0 AB	3.9 ABC	48.5 ABC	0.74 CD	3.06 B	0.58 B	7.05 CD
3	9.0 A	35.2 A	7.2 A	90.4 A	1.64 ABC	6.23 AB	1.28 AB	16.03 ABC
4	8.7 A	33.5 A	6.9 A	83.9 A	2.09 A	8.16 A	1.70 A	20.56 A
5	6.7 AB	25.8 AB	5.3 ABC	56.9 ABC	1.23 ABCD	4.88 AB	1.04 AB	10.43 BCD
6	6.4 AB	25.0 AB	4.8 ABC	58.3 ABC	1.64 ABC	6.41 AB	1.26 AB	15.29 ABC
7	6.1 AB	22.9 AB	4.9 ABC	51.8 ABC	1.45 ABCD	5.28 AB	1.18 AB	11.91 ABCD
8	7.7 A	32.5 A	6.9 A	71.48 AB	1.96 A	8.34 A	1.74 A	18.08 AB
9	3.3 B	11.9 B	2.41 C	28.1 C	0.66 D	2.4 B	0.49 B	5.74 D
10	3.2 B	12.4 B	2.5 C	29.1 C	0.88 CD	3.41 B	0.71 B	7.85 CD

Means ± SD not assigned the same letter within each column indicate significant difference ( $p < 0.05$ ,  $n = 8$ ).

### 3.5. Pearson correlations and yield component analysis

Pearson coefficients showing overall correlations between yield and yield components and some morphological traits of the ten cultivars (Table 8.8) indicate that safflower yield is tightly correlated with the plant height and its dry matter, but not correlated with the number of days to full flowering. Concerning the yield components, oil yield per ha was highly ( $p < 0.001$ ) correlated with the number of capitula per plant, the number of achenes per capitulum and the mass per thousand achenes, but was not correlated with the oil concentration. The number of capitula per plant was found to exhibit a high positive correlation with TAM, while being highly negatively correlated with the oil concentration and was not correlated with the number of achene per capitulum. TAM and achenes capitulum<sup>-1</sup> were significantly and positively correlated to each other. Oil concentration had significant negative correlation with TAM and capitulum plant<sup>-1</sup>. Plant height and its DM were tightly correlated to each other, and both are highly correlated with number of capitula per plant and TAM, and negatively correlated with the oil concentration. The number of days to full flowering was not correlated with any of the yield components.

When cultivars were analysed separately (Table 8.9) Pearson correlation reveals that oil yield was significantly correlated with plant height in cultivars 4-10, and with plant DM in

cultivars 1, 4, 5, 8, 9, 10, and was correlated with the number of days to full flowering in cultivar number 9 only. Oil yield per ha was positively correlated with the number of capitula per plant in cultivars 1, 4, 5, 9, and 10, while the correlation with this yield component was significant and negative in cultivar 2. The oil yield was tightly correlated with TAM in cultivars 1, 4, 7, 8, 9, 10. Oil yield had significant negative correlation with oil concentration in cultivar 9, otherwise the other cultivars showed no significant correlations. Interestingly plant dry mass was highly and positively correlated with the number of capitula per plant in all cultivars, and with TAM in most of them (Table 8.10), but negatively correlated with the oil concentration of most of the cultivars under investigation. Also, the two yield components TAM and number of capitula per plant are significantly positively correlated with each other. When a significant correlation was found between the oil concentration and any of the traits in any cultivar; it was found to be negative.

Pearson correlations are obviously misleading, as they do not allow the quantitative contribution of the variation of a given yield component to final yield to be elucidated. Therefore  $c_i$  coefficients, based on the covariance of the log-transformed yield components with the log-yields, were calculated to quantify the contribution of each component to the yield variability (Piepho, 1995) and were done for each cultivar individually and for the ten cultivars under investigation together. The yield component analysis of the ten pooled cultivars reveals that the major yield component influencing the oil yield was the number of achene per capitulum followed by the number of capitulum per plant followed by TAM (Table 8.11). The plant density and oil concentration were negatively associated with oil yield.

Considering the influence of the  $c_i$  of two main yield components ( $\text{Capitula plant}^{-1}$ ,  $\text{achenes capitulum}^{-1}$ ) on the variance of the oil yield of each cultivar individually (excluding TAM for simplicity of grouping), it can be observed that the ten cultivars under investigation can be divided into three groups; one group in which the number of capitulum per plant is the dominant yield component (cultivars 4, 5, 7, and 10), second where number of achenes per capitulum dominates (cultivars 2, 3, and 6), and finally a group where both of these yield components have a similar impact on oil yield (cultivars 1, 8, and 9).

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**Table 8.8:** Pearson coefficients among variables determining oil yield of the ten safflower cultivars (pooled).

	Oil yield (kg ha <sup>-1</sup> )	Plant height (cm)	Plant DM (g plant <sup>-1</sup> )	Days to full flowering	Capitulum plant <sup>-1</sup>	Achene capitulum <sup>-1</sup>	TAM <sup>1)</sup>
Plant height (cm)	0.377***						
Plant DM (g plant <sup>-1</sup> )	0.418***	0.646***					
Days to full flowering	0.022	0.217	0.016				
Capitula plant <sup>-1</sup>	0.414***	0.678***	0.888***	-0.014			
Achene capitulum <sup>-1</sup>	0.731***	0.042	0.117	0.018	-0.001		
TAM <sup>1)</sup>	0.582***	0.505***	0.725***	0.075	0.610***	0.241*	
Oil concentration (%)	-0.141	-0.531***	-0.602***	-0.181	-0.565***	-0.113	-0.508***

\* indicate significant at p<0.05, \*\* indicate significant at p<0.01, \*\*\* indicate significant at p<0.001, <sup>1)</sup> Thousand achene mass

## Chapter 8 Screening of safflower cultivars

**Table 8.9:** Pearson coefficients between oil yield and traits determining oil yield of each safflower cultivar.

Cultivar	Plant height (cm)	Plant DM (g plant <sup>-1</sup> )	Days to full flowering	Capitulum plant <sup>-1</sup>	Achene capitulum <sup>-1</sup>	TAM <sup>1)</sup>	Oil concentration
1	0.374	0.839**	0.484	0.862**	0.882**	0.877**	-0.578
2	-0.474	-0.611	0.252	-0.724*	0.781*	-0.543	0.607
3	-0.209	-0.579	-0.274	-0.461	0.500*	-0.343	0.658
4	0.905**	0.915**	0.013	0.916**	0.703	0.896**	-0.488
5	0.890**	0.754*	0.077	0.846**	-0.172	0.652	-0.417
6	0.731*	0.520	-0.001	0.489	0.703	0.636	-0.232
7	0.723*	0.502	0.320	0.581	0.074	0.769*	-0.566
8	0.908**	0.726*	-0.110	0.588	0.710*	0.944***	0.122
9	0.758*	0.921**	0.974***	0.866**	0.703	0.914**	-0.818*
10	0.862**	0.854**	-0.144	0.840**	-0.110	0.717*	-0.610

\* indicate significant at p<0.05, \*\* indicate significant at p<0.01, \*\*\* indicate significant at p<0.001, <sup>1)</sup> Thousand achene mass

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**Table 8.10:** Cultivars showing significant Pearson coefficients among traits determining oil yield.

	Plant height (cm)	Plant DM (g plant <sup>-1</sup> )	Days to full flowering	Capitula plant <sup>-1</sup>	Achene capitulum <sup>-1</sup>	TAM <sup>1)</sup>
Plant DM (g plant <sup>-1</sup> )	2***, 4***, 5*, 7**, 10**					
Days to full flowering	3*, 9*	2*, 9***				
Capitula plant <sup>-1</sup>	2**, 4***, 5***, 7***, 10**	1***, 2**, 3**, 4***, 5***, 6***, 7***, 8*, 9*, 10***	1*, 9*			
Achene capitulum <sup>-1</sup>	6*, 9*	1*, 3-, 6*, 9*		3-**, 6*		
TAM <sup>1)</sup>	4***, 7***, 8*, 9*	3***, 4***, 5**, 6**, 7***, 8**, 9*	9**	1*, 3**, 4, ***, 5*, 6**, 7***, 8*, 9**	1*, 3-*	
Oil concentration (%)	7-***, 9-***, 10-*	1-**, 2-, 3-**, 6-, 7-***, 9-, 10-**	9-*	1-*, 6-, 7-**, 10-*	9-*	3-*, 4-*, 7-**, 9-*

\* indicate significant at p<0.05, \*\* indicate significant at p<0.01, \*\*\* indicate significant at p<0.001, when minus (-) came before the stars indicates that the correlation was negative, <sup>1)</sup> Thousand achene mass

## Chapter 8 Screening of safflower cultivars

**Table 8.11:** Estimates of variation coefficients (VC) of yield, SD and variance of log-yields, and  $c_i$  coefficients for yield components of the ten safflower cultivars and pooled (data were transformed to the natural logarithms, calculations according to Piepho, 1995).

Cv.	VC	SD	Variance	Ci for yield components				
	Oil yield (%)	Log-yield ( $\times 100$ )	Log-yield ( $\times 100$ )	Plant per $m^2$ ( $\times 100$ )	Capitula plant <sup>-1</sup> ( $\times 100$ )	Achenes capitulum <sup>-1</sup> ( $\times 100$ )	TAM <sup>1)</sup> ( $\times 100$ )	Oil concentration ( $\times 100$ )
1	22.73	23.27	5.41	-7.62	7.01	5.19	1.72	-0.93
2	28.66	32.17	10.35	3.93	-6.24	11.93	-1.69	2.68
3	14.93	15.31	2.35	0.96	-2.69	4.31	-1.37	1.14
4	42.89	46.51	21.63	-16.09	21.00	11.16	8.44	-2.88
5	23.29	25.11	6.30	-5.12	9.08	-0.67	4.23	-1.14
6	33.44	31.76	10.09	-5.97	4.91	8.96	3.52	-1.31
7	26.06	28.64	8.20	-0.48	8.13	0.11	2.59	-2.32
8	51.69	52.85	27.93	-10.98	7.60	15.63	15.24	0.34
9	42.28	46.70	21.81	-5.11	10.86	12.76	8.72	-5.50
10	30.73	33.57	11.27	-5.02	14.13	0.13	3.40	-1.37
Pooled	47.21	49.29	24.30	-5.00	7.21	17.66	5.56	-1.15

<sup>1)</sup> Thousand achene mass

### 4. Discussion

#### 4.1. Cultivars origins

Interestingly, the high-yielding safflower cultivars (3 and 8) were originated from Romania and Poland respectively, while the Canadian cultivar number 2, and unexpectedly the German cultivars number 9 and number 10 were the less yield producing cultivars under our experimental conditions.

#### 4.2. Pearson Correlations between yield, and growth and yield traits

Safflower yield was found to be correlated with the plant height (Digming and Yuguang, 1993, Tunctürk et al., 2005) and its dry matter (Tabrizi, 1999) indicating that large plants produce more capitula per plant and TAM which agrees with our results (Table 8.8, 8.10). It was reported that achene and oil yield is correlated with number of capitula per plant (Tunctürk et al., 2005, Tabrizi 2000, Malleshappa et al., 2003), number of achene per capitula (Tunctürk et al., 2005, Camas et al., 2005) and the mass per thousand achene (Camas et al., 2005), but was not correlated with the oil concentration (Malleshappa et al., 2003, Tabrizi, 2000), which agrees with our findings (Table 8.8, 8.10). The number of capitula per plant was found to be positively correlated with TAM (Camas et al., 2005, Tabrizi 2000), and negatively correlated with the oil concentration (Tabrizi, 2000, Malleshappa et al., 2003, Tunctürk et al., 2005). In contrary with our results, Camas et al., (2005) found that, the number of capitula per plant and the number of achenes per capitulum are positively correlated with each other. In the same line with our findings, TAM and achenes capitulum<sup>-1</sup> were reported to be positively correlated to each other (Camas et al., 2005, Malleshappa et al., 2003). The negative correlation found in our investigation between oil concentration and TAM was previously documented (Camas et al., 2005, Tunctürk et al., 2005).

#### 4.3. Nutrients issues

Apparently, there are no published data concerning the nutrient status in the young mature blade in safflower for optimal growth and achene yield. But according to the nutrient experiments done in the greenhouse of this work, the N, P, and K content in the YMB of the ten cultivars used in the field were optimal and not limiting for normal growth and yield. The only significant difference was found in the Mg content of the YMB of cultivar 1 and cultivar 10 and may be discussed in terms of difference plant demand or metabolism (Marschner, 1995). Although the nutrient availability was the same for all cultivars under

study, the nutrient efficiency (Efficiency ratio and Utilization index) may differ between cultivars indicating the differences in the use efficiency between different cultivars subjected to the same nutritional conditions (Baligar et al., 2001) providing data which can be used in further studies concerning the nutrient use efficiency of these cultivars under investigation.

#### **4.4. Yield Components and their development as affected with climatic conditions**

Oil yield can be analysed in terms of its yield components into number of branches per unit area (related to number of capitulum per plant), number of achenes per capitulum, mean achene mass, and the oil concentration. These components develop sequentially, with later-developing components under control of earlier-developing ones (Dofing and Knight, 1993). Branching is one of the first developmental processes; it occurs during early growth and mainly depends on sowing density and the availability of water and nitrogen (Simance et al., 1993). Development of florets or capitula happens during rapid vegetative growth, then, competition for limiting resources may take place between vegetative and floral organs (Miralles et al., 2000). Later, achene filling is maintained by a high contribution from assimilation before and immediately after anthesis and remobilization of vegetative reserves (Royo et al., 1999). The compensation of yield components occurs as a result of competition for limited resources (Miralles et al., 2000), and environmental stresses (Moragues et al., 2006). Therefore a reduction in a certain yield component is due to stresses during the time of development of that component. Accordingly, analysing our data; Pearson correlation reveals that the oil yield of cultivar number 3 which produced significantly higher oil yield than other cultivars has significant positive correlation with number of achene per capitulum, but has no significant correlation with the other yield components (Table 8.8, 8.10). Also the yield component analysis shows that the major positively influencing yield component on oil yield of this cultivar is the number of achene per capitulum. In the same line, cultivar number 8 produced significantly high oil yield, shows the same positive significant correlation between oil yield and number of achene per capitulum without significant correlation with the other yield components except the TAM which correlates tightly with the oil yield of this cultivar. The yield component analysis shows that both the number of achene per capitulum and the TAM are the major yield components influencing the oil yield of this cultivar in the same importance. Analysing the correlations between oil yield and the yield components of the low yielding cultivars; it can be observed that the oil yields of cultivars 9 and 10 are tightly correlated with the number of capitula per plant and TAM, while the number of achene per capitulum shows no significant correlation with oil yield of both cultivars. The

yield component analysis shows that the number of capitula per plant contributes highly to the variation of oil yield of cultivars 9 and 10, but the number of achene per capitulum and the TAM has relatively equal contribution to the variation of the yield in cultivar 9. The yield of the low yielding cultivar number 2 shows positive correlation with the number of achene per capitulum as what found in the high yielding cultivars, and also shows negative correlation with the number of capitulum per plant in contrary with the other two low yielding cultivars. Plant height and DM as selecting traits didn't show consistent correlation with the oil yield to differentiate cultivars to high- and low-yielding cultivars. The comparison of means shows more clearly, that the high yielding cultivars (3 and 8) share with high number of achene per capitulum, while the low yielding cultivars (2, 9, and 10) share with low number of achene per capitulum. Accordingly, it seems that the number of achene per capitulum may be the important yield component determining the yield of the cultivars which compensate with the number of capitula per plant (number of branches). Consequently, the cultivars, which produce less number of branches, enhance the plant to produce more achenes per capitulum and mature quickly avoiding the wet period at maturity stage which reduces achene yield and achene quality. But the number of the days to full flowering didn't show clear trend to separate low and high yielding cultivars (Table 8.4), which may indicate that the period after full flowering (achene filling and maturity) is the critical period which must be given more attention to select the most suitable cultivars in this humid area. Selecting for achene yield under variable moisture availability is difficult, particularly in the wet climate of the northern part of Germany, where rain of different severity may occur any time of the growing period especially at maturity.

The data obtained in this investigation indicate that in the ten cultivars when pooled, the number of achene per capitula is not correlated with the number of capitulum per plant or the oil concentration, but was significantly correlated with the TAM. On the other hand the TAM is negatively correlated with the oil concentration, presumably because the contribution of the achene shell to the achene mass is large (Camas et al., 2005). Accordingly, to achieve a high oil yield, however, it is necessary for the factors which affect these yield components to be balanced. The oil yield may be curtailed by unfavourable conditions at the time of seed filling and oil formation, in which TAM and oil concentration will be low, therefore these components should be provided optimum conditions at their stages of formation by a timely and correct performance of cultural practices.

### 4.5. Influence of climate of northern part of Germany on safflower production

Safflower is a crop sensitive to temperature and photoperiod, needing a period of low temperature and short days for vegetative growth and high temperature and long days for reproductive growth (Dajue and Griffee, 2001, Li, et al., 1997). Under production practice, a high harvest occurs when the seedling stage of safflower is under short day conditions, and thus it could grow with luxuriant roots and foliage, then, turns into a long day condition in order to promote it to flower and fruit (Li et al., 1997). It was found that an approximately fourteen-hour day is considered necessary to initiate flowering (Esendal, 1997), and the crop was considered a day-neutral plant, being equally productive in long days as in short days (Weiss, 2000, Cosentino et al., 1997, Esendal, 1997), whereas other researchers described safflower is a long-day sun-loving plant (Li et al., 1997, Armah-Agyeman et al., 2002). But different cultivars have different reactions to the duration of sunshine (Li et al., 1997), and varieties may show adaptation to specific photoperiod when grown at the limits of their normal range (Esendal, 1997). Under our conditions, we have chosen the period of cultivation to get as much as possible day length and sunshine in both years in the area of study (Table 8.2). The cultivars were sown at conditions of 14 h day duration (in middle of April) and cultivated in the middle of September which has around 13 h day length. The longest days (17 h) in the area of study were achieved in the vegetative stage before and within flowering and decreased in the period of seed filling and ripening. The daily average sunshine duration during the experiment was much less than the length of the day (5.1, and 7.0 h in 2004 and 2005 respectively), and was less than this average at the seed filling and ripening stage in both years which affect the oil formation and can be a cause of the reduced oil concentration in all cultivars under investigation.

However, safflower is adapted to the climate of warm areas as a tropical crop (Weiss, 2000), some cultivars have an extensive adaptability to temperature, and can be planted in the temperate zone (Li et al., 1997). Concerning the growing temperature, it would appear that temperature is more important than day-length (Esendal, 1997). Safflower emerges at soil temperatures above 4°C, and it grows much more rapid (3-4 days after sowing) at temperature of 15°C or greater and slow at minimum temperature, requiring up to three weeks to emerge (Li, 1997, Kaffka et al., 2001). The weekly average temperatures at sowing in our experiments were 10.5°C and 9.0°C in successive years respectively, so it was suitable for achenes to germinate. The minimum temperatures reached in both seasons were 4.7 and 2.1°C in 2004 and 2005 respectively and were during the rosette and stem elongation stages, but researchers found that in the seedling stage, most safflower varieties can tolerate tem-

peratures below -7 °C during its first stages of development (Esendal, 1997), then it becomes susceptible during flowering (Weiss, 2000; Mündel et al., 1994). It can be considered that the main economic facets of temperature to be considered are the seedling's tolerance to low temperatures and susceptibility to high temperatures at flowering (Esendal, 1997). Under low temperature climates the rosette and the vegetative growth periods of safflower are prolonged, so the high yield could be obtained, because of the two important components—the number of capitulum per plant and the number of achenes per capitulum (Raghunatham, et al., 1989, Li et al, 1997). It was found that range of 24-32°C is considered most favourable (Weiss, 2000); and regarded as the optimum temperatures for flower fertility (Knowles, 1972), and if temperature at maturity was below the optimal, maturity interval is extended (Esendal, 1997). Concerning our experimental conditions, the temperature was suitable at sowing and optimal at vegetative stage to produce high vegetative growth and optimal flowering, but the temperatures at seed filling and ripening were not enough for normal maturity (12.3 °C, and 16 °C as average of the period between full flowering and harvest in 2004 and 2005 respectively), and the maturity period was extended by the cold conditions to around one month in both years.

As a crop having a particular demand on photoperiod, and temperature; and since time of sowing basically governs the temperature and photoperiod range at which plants grow, suitable sowing time must be selected to meet its demand on duration of sunshine and temperature in various growing stages, and the high yield would be obtained, otherwise, the yield would be decreased (Li et al, 1997, Weiss, 2000). In the Washington area, grower's plant safflower in late March to early April, or whenever soil temperatures are above 5°C, but in other safflower growing areas of the United States, growers grow the crop from late April to early May, because late plantings may not mature before rainfall. If safflower is planted in April in the Pacific North-west (PNW), it takes 1 to 2 weeks to germinate, and after emergence, it stays 2 to 4 weeks in the rosette stage, so early growth and development is slow, but growth is rapid in the stem elongation stage, and flower buds (heads) form from mid to late June, then flowering occurs by mid to late July and may continue over an extended period (Armah-Agyeman, 2002). In our experiment, the growing period was chosen to meet the highest sum of temperature degrees and sunshine duration in the area of study, and to avoid wet soil before sowing. If the sowing time was earlier than middle of April, to meet maturity in late July or in August before heavy rain, the soil may be wet and its temperature may be below 4°C. In consequence, the germination will be delayed, or the rosette stage period will be extended leading to the same results of extended growth period.

It was reported that, when safflower is spring-sown in warm areas of the U.S.A., it matures in around 120 days, but in cooler areas, it needs 145 days (Esendal, 1997). Although, under our experimental conditions, safflower cultivars were sown in the year time were they can receive as much sunshine and temperature as possible, the growing period was much extended and was 170 days in 2004, and 161 days in 2005.

The annual rainfall in the our experimental area was 772.9 and 649.2 mm in 2004 and 2005 respectively, half of it was during the experimental period (377.5 and 293.9 mm in successive seasons) and also the rain continued to fall even after full flowering and in the seed filling and maturity stage. As a crop originated from the Middle East (Knowels, 1976), safflower is adapted to areas which has winter and spring rainfall and dry summer (Yau et al., 1999), and 600 mm of annual rainfall seems to be adequate to obtain high yield (Weiss, 2000, Cosentino et al 1997, Uslu et al., 2002), but the majority of this must fall before flowering if achene quality and yields are not to be adversely affected (Weiss, 2000, Esendal, 1997). It was observed that in early growth stages, safflower is tolerant to high atmospheric humidity (Kaffka et al., 2001), but it is sensitive to heavy rain especially in the later stage of crop, therefore plants need dry atmospheric conditions during flowering and seed filling (Armah-Agyeman et al., 2002). The relative humidity in the experimental area ranged from 63.9 - 83.7% as the minimum and maximum weekly average in 2004, the same figure in 2005 was 64.3 - 79.4%. At maturity period (last three weeks before harvest), RH was 77.5% and 70% in the two successive seasons, which affects the maturity process and increase the susceptibility of the crop to diseases. It was observed that persistent rain or periods of high humidity during flowering and seed-filling adversely affected pollination, and produced discoloured and small achenes (Weiss, 2000, Esendal, 1997), and reduced achene yield and oil concentration (Armah-Agyeman, et al., 2002). The previous mentioned factors explain the reasons why TAM and oil concentration was largely reduced in all cultivars under this investigation in both seasons (Reinbrecht et al., 2005).

It was found that cultivars 9 and 10 had high incidence with head rot much more than the other cultivars (40%) which could be another cause for the severe reduction of achene yield of these two cultivars (data are not shown). After flower buds form, prolonged rain or fog cause head rot to appear on the buds or flowers (Kaffka et al., 2001). It was reported that at all stages of growth, excessive rainfall or humidity increases damage from fungal diseases (Weiss, 2000, Esendal, 1997). In periods of higher than normal rainfall, fungal diseases such as root rot (*Phytophthora sp.*), leaf spot (*Altenaria carthami*), bacterial blight (*Pseudomonas syringae*), plant wilts (*Fusarium sp.*, *Verticillium albo-artum*), and head rot (*Sclerotinia*

*sclerotiorum*, *Botrytis cinerea*) can cause serious losses. (Armah-Agyeman, et al., 2001, Esendal, 1997, Kaffka et al., 2001, Mündel et al., 2004). There are no disease resistant cultivars and the disease cannot be controlled economically (Kaffka et al., 2001). Because of these and other disease limitations, safflower production is limited to locations having a rain- and fog-free climate during most of the growing season, particularly in the late summer and early fall (Kaffka et al., 2001).

### 5. Conclusion

Although two years field experiment are not enough to analyse the performance of safflower adaptability to certain region, it can be concluded that, the most adaptive cultivars which produce significantly higher yield are PI-209286 originated from Romania, and cultivar CART-19/89 originated from Poland, while the less adaptive cultivars were DO-13/03 and DO-15/03 with German origin in addition to the Canadian cultivar PI-572475/Saffire. Under our experimental conditions, the most striking yield component that may contribute to the high yield production according to the comparison of means, Pearson correlation with oil yield and yield component analysis is the number of achene per capitulum, which can be used as a base for selecting cultivars adaptive to the humid cold area of the northern part of Germany. The growth period which influences this yield component must be given attention to improve yield under these conditions. This yield component is correlated with TAM, but the negative correlation between TAM and oil concentration must be balanced by tuning the date of sowing and the growing practice or through breeding of the adaptive cultivars. More field trials tuning the date of sowing selecting the high yielding cultivars found in this investigation are needed to improve achene quality and oil concentration.

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## Summary

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### Summary

Safflower represents an important oil crop internationally and may have a certain production potential under German conditions, particularly in organic farming where the putatively low nutrient requirement is highly welcomed. Current knowledge regarding the nutrient requirements of safflower as compared to similar oil crops is limited. However safflower was held worthy under German conditions in the beginning of this century, it was thus the aim of this study to determine the response of safflower (*Carthamus tinctorius* L.) as compared to sunflower (*Helianthus annuus* L.) in terms of growth, yield, and nutrient use efficiencies with respect to NPK supply in pot experiments using soil mixture. A further nutrient solution experiment with respect to P was conducted. A field experiment was done to screen ten safflower cultivars ((1) Sabina, (2) PI-572475/Saffire, (3) PI-209286, (4) PI-253518, (5) PI-253555, (6) BS-62915, (7) BS-62924, (8) CART-19/89, (9) DO-13/03 and (10) DO-15/03) for yield under low input organic farming under northern German conditions.

Both species responded strongly to increasing N supply with respect to plant growth and yield. Growth and yield of safflower increased up to 1 g N per pot for safflower, while the optimum for sunflower was 2.0 g N per pot. Safflower out-yielded sunflower at low N supply, while at high N level the opposite was observed. Pearson coefficients indicate that in safflower, yield is tightly correlated with the number of capitula per plant and the mass per achene, while these two yield components are tightly correlated to each other. To the contrary, sunflower yield was most tightly correlated with the number of achenes per capitulum, followed by the mass per achene. Path coefficient analysis considering the most important traits determining oil yield revealed that in sunflower, the number of achenes per capitulum exerts a strong direct effect on oil yield, and compensatory effects with the number of capitula per plant and the number of achenes per capitulum are not important. For safflower direct effects of the achene and leaf N content as well as the leaf dry matter are small, and mediated principally via the indirect effect on the number of achenes per capitulum. Both species accumulated similar amounts of N per pot at equivalent N supplies, but safflower was better N accumulator (concentrator) than sunflower due to safflower's less dry matter. Safflower utilizes absorbed N more efficiently than sunflower to produce achene yield at suboptimal N supply in terms of efficiency ratio and utilization index, but the opposite holds true at optimal and high supply. The efficiency to use accumulated N for dry matter and achene production interpreted in terms of Michaelis-Menten kinetics reveals the low  $C_{\min}$  and  $K_m$  value in safflower compared to sunflower. It can be concluded that in terms of N availability safflower represents a low input crop and outperforms sunflower with respect to achene yield on soils low in available N.

Both species responded strongly to increasing P levels in terms of plant growth and yield. Growth and yield of safflower increased up to 1 g P per pot, while the optimum for

## Summary

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sunflower was 0.5 g P per pot only. Supply of P affected safflower yield mainly through increasing number of capitula per plant and number of achenes per capitulum was reduced at very low P supply. Sunflower yield was improved with increasing P supply through increased number of achenes per capitulum only. Yield component analysis reveals that, oil yield in safflower was affected by P deficiency mainly due to number of capitulum per plant followed by the number of achenes per capitulum followed by single achene mass (SAM), but contribution of oil content to overall yield variation was insignificant. Sunflower major yield component influencing oil yield was number of achenes per plant, followed by SAM without a significant contribution of the oil content. Pearson correlations, followed by path coefficient analysis indicate that in sunflower only total N accumulated was found to be most important, while in safflower the total amount of P and N accumulated showed the highest standardized partial regression coefficient. Sunflower accumulated much more P (mg/pot) than safflower at all equivalent P supplies especially at low levels, but both accumulated the same P amounts at their individual optimal supplies. Uptake efficiency ( $\text{mg P accumulated} / (\text{mg P provided})^{-1}$ ) was higher in sunflower than safflower at all equivalent P supplies including their optimal levels. Agronomic efficiency interpreted as g P required producing fixed amount of achenes (in soil experiment), or DM (in nutrient solution experiment) was higher in safflower than sunflower at optimal and suboptimal P supply indicating the superiority of sunflower in term of the efficiency to use external P supply to produce achenes and DM than safflower. Sunflower was much more efficient at their optimal and suboptimal P supplies to utilize absorbed P than safflower in term of efficiency ratio ( $\text{g achene} / (\text{g P accumulated})^{-1}$ ) and utilization index ( $\text{g achene} / (\text{g P} / (\text{g DM})^{-1})$ ). From the nutrient solution experiment, the DM response curves based on accumulated P ( $\text{mg P pot}^{-1}$ ) (efficiency ratio (ER)), and P concentration in DM ( $\text{mg P} / (\text{g DM})^{-1}$ ) (utilization index (UI)) interpreted in terms of Michaelis-Menten kinetics reveals that  $K_m$  didn't differ for both species in terms of ER, but sunflower had less  $K_m$  value in term of UI. It can be concluded that safflower has a high requirement for P with respect to growth and yield; sunflower is more efficient than safflower in term of uptake and utilization of P at optimal and suboptimal P supplies indicating that safflower cannot be considered as a low nutrient input crop in terms of phosphorus.

Both species responded strongly to increasing K supply with respect to plant growth and yield. Growth and yield of safflower increased up to 1 g K per pot, while the optimum for sunflower was 3.0 g K per pot. Safflower out-yielded sunflower at low K supply, while at high K level the opposite was observed. The number of capitula in safflower was only slightly affected by K supply and the number of achenes per capitulum was only reduced under severe K deficiency, while single achene mass increased with increasing K supply. To the contrary, in sunflower, the number of achenes per capitulum strongly responded to the K supply, as did the single achene mass. Oil yield in safflower was affected by K deficiency mainly due to reduced achene yield, not oil concentration. To the contrary, oil yield in sun-

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flower was severely affected by low K supply due to both reduced achene yield and lower oil concentration. Multiple regression analysis indicate that in sunflower, the stem DM and the total amount of K accumulated in DM was most important, while in safflower the total amount of K and N accumulated had the highest impact. Both species accumulated similar amounts of total K in whole shoots per pot at low, equal K supply, except at extremely low K levels where safflower out-yielded sunflower. K concentration in safflower tissues was significantly lower than in sunflower at optimal and suboptimal K levels. Safflower utilizes absorbed K more efficiently than sunflower to produce achene yield at suboptimal and optimal K supply in terms of efficiency ratio, but only at suboptimal K availability when considering the utilization index. In terms of Michaelis-Menten (MM) kinetics addressing nutrient response curve to use K for growth and yield,  $K_m$  was higher in sunflower than in safflower, while the K accumulation required initiating yield formation ( $C_{min}$ ) in safflower was significantly lower. Similarly, safflower required much less external K to produce a given amount of achenes than sunflower at low and optimal K supplies. It can be concluded that in terms of K availability safflower represents a low input crop and outperforms sunflower on soils low in available K.

In the field experiments, the highest oil yielding cultivars were cultivar 3, and cultivar 8, while the least yielding cultivars were cultivars 2, 9, and 10. Yield component analysis reveals that the achene capitulum<sup>-1</sup> had the major influence on the variation of the oil yield of the ten cultivars under study (pooled) followed by capitula plant<sup>-1</sup> followed by TAM, while the oil concentration had slightly negative influence, and the plant density influencing oil yield negatively. Oil yield ha<sup>-1</sup> was strongly reduced even for the best yielding cultivars due to low achene yield and low oil concentration. As these two yield components are largely influenced by the environmental conditions at maturity, the required temperature and the sunshine duration needed for safflower to perform optimally for achene filling and oil formation was not reached. In addition to high humidity and continuous rainfall at period of maturity affected achene quality and increased disease incidence causing reduction in oil yield. Although two years are not enough to analyse the performance of safflower adaptability to certain region, cultivar 3, and 8 may have a production potential in Northern Germany, but further trials using the ten cultivars are needed. The most striking contribution from yield components to the variation of yield came from the number of achene per capitulum which must be given attention to tune safflower cultivation to provide the period of formation of this yield component more suitable climatic conditions in further research at the area of study.

## Zusammenfassung

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### Zusammenfassung

Saflor zählt international zu den wichtigen Ölfrüchten. In Deutschland könnte Saflor, bedingt durch den vermuteten niedrigen Nährstoffbedarf, im biologischen Landbau eine gewisse Bedeutung erlangen. Zur Zeit ist der tatsächliche Nährstoffbedarf jedoch unzureichend charakterisiert. Ziel dieser Studie war es daher, die Nährstoff-, Ertrags- und Wachstumseffizienz von Saflor (*Carthamus tinctorius* L.) mit Sonnenblume (*Helianthus annuus* L.) in bezug auf die Versorgung mit N, P und K im Gefäßversuch zu vergleichen. Ergänzend wurde ein Nährlösungsversuch zur näheren Charakterisierung der P-Effizienz durchgeführt. Zwei Feldversuche fanden auf dem Versuchsgut Lindhöft (Breite: 54,45, Länge: 9,97, Höhe: 15 m) auf Lehm-Sandboden statt. Zehn Saflorsorten (1) Sabina, (2) PI-572475/Saffire, (3) PI-209286, (4) PI-253518, (5) PI 253555, (6) BS-62924, (8) CART-19/89, (9) DO-13/03 und (10) DO-15/03 wurden nach den regeln des biologischen Landbaus angebaut.

Beide Pflanzenarten reagierten stark auf ein erhöhtes N-Angebot. Wachstum und Ertrag von Saflors waren bei N-Gaben von 0,1 g pro Gefäß am höchsten, bei Sonnenblume war dies bei 2,0 g N der Fall. Bei Saflor zeichnet sich ein besserer Ertrag bei niedrigen N-Gaben im Vergleich zu Sonnenblume ab. Das Entgegengesetzte wurde bei hohem N-Niveau beobachtet. Bei der Ertragskomponentenanalyse (YCA) zeigte sich der größte Einfluss durch die N-Versorgung auf die Anzahl der Körbe (Blütenköpfe) pro Pflanze, gefolgt vom Tausendkorngewicht (TKG). Dagegen wurde beim Sonnenblumenertrag der größte Einfluss auf die Samenanzahl pro Korb gefolgt vom Tausendkorngewicht festgestellt. Beide Arten bekamen eine gleiche N-Versorgung, hatten die gleiche N-Akkumulation an N. Verstehe ich nicht. Die geringere Trockensubstanzproduktion von Saflor gestattete eine besserer N-Akkumulator (im Sinne höherer N-Konzentrationen) als Sonnenblume. Bei beiden Pflanzenarten hatten die gleiche Aufnahmeeffizienz ( $\text{mg N aufgenommen} / \text{mg N Angebot}$ )<sup>1)</sup> bei niedrigem und hohem N-Angebot. Die N-Ausnutzungseffizienz (efficiency ratio, utilization index) hinsichtlich des Samenertrages ist bei niedrigem N-Angebot bei Saflor höher als bei Sonnenblumen. Bei optimalen und hohem N-Angebot wurden entgegengesetzten Effekte beobachtet. Saflor benötigt weniger 'external N' bei der niedrigen N-Stufe für die Produktion einer bestimmten Samenmenge als Sonnenblume, während bei dem jeweiligen optimalen N-Angebot das 'external N requirement' gleich war. Für die Interpretation der Nutzungseffizienz wurden die Michaelis-Menten Gleichung für die Nährstoffaufnahme, sowie für den Samen- und Trockenmasseertrag benutzt. Im bezug auf

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die N-Ausnutzung bestätigt die MM-Kinetik die höhere Effizienz des Saflors bei niedrigem  $K_m$  in bezug auf die TM und des Samenertrags. Der  $c_{min}$ -Wert hinsichtlich des Samen- und Ölertrages war bei Saflor geringer. Abschließend kann in bezug auf die N-Versorgung gesagt werden, dass Saflor hinsichtlich des Samen- und Ölertrages mit einem geringen N-Angebot auskommt als Sonnenblume.

Beide Sorten reagierten stark auf die Steigerung der P Gaben. Wachstum und Ertrag des Saflors waren bei einer P-Gabe von 1,0 g maximal, bei Sonnenblume war dies bei 0,5 g P der Fall. Die Sonnenblumen weisen bei niedrigen und hohem P-Angebot einen höheren Ertrag auf. Der Ölertrag bei Saflor reagierte auf P-Mangel durch die Anzahl der Körbe pro Pflanze, gefolgt von der Samenanzahl pro Korb, sowie der Samenmasse. Der Einfluss unterschiedlicher Ölkonzentrationen war hinsichtlich der Ertragsveränderung bedeutungslos. Der Einfluss der P-Versorgung auf den Ölertrag von Sonnenblume manifestierte sich insbesondere durch den Einfluss auf die Anzahl der Samen pro Pflanze, gefolgt vom Tausendkorngewicht, während die Ölkonzentration wiederum ohne Einfluss war. Die P-Akkumulation ( $\text{mg P Topf}^{-1}$ ) war bei gleicher P-Versorgung bei Sonnenblume höher als bei Saflor, insbesondere bei niedrigem P-Angebot. In der jeweiligen optimalen Versorgungsstufe akkumulierten die Kulturen die gleichen P-Menge. Sonnenblume hatte eine höhere Aufnahmeeffizienz ( $\text{mg P aufgenommen (mg P Angebot)}^{-1}$ ) im vergleich zu Saflor. Dies fand sich bei allen P-Versorgungsstufen. Im Nährlösungsversuch wies Sonnenblume, bei gleichem P-Angebot, eine höhere P-Akkumulation ( $\text{mg P Topf}^{-1}$ ) sowie Ausnutzung ( $\text{mg P aufgenommen (mg P Angebot)}^{-1}$ ) auf. Sonnenblume zeigte bei optimaler P-Versorgung eine höhere Effizienz (efficiency ratio, utilization index). Bei sehr niedrigem P-Angebot zeigt Saflor ein höheres 'efficiency ratio' als Sonnenblume, was man als Verdünnungseffekt ansehen kann, wenn wir es als 'utilization index' interpretieren. Die P-Verwertung von Sonnenblume, bewertet als efficiency ratio und utilization index, ist bei moderatem Angebot höher als bei Saflor Letzteres benötigt ein höheres externes P-Angebot als Sonnenblume bei optimaler und niedriger Versorgung um einen bestimmten Samenertrag zu erzeugen. Im Nährlösungsversuch wies Saflor ein höheres 'external P requirement' auf als Sonnenblume um eine bestimmte Trockenmasse zu produzieren. Im Nährlösungsversuch wies die Trockensubstanzkurve von Sonnenblume einen geringeren  $K_m$  auf als bei Saflor. Abschließend kann in bezug auf die P-Versorgung gesagt werden, dass Saflor einen höheren Bedarf als Sonnenblume hat, wobei letztere eine höhere Effizienz besitzt.

Beide Sorten reagierten stark auf die Steigerung der K-Gaben. Wachstum und Ertrag des Saflors waren bei Gaben von 1,0 g K pro Gefäß maximal, bei Sonnenblume war dies bei 3,0

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g K der Fall. Bei Saflor zeichnet sich ein besserer Ertrag bei niedrigen K-Gaben im Vergleich zu Sonnenblume ab. Das Entgegengesetzte wurde bei hohem K-Niveau beobachtet. In bezug auf die K-Mangelversorgung war der Ölertrag bei Saflor niedriger, wegen des verringerten Samenertrags. Auf die Ölkonzentration hatte dies keinen Einfluss. Bei der Ertragskomponentenanalyse zeigt sich, dass der Samenertrag durch das Gewicht pro Same beeinflusst wird, gefolgt von der Anzahl Körben pro Pflanze. Die Zahl der Samen pro Korb hatte einen geringfügig negativen Einfluss auf den Samenertrag. Im Gegensatz dazu reagierte der Ölertrag von Sonnenblume besonders stark auf eine niedrige K-Versorgung, welches mit dem verringerten Samenertrag und der niedrigen Ölkonzentration korrespondiert. Die Ertragskomponentenanalyse zeigt, dass der Samenertrag durch die Anzahl der Samen pro Korb beeinflusst wird, gefolgt vom Tausendkorngewicht. Beide Arten akkumulierten bei niedrigem K-Angebot ähnliche Mengen. Unterschiede gab es nur bei extrem niedrigem Kalium, da zeigte Saflor eine höhere K-Akkumulation. Saflor hat bei allen K-Versorgungsstufen eine höhere Ausnutzung an K (interpretiert als 'efficiency ratio') hinsichtlich des Samenertrages als Sonnenblume. In bezug auf den 'utilization index' war Saflor nur bei der niedrigen Versorgung überlegen. Saflor benötigt bei allen K-Versorgungsstufen deutlich weniger 'external K' um einen bestimmten Samenertrag zu erzeugen. Der aus der Ertragskurve abgeleitete  $K_m$  war bei Sonnenblume höher als bei Saflor, wogegen für den  $c_{min}$  gegenteilige Beobachtungen gelten. Dies zeigt, dass Saflor eine höhere K-Effizienz hinsichtlich des Ölertrag besitzt.

Im Feldversuch hatte die Sorte 3 und 8 den höchsten Ölertrag, dagegen hatten die Sorten 2, 9 und 10 den geringsten. Die Ertragskomponentenanalyse über alle Sorten ('pooled') zeigt, dass die Samenanzahl pro Korb den Haupteinfluss auf den Ölertrag bei allen 10 Sorten hat, gefolgt von der Korbanzahl pro Pflanze, und dem Tausendkorngewicht (TKG). Die Ölkonzentration war schwach negativ mit dem Ölertrag assoziiert, wie auch die Pflanzendichte. Selbst für die Sorten mit den höchsten Erträgen (Sorte 3 und 8), war aufgrund des geringen Samenertrages und der niedrigen Ölkonzentration der Ölertrag niedrig. Diese zwei Ertragskomponenten sind durch die Witterung während der Samenreife beeinflusst. Die Samenreife benötigt warme Temperaturen und längere Sonnenscheindauer für die optimale Samenbildung und Ölanreicherung, dies wurde in Norddeutschland nicht erreicht. Zusätzlich wurde die Samenqualität durch hohe Luftfeuchtigkeit, anhaltenden Regen und daraus folgendem Krankheitsbefall negativ beeinflusst. Die Anzahl an Samen pro Korb ist die Ertragskomponente, welche den größten Effekt auf den Ertrag hatte. Darauf sollte bei der Züchtung angepasster Genotypen die größte Beachtung gelegt werden.

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### Curriculum vitae

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