African Crop Science Journal, Vol. 20, No. 4, pp. 225 - 237
ISSN 1021-9730/2012 $4.00


# EFFECT OF NITROGEN ON SAFFLOWER PHYSIOLOGY AND PRODUCTIVITY

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*(Received 29 August, 2012; accepted 2 October, 2012)*

## ABSTRACT

Safflower (*Carthamus tinctorius* L.) is an oil-crop suitable for semi-arid agriculture, but its physiological response to agronomic inputs has yet to be fully evaluated. The effect of fertiliser on the physiology and production of Safflower grown in pots filled with standard grade perlite inside a semi-controlled glass house was studied. Plants were initially irrigated with 20-100 ml standard hydroponic solution and then one month after germination plants were irrigated using 50-200 ml complete Hoagland's solution, supplemented with one of eight levels of nitrogen (0, 25, 50, 75, 100, 125, 150, 175 kg N $ha^{-1}$) in the form of ammonium nitrate. Elevating nitrogen to 100 kg N $ha^{-1}$ significantly increased the assimilation rate by about 42 %, transpiration rate by 32%, stomatal conductance by 52% and LAI by 42% compared with the control. The above ground dry weight, seed yield and Water Use Efficiency (WUE) increased incrementally with increases in nitrogen rate. Above ground dry weight increased by an average of 42%, seed yield by 76% and WUE by 41% at harvest compared with the control. This study clearly demonstrates how nitrogen fertilisation can affect physiology of Safflower leading to improvement in seed yield.

*Key Words*: Stomatal conductance, transpiration, water use efficiency

## RÉSUMÉ

Le carthame (*Carthamus tinctorius* L.) est une culture oléagineuse appropriée à l'agriculture semi aride, mais sa réponse physiologique aux intrants agronomiques reste à évaluer. L'effet de fertilisants sur la physiologie et la production du carthame cultivé en pots rempli de perlites de niveau standard à l'intérieur d'une serre semi contrôlée était étudié. Les plants étaient initialement irrigués avec 20-100 ml de solution hydroponique standard et, ensuite, un mois après la germination des plants étaient irrigués avec 50-200 ml de solution de Hoagland complète et un supplément de huit niveau d'azote (0, 25, 50, 75, 100, 125, 150, 175 kg N $ha^{-1}$) sous forme du nitrate d'ammonium. L'utilisation de la dose de 100 kg N $ha^{-1}$ a significativement accru le taux d'assimilation d'environ 42 %, le taux de transpiration de 32 %, la conductance stomatale de 52 % et l'index de la surface foliaire de 42 % en comparaison avec le témoin. Le poids sec de la biomasse aérienne, le rendement en grain et le Water Use Efficieny (WUE) avait augmenté avec la dose d'azote. Le poids sec de la biomasse aérienne a en moyenne augmenté de 42% pendant que le rendement en grains et le WAE ont augmenté à la récolte de 76% et 41% , respectivement, en comparaison avec le témoin. Cette étude démontre clairement comment la fertilisation azotée peut affecter la physiologie du carthame conduisant à l'amélioration du rendement en grains.

*Mots Cles*: Conductance stomatale, transpiration, water use efficiency

## INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a multipurpose crop, belonging to the family Compositae or Asteraceae. It has exhibited potential medical, pharmaceutical and cosmetic importance all over the world (Berglund *et al*., 2007; Emongor, 2010; Mündel and Bergman, 2010). Safflower is currently grown mostly for edible oil, considered as one of the best for human consumption due to high quantities (70 - 75%) of poly-unsaturated (Linoleic acid ) or mono-unsaturated fatty acid (Oleic acid) (Nimbkar and Singh, 2005).

Historically, the crop is restricted to the middle east, parts of Asia and Africa and over time it has been adapted to the semi-arid climatic condition of western United States (Lartey *et al.*, 2005). In North America, it is ranked as one of the most superior alternative oil crop (Johnston *et al.*, 2002). It is being grown in over 60 countries in the world, but about 50% of world production remains in India (Omidi *et al.*, 2009). In general, the crop has adapted to areas receiving low rainfall with a low humidity during flowering and seed maturation stages (Knowles, 1976).

Safflower has a reputation as a drought tolerant crop, in comparison with small grain and other oil crops mainly because of its ability to capture the water from depths of up to 2.5 - 3 m (Berglund *et al.*, 2007; Burgener *et al*., 2004). It exhibits reasonable production under low fertility conditions and has reasonable production potential under low input situations (Abbadi *et al.*, 2008).

The importance of nitrogen fertilisation for Safflower grown in the field (Zaman and Das, 1990), and its physiological response (Dordas and Sioulas, 2008) has been investigated in only a limited manner. These studies showed that elevated nitrogen levels to 200 kg N ha$^{-1}$, in the form of $(NH4)_2\ SO_4$, increased leaf nitrogen concentration and chlorophyll content leading to an increase in photosynthetic rate and stomatal conductance. Such increased photosynthetic rate and stomatal conductance resulted in enhanced water use efficiency and net increased seed yield.

Total biomass as an indicator of photosynthetic product translocation and accumulation has been used in Safflower and an increase of nitrogen fertilisation up to 200 kg N ha$^{-1}$ has given a substantial increase in total above ground biomass at both anthesis and harvest. In $C_3$ plants, the photosynthetic capacity is limited by nitrogen per unit leaf area (Evans, 1989) and total net assimilation is strongly related to leaf area index (LAI) and, thereby, is directly associated with plant biomass accumulation.

A multi-year study of Safflower at four levels of nitrogen (0, 40, 80 and 120 kg N ha$^{-1}$) concluded that plant growth at different stages from elongation until maturity did not affect LAI, chlorophyll content, plant organ nitrogen concentration and biomass (Yau and Ryan, 2010). However, in other oil crops such as sunflower (*Helianthus annus* L.), nitrogen fertiliser, enhanced growth and productivity through increasing the LAI and canopy development (Gimenez *et al.*, 1994).

Nitrogen levels have been shown to affect Safflower crop component and growth performance. Siddique and Oad (2006) considered 120 kg N ha$^{-1}$ as the optimum level for production of maximum seed yield by significantly producing greater branches, heavier seed weight and more seeds per plant. While more heads per plant and taller plants were recorded in plots treated with 180 kg N ha$^{-1}$, these did not produce any further significant increases in yield. Strasil and Vorlicek (2002) found that neither the one thousand seed weight nor number of capitula per plant and consequentl, seed yield in three varieties of Safflower (Gila, cw-74 and Sironaria) at two sites under three rates of nitrogen (0, 40 and 80 kg N ha$^{-1}$) were significantly affected by nitrogen fertiliser.

Studying the effects of nitrogen in the field or in potting compost is always complicated by the effect of soil residual nitrogen stores. The use of hydroponic or semi-hydroponic systems, however, allows nitrogen supply to be controlled more precisely and there are no reports in the literature of the effect of nitrogen fertiliser on Safflower grown in pots using perlite. This study investigated the effects of nitrogen fertiliser on the growth, physiology and yield of Safflower using perlite as the growth medium.

## MATERIALS AND METHODS

The experiment was carried out in a semi-controlled glasshouse at Plymouth University, UK. Safflower seeds (Richter Lemon Yellow) were germinated in an incubator using a fluctuating 12 hr/12 hr temperature of 12 and 20-

24 °C. After 3 days, germinated seed (radicle emerging) were planted in 30 cm (height) and 11.24 cm (diameter) pots filled with standard grade perlite.

The experimental design was a randomised block with four replicates. Each replicate comprised of 48 pots. Plants were watered with 20-100 ml of a standard hydroponic solution (Vita Link Max Grow (soft water) A and B) every 3-5 days, for 28 days. Thereafter, plants were irrigated using 50-200 ml of complete Hoagland's solution, minus nitrogen (Hershey, 1995), every week.

Eight levels of ammonium nitrogen salt solution were prepared by dissolving 0, 0.46, 0.91, 1.34, 1.8, 2.28, 2.64, 3.12 g ammonium nitrogen in 2.8 litre of Hoagland solution, and 200 ml of the appropriate solution per pot in 4 doses, at monthly intervals to give the final equivalent of 0, 25, 50, 75, 100, 125, 150 and 175 kg N $ha^{-1}$.

Between fertigations, irrigation water was applied according to plant demand. Air temperature and humidity were logged using a Tiny Tag data logger (mean maximum and minimum temperature were 30 and 6 °C during the growing season and the mean maximum and minimum humilities were 100 and 4.6%, respectively).

Light was supplemented using SonT Sodium Vapour lamps during the winter months to maintain a 12 hr photoperiod, and the photosynthetic active radiation (PAR) was measured on both sunny and cloudy days, and PAR ranged between 800-1000 µmol $m^{-2}$ $s^{-1}$.

The volume of irrigation water was recorded on each occasion so that total volumes of water used was known. The instantaneous water use efficiency (WUE) was calculated on an economic yield basis by dividing the biomass production at both anthesis and harvest by the amount of water consumed during the growing periods (Conley *et al*., 2001).

At 50% anthesis, physiological parameters, namely, assimilation rate (A), transpiration rate (E), stomatal conductance (gs), and sub-stomatal $CO_2$ (Ci) were measured on the youngest, three top expanded leaves of three plants in each replicate for each treatment, randomly. This was done using a LCi portable photosynthesis system equipped with a 6 $cm^2$ chamber with the spectral response from the Lamp operated at 11V DC. After one day, half of the plants used for photosynthesis measurement were harvested and used for measuring leaf area index (LAI), chlorophyll content, above ground biomass and nitrogen content. The leaf area of three plants were measured using a Delta-T Image Analysis System-type (DIAS) ™. The LAI was calculated by dividing the leaf area by the surface area of pot (Bréda, 2003).

Three subsamples of leaves were extracted with acetone 80% (v/v) and the amount of chlorophyll was determined using a spectrophotometer at wavelength 645 and 663 nm on a fresh weight basis (Porra, 2002). The remaining plants were separated into stems + branches, leaves, capitula and dried at 80 °C for 48 hr in a Gallenkamp drying oven until constant weight and above ground dry weight recorded. These dried organs were ground and nitrogen concentrations measured using Kjeldahl (Bremner *et al*., 1996). At harvest, above ground dry weight, seed yield and yield components (number of capitula per plant, seeds per plant, seed weight per plant and 1000 weight) were determined on three plants from each treatment and each replicate.

Statistical analyses of data were performed using Minitab v. 15 using two-way ANOVA. Significant differences between treatments were determined using least significant differences (LSD) at the 0.05 level. Correlation coefficient analyses between parameters were also carried out using Sigma Plot version 12.0.

## RESULTS

**Gas exchange.** Safflower leaf assimilation rate (A) at anthesis increased incrementally, with each increase in nitrogen supplied ($P<0.05$), up to 100 kg N $ha^{-1}$ where the average assimilation rate was 42% higher than the control (Fig. 1) . Stomatal conductance (gs) and Transpiration rate (E) also showed incrementally increasing response curves to nitrogen (Figs. 2 and 3), and there were significant ($P<0.05$) increases at anthesis. The application of 100 kg N $ha^{-1}$ increased the stomatal conductance by 52% and led to an increase in mean transpiration rate by

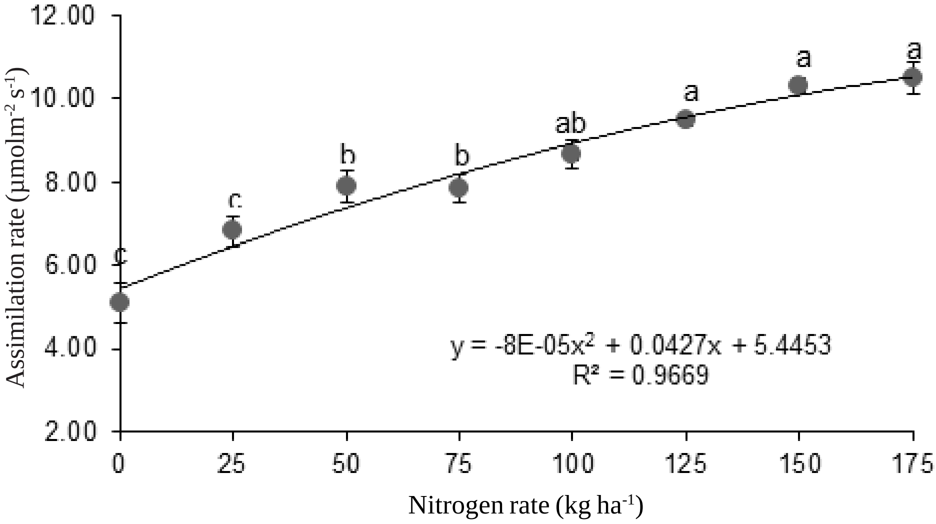


Figure 1. Leaf assimilation rate at anthesis under different levels of N fertiliser in Safflower. Points with the same letter are not significantly different from each other.

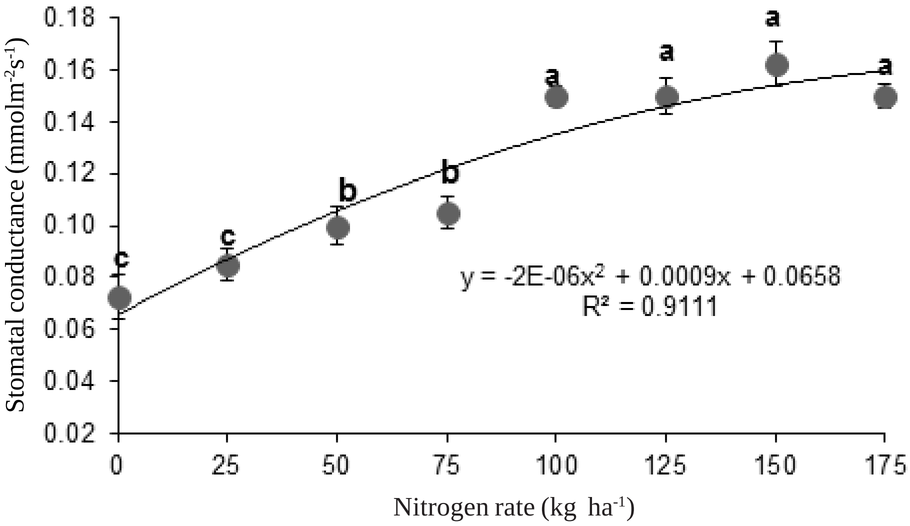


Figure 2. Leaf stomatal conductance under different levels of N fertiliser at anthesis in Safflower. Points with the same letter are not significantly different from each other.

32%, compared with the control. In contrast, the sub-stomatal concentration of $CO_2$ decreased incrementally by the increase in N application (Fig. 4).

**Water use efficiency (WUE).** Water use efficiency of Safflower from sowing to harvest (Fig. 5) was higher in the fertilised treatments than in the control. Under the highest nitrogen rate of 175 kg N $ha^{-1}$, WUE increased by 41% compared with the control.

**Leaf area and leaf area index.** The leaf area index (LAI) of Safflower increased incrementally with nitrogen levels ($P < 0.05$), reaching a plateau of about LAI 6.27 for the three highest levels of

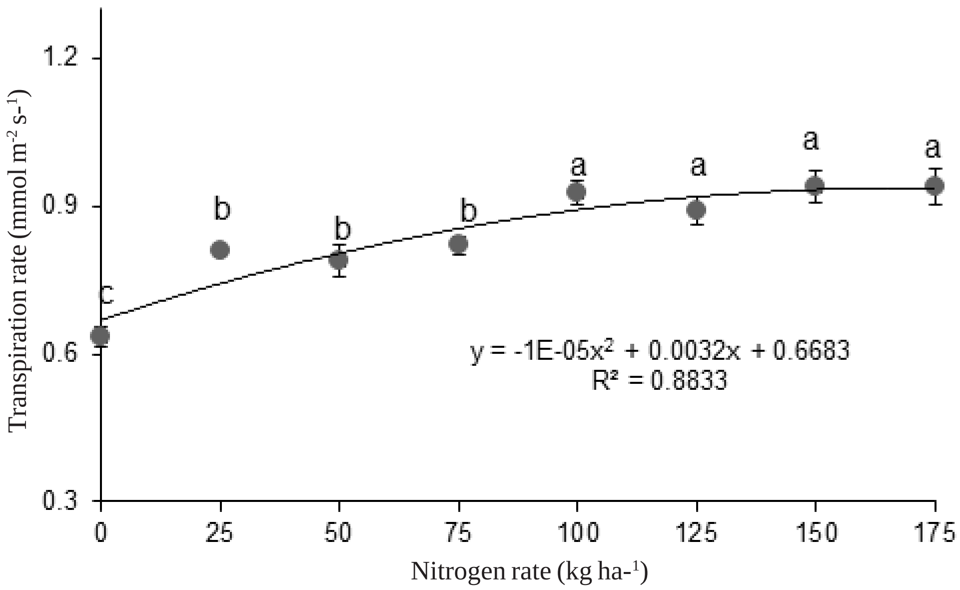


Figure 3. Leaf transpiration rate at anthesis under different levels of N fertiliser in Safflower. Points with the same letter are not significantly different from each other.

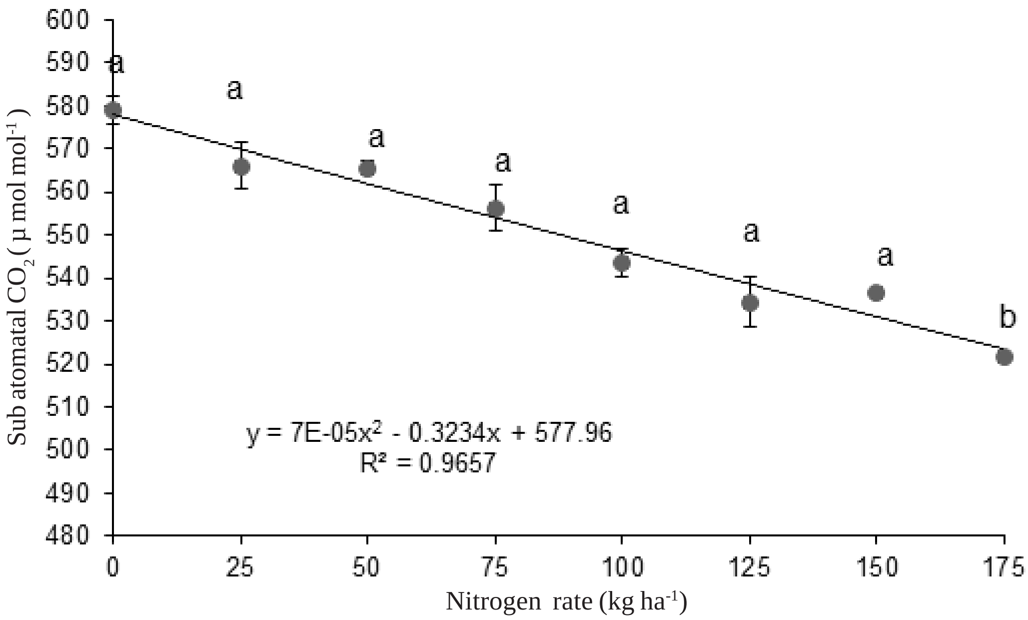


Figure 4. Leaf sub stomatal $CO_2$ concentration under different levels of N fertiliser at anthesis in Safflower. Points with the same letter are not significantly different from each other.

nitrogen (Fig. 6). Application of 125 kg N $ha^{-1}$ increased the LAI by 42% compared with the control.

**Chlorophyll content.** Chlorophyll a, b and total chlorophyll content at anthesis were significantly ($P<0.05$) influenced by nitrogen treatment (Table 1). The 150 kg N $ha^{-1}$ rate increased chlorophyll

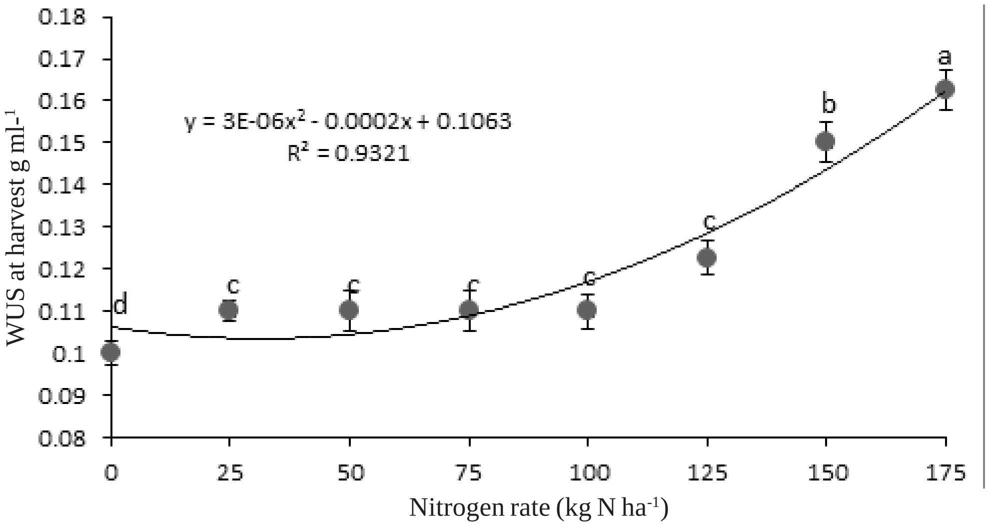


Figure 5. Safflower plant water use efficiency from sowing to harvest under different levels of N fertiliser.

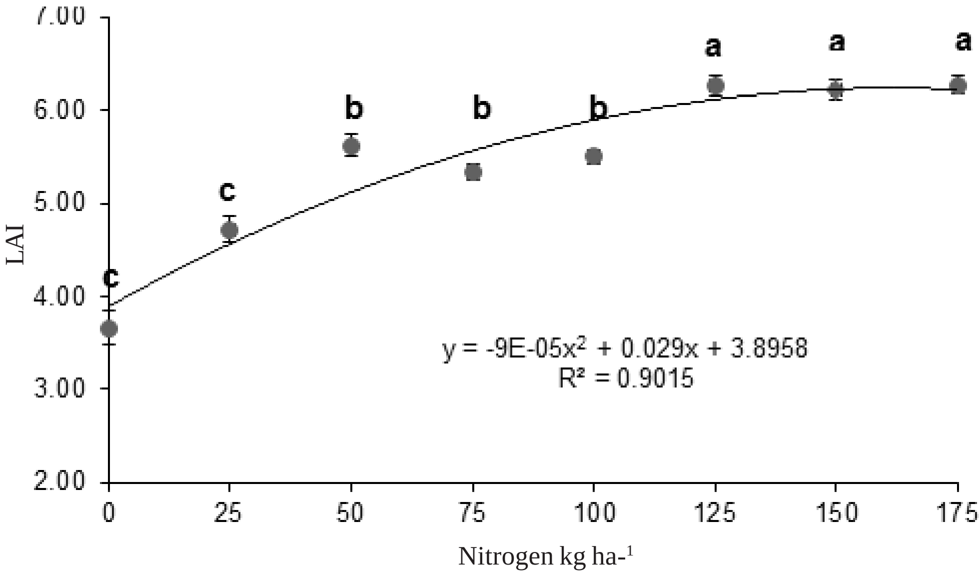


Figure 6. Safflower leaf area index at anthesis under different levels of N fertiliser. Points with the same letter are not significantly different from each other.

content by 55% compared with the control. No significant increment in chlorophyll a, b and total chlorophyll was observed with amount of nitrogen above 150 kg N $ha^{-1}$.

**Nitrogen concentration.** At anthesis, the nitrogen content of the whole plants was significantly ($P<0.05$) affected by nitrogen fertilisation (Table 2). Overall, 100 kg N $ha^{-1}$ increased total plant nitrogen by 40%; however, there were no significant increases in total above ground nitrogen content.

Nitrogen influenced Safflower plant organs differently. Stems and branches responded to nitrogen level with a significant increment in nitrogen content. The capitula responded over

TABLE 1. Plant chlorophyll content (mg g $^{-1}$ leaf fresh weight) under eight N levels at anthesis in Safflower

| N rate ( kg N. ha$^{-1}$) | Chlorophyll a | Chlorophyll b | Total chlorophyll |
|---|---|---|---|
| 0 (control) | 0.58 | 0.40 | 0.98 |
| 25 | 0.85 | 0.48 | 1.34 |
| 50 | 0.85 | 0.51 | 1.40 |
| 75 | 1.05 | 0.58 | 1.64 |
| 100 | 1 .21 | 0.60 | 1.83 |
| 125 | 1.22 | 0.58 | 1.83 |
| 150 | 1.42 | 0.78 | 2.10 |
| 175 | 1.42 | 0.79 | 2.20 |
| P | 0.000 | 0.000 | 0.000 |
| LSD (0.05) | 0.25 | 0.13 | 0.31 |

TABLE 2. Percentage of nitrogen concentration (g 100 g$^{-1}$ g dry weight) of various Safflower plant parts under varying N levels at anthesis

| N rate ( kg N ha$^{-1}$) | Stem and branch | Leaf | Capitula |
|---|---|---|---|
| 0 (control) | 0.24 | 1.28 | 0.73 |
| 25 | 0.25 | 1.56 | 0.82 |
| 50 | 0.25 | 1.74 | 0.89 |
| 75 | 0.25 | 1.88 | 0.99 |
| 100 | 0.28 | 2.14 | 1.09 |
| 125 | 0.31 | 2.21 | 1.08 |
| 150 | 0.30 | 2.16 | 1.20 |
| 175 | 0.33 | 2.34 | 1.22 |
| P | 0.000 | 0.000 | 0.000 |
| LSD (0.05) | 0.03 | 0.31 | 0.43 |

most of the range of nitrogen applied. Leaves were less responsive, with highest nitrogen content achieved at 100 kg N ha.$^{-1}$

**Yield components.** Nitrogen significantly ($P< 0.05$) influenced Safflower above ground biomass at both anthesis and harvest stages; with the seed yield and yield components at harvest stage only (Table 3). The above ground biomass increased by an average of 46 and 42%, respectively, at anthesis and harvest, with a nitrogen application of 175 kg N ha$^{-1}$, compared with the control. Seed yield showed a similar pattern of response to nitrogen levels. Application of 175 kg N ha$^{-1}$ increased seed yield by an average of 74%, compared with the control. The yield components (the number of capitula per plant, the number of seed per plant and the seed weight per plant) were also increased by nitrogen fertilisation (Table 3). The number of capitula was increased by an average of 51%; while seeds and seed weight per plant were enhanced by an average of 76% at 175kg N ha$^{-1}$, compared with the control.

**Correlation.** Positive correlation was found between A, gs, E, WUE, N concentrations and leaf chlorophyll content at anthesis, and LAI (Table 4); while E and A had negative relationships with Ci for the highest N-grown plants. As the E and A of the leaves decreased under low nitrogen grown plants and the control, the $CO_2$ concentration in the sub-stomatal cavity of the leaf increased. Also, there were negative

TABLE 3. Above ground biomass at anthesis and harvest (g $pl^{-1}$), seed yield (g $pl^{-1}$) and yield components at harvest (number of capitula $plant^{-1}$, number of seed $plant^{-1}$) of safflower under eight levels of nitrogen

| Treatments (kg N $ha^{-1}$) | Above ground dry weight at anthesis | Above ground dry weight at harvest | Seed yield | Capitula number | Seed number |
|---|---|---|---|---|---|
| 0 | 8.65 | 8.46 | 0.21 | 3.00 | 6.00 |
| 25 | 9.79 | 8.89 | 0.22 | 3.50 | 7.00 |
| 50 | 11.93 | 9.71 | 0.29 | 3.50 | 9.75 |
| 75 | 12.24 | 11.30 | 0.26 | 3.25 | 9.25 |
| 100 | 13.20 | 10.85 | 0.60 | 3.38 | 16.00 |
| 120 | 13.88 | 13.54 | 0.71 | 4.13 | 18.25 |
| 150 | 13.80 | 13.04 | 0.82 | 4.25 | 22.50 |
| 175 | 16.09 | 14.72 | 0.89 | 4.00 | 24.50 |
| LSD (0.05) | 1.76 | 1.77 | 0.12 | 0.89 | 2.66 |

correlations between above ground biomass at harvest and E at anthesis and WUE at harvest was not significantly correlated with the LAI at anthesis.

Altogether, in this study seed yield had positive correlations with all of the physiological parameters, WUE, N concentration, leaf chlorophyll content, LAI and above ground biomass.

## DISCUSSION

This experiment indicated that Safflower leaf assimilation rate at anthesis increases with increasing levels of nitrogen fertiliser (Fig. 1). At 100 kg N $ha^{-1}$ the assimilation rate was found to be increased by an average of 42%, compared with the control with no significant incremental increase in plant photosynthesis above this level. This indicates that this amount was critical for plants to achieve maximum assimilation rate. Maximum assimilation rate achieved at anthesis was previously reported in field-grown plants with an average of 51% increase in assimilation rate (Dordas and Sioulas, 2008), and such an increment was about twice higher than the control under the highest nitrogen level.

Increased assimilation rate under nitrogen fertilisation is commonly reported for other crops (Ciompi *et al*., 1996) and it is considered an essential aspect of the crop's physiological response to limiting factor improvement. Nitrogen availability typically increases the photosynthetic capacity of $C_3$ plants because of increased investment in the levels of proteins of the Calvin cycle (Rubisco) and thylakoids, which are directly related to increases in the nitrogen content of the leaf (Evans, 1989). Thus, photosynthetic enhancement can be interpreted as a result of improvement in leaf nitrogen status and the consequent nitrogen investment in photosynthetic protein components.

In contrast, nitrogen deficiency reduced initial activity of Rubisco by nearly 20%, and slightly increased starch about 25% in upper canopy leaves for field grown sunflower. This led to reduced initial photosynthesis, but eventually led to an increase in the intercellular $CO_2$ such that the rate of assimilation was maintained per unit of leaf nitrogen (Fredeen *et al*., 1991).

Stomatal conductance increased and led to an increased transpiration rate at anthesis. Application of 100 kg N $ha^{-1}$ increased stomatal conductance by 52% and transpiration rate by 32%, respectively. Stomatal conductance was up to two times higher under the highest nitrogen level compared with the lowest at anthesis; and this parameter increased by an average of 27% in field grown Safflower (Dordas and Sioulas, 2008). This achievement could be due to the contribution of transpiration rate and stomatal conductance on photosynthetic assimilation rate and there is a clear linear relationship between assimilation rate and transpiration rate as well as between assimilation rate and stomatal conductance. This result is strongly supported

TABLE 4. Correlation coefficients among physiological characteristics and above ground biomass at anthesis, above ground biomass and seed yield at harvest

| | LAI | A | E | gs | ci | Total chlorophyll | Total nitrogen content | Above ground biomass at anthesis | Above ground biomass at harvest | Seed yield | WUE at harvest |
|---|---|---|---|---|---|---|---|---|---|---|---|
| LAI | | | | | | | | | | | |
| A | 0.964 *** | | | | | | | | | | |
| E | 0.891 ** | 0.942 *** | | | | | | | | | |
| gs | 0.937 *** | 0.933 *** | 0.925 *** | | | | | | | | |
| ci | 0.882 ** | 0.910 ** | 0.903 ** | 0.928 *** | | | | | | | |
| Total chlorophyll | 0.899 ** | 0.911 ** | 0.937 *** | 0.937 *** | 0.966 *** | | | | | | |
| Total nitrogen content | 0.928 *** | 0.975 *** | 0.953 *** | 0.977 *** | 0.971 *** | 0.979 *** | | | | | |
| Above ground biomass at anthesis | 0.925 *** | 0.960 *** | 0.877 *** | 0.891 *** | 0.961 *** | 0.955 *** | 0.950 *** | | | | |
| Above ground biomass at harvest | 0.942 *** | 0.964 *** | 0.551 ns | 0.911 ** | 0.979 *** | 0.970 *** | 0.956 *** | 0.962 *** | | | |
| Seed yield | 0.802 ** | 0.912 ** | 0.841** | 0.914 ** | 0.948 *** | 0.918 ** | 0.917 ** | 0.890 ** | 0.940 *** | | |
| WUE at harvest | 0.665 ns | 0.900 ** | 0.711 * | 0.7029 * | 0.855 ** | 0.848 ** | 0.749 * | 0.802 ** | 0.880 ** | 0.743* | |

ns = non-significant (P< 0.05); * Significant at P< 0.05 level of probability; ** significant at P< 0.01 level of probability; *** significant at P< 0.001 level of probability. LAI = leaf area index; A = Assimilation rate; E = Transpiration rate; gs = Stomatal conductance; ci = Sub-stomatal CO2; WUE = water use efficiency by previous studies (Fredeen *et al*., 1991; Broadley, 2000; Cechin and Fátima Fumis, 2004; Zhao *et al*., 2005; Del Pozo *et al*., 2007).

A negative correlation between assimilation rate and sub-stomatal concentration of $CO_2$ was obtained. This finding was interpreted as a result of investment of carbon in photosynthesis with nitrogen supplied and led to a decline in the intercellular $CO_2$, which corresponded with that reported previously for Safflower (Dordas and Sioulas, 2008). It was observed that in Safflower plants, the highest assimilation rate was in the N fertilised treatments, related to higher stomatal conductance and transpiration rate leading to the highest water use. This also led to higher biomass production rates and, therefore, an improved WUE, which showed an average increase of about 41% from sowing to harvest under the highest nitrogen rate compared with the control. This can be interpreted as a result of greater increase in the above ground dry matter accumulation relative to the increased water transpired, thus, increasing as a consequence. This is supported by Dordas and Sioulas (2008) for Safflower and has been previously recorded in other crops and plant species (Ciompi *et al*., 1996). Cechin and Fumis (2004) found that increases in transpiration rate in high nitrogen grown sunflower plants did not result in lower WUE because of a higher rate of photosynthesis.

In response to N fertilisation, leaf area index of Safflower behaved like many other $C_3$ plants with a strong response to nitrogen fertiliser and at 125 kg N $ha^{-1}$, LAI was increased by 42% compared with the control. LAI reached a plateau of 6.27 for the highest levels of nitrogen and was interpreted as plant growth acclimation in term of leaf growth with nitrogen availability. LAI was tightly correlated with assimilation rate and this was interpreted as improved growth through increasing the number of cells and their size as a result of increased photosynthetic rate caused by increased nitrogen as previously established (Lea *et al*., 2001). Similar findings were reported for field grown sunflower (Gimenez *et al.*, 1994; Cechin and Fumis, 2004) but increasing nitrogen from 0 to 120 kg N $ha^{-1}$ did not change LAI relative to the control and as a result above ground biomass in field grown Safflower. Safflower growth in term of LAI in this study was in a smaller range (between 3.66 to 6.27) compared to that produced in field grown Safflower which ranged between 19 to 20 under Mediterranean conditions (Yau and Ryan, 2010). This is probably due to the field grown plants being able to explore a greater root growth which consequently led to a greater vegetative growth. Differences can also exist between cultivars used.

The higher concentration of nitrogen in the leaves of Safflower led to a higher chlorophyll content. This was also previously reported for Safflower and a linear relationship between leaf chlorophyll content and plant nitrogen concentration was reported at anthesis. Consequently, the positive relationship between leaf chlorophyll content and leaf assimilation rate at anthesis was observed. This is in agreement with Lawlor *et al*. (2001) and other findings revealing that the structures involved in light harvesting in photosynthesis that capture the photon energy are a chlorophyll protein complex.

In contrast, Yau and Ryan (2010) reported that none of the four nitrogen levels (0, 40, 80 and 120 kg N $ha^{-1}$) in the form of ammonium sulphate had significant increment of changes in the nitrogen concentration of different organs of Safflower and as a result leaf chlorophyll content as well the consequent biomass was not affected significantly when tested in field grown multi-year experiments (Yau and Ryan, 2010).

The assimilation rate factors mentioned above led to a higher biomass production, both at anthesis and harvest, when nitrogen was increased. This is reported as typical elsewhere (Huber *et al*., 1989; Cechin and de Fátima Fumis, 2004; Zhao *et al*., 2005) and indicated that the total plant dry matter production was strongly affected by nitrogen fertiliser and paralleled shoot/leaf dry matter accumulation. In this study, Safflower plants given adequate nitrogen were up two times heavier than the control. Similar results have been obtained for field grown Safflower. Both increasing assimilation rate and biomass led ultimately to increases in seed yield. This was interpreted as a result of improved leaf nitrogen status by nitrogen fertiliser, and clearly demonstrated the role of nitrogen in photosynthesis enhancement and photo-synthetic product partitioning. In contrast with this result, Siddiqui and Oad (2006) stated that field grown Safflower seed yield responded to nitrogen with each additional increment in nitrogen rate and peaked at 120 kg N $ha^{-1}$ with no significant response with further increase in nitrogen fertiliser.

Increases in seed yield were always associated with an increase in heads (capitula) per plant, and thereby seed number and this is reported elsewhere too (Abbadi *et al.*, 2008) and seed number positively correlated with capitula number per plant (r=0.946). This generally agrees with previous studies ( Tuncturk and Yildirim, 2004; Dordas and Sioulas, 2008). Safflower seed yield increased but was associated with both heads per plant and seeds per head, when fertilised with 100 kg N $ha^{-1}$ at three stages of sowing, early elongation and early flowering (Soleimani, 2010).

Seed yields per plant obtained in this study were much lower than expected; this was possibly because of mean relative humidity (about 50%) associated with an average low temperature (17 $^{o}$C) during the flowering period, which may have affected head fertility. Safflower is typically grown in arid or semi-arid regions of the world (Johnston *et al*., 2002) with a hot and dry climate, especially during flowering (Knowles, 1976; Dajue and Munde, 1996) and moist weather during flowering, as experienced in the UK, even in a glasshouse, appears to reduce seed set. In the current experiment, unfortunately, due to insufficient quantities of seed obtained at harvest, seed oil content and fatty acid were performed on a sample pooled from all replicates, so it was difficult to make a decision about the effect of nitrogen supplied on seed oil composition. However, seed oil content was reduced slightly by nitrogen fertilisation and seed oil profile was not changed significantly (data not shown).

## CONCLUSION

Safflower seed yield shows a positive and incremental response to nitrogen. The combination of increases in both assimilation rate and leaf area contributed to the increase in seed yield. It is noticeable that seed yield response continued to increase while leaf area and assimilation rate slowed down. This indicates that at high nitrogen rates, there is an effect on partitioning of plant biomass to the seed component. Water use per unit weight of seed increased as nitrogen rate increased indicating that there is a water use cost associated with increasing yield by nitrogen fertiliser.

## ACKNOWLEDGEMENT

The Iraqi Ministry of Higher Education through a PhD scholarship financially supported this study and University of Plymouth are gratefully acknowledged. Also, thanks to Stirling University for the help in conducting seed chemical analysis.

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