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Yield Components, Leaf Pigment Contents, Patterns of Seed Filling, Dry Matter, LAI and LAID of Some Safflower (*Carthamus tinctorius* L.) Genotypes in Iran

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Abstract: In order to assess the genotypic variation among yield components and different physiological parameters and their relationships with safflower seed yield, six safflower genotypes were grown in Pakdasht, Iran in a randomized complete block design with four replications, during 2003-2004 growing season. Among the genotypes, chlorophyll a, chlorophyll b, chlorophyll a+b, total carotenoids contents, chlorophyll a/chlorophyll b ratio and Chlorophyll a+b/total carotenoids ratio ranged from 0.78 to 1.10, from 0.54 to 0.71, from 1.37 to 1.71, from 0.09 to 0.13 mg g⁻¹, from 1.33 to 1.68 and from 13.52 to 14.82, respectively. Negative relationships existed between seed yield and pigment contents. There were significant yield differences among genotypes and varied from 2452.60 to 3897.20 kg ha⁻¹. A diverse range of capitulum diameter (24.08-28.91 mm), seed weight/capitulum (1.18-2.04g), number of seeds/m² (8704.5-13165.4), number of capitula/plant (16.38-23.27), number of seeds/capitulum (35.65-41.90) and 1000-seed weight (29.94-50.60 g) was recorded. Genotypes differed in HI and the HI values ranged from 21.83% (LRK-262) to 29.62% (IL.111). In the studied set of 6 safflower genotypes, total biomass and LAI peaked around after full flowering and at the beginning of flowering, respectively. Zarghan-279 (with the greatest LAID) had 25% longer LAID than LRV.51.51 (with the lowest LAID). Differences among genotypes for rate of seed filling and effective seed filling duration were significant and differences in seed yield could be attributed to differences in the rate of seed filling. The results of this experiment indicate that physiological parameters including rate of seed filling, rapid leaf formation and expansion and delayed plant senescence are the characteristics of high-yielding safflower. Also, higher dry matter accumulation, HI, seed weight/capitulum, 1000-seed weight and capitulum diameter were found to be closely related to high-yield genotypes.

Key words: Safflower, yield and yield components, pigment contents, seed filling rate and duration, LAID, dry matter

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) a strongly tap-rooted annual plant and resistant to saline condition and to moisture stress, is a member of the family Compositae or Asteraceae, ranks eighth after soybean, groundnut, rapeseed, sunflower, sesame, linseed and castor crops grown world-wide. India, Mexico, USA, Ethiopia, Argentina and Australia together account for 99 and 87% of the world safflower area and production,

respectively (Dwivedi *et al.*, 2005). The world average yield of safflower is much lower (0.72 t ha⁻¹) than those reported for soybean (2.34 t ha⁻¹), rapeseed (1.51 t ha⁻¹), groundnut (1.37 t ha⁻¹) and sunflower (1.14 t ha⁻¹). The seeds contain 30% oil, 20% protein and 35% crude fiber. The seeds are also a rich source of minerals (Zn, Cu, Mn and Fe), vitamins (Thiamine and B-carotene) and the tocopherols (alpha, beta and gamma) (Velasco *et al.*, 2005). Safflower leaves, petals and seeds have tremendous medicinal and therapeutic significance and

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petals are also used for extracting dye for coloring cloths and foodstuffs (Danisova and Sarooka, 1994; Varma *et al.*, 1997; Zhaomu and Lijie, 2001; Carvalho *et al.*, 2006). The green safflower fodder is highly palatable and comparable to other forage legumes, grasses and cereals in crude protein and total digestible nutrients (Lachover and Kostrinski, 1965). The Relative Feed Value (RFV) of safflower forage from the bud, bloom and seed fill growth stages are generally above the standard value (RFV = 100) for full bloom alfalfa forage (Wichman *et al.*, 2001).

Safflower breeders and physiologists have long been intrigued by the prospect of increasing seed and oil yield. In fact, to enhance safflower yield improvement in the future, physiological understanding of yield improvement is essential.

The yield of safflowers can be divided into several components. Seed weight, plant height, first branch height, number of branch, capitulum diameter, number of seed per capitulum and number of capitulum per plant are the main parameters which are determined at different experiments (Gonzalez *et al.*, 1994; Pascual-Villalobos and Alburquerque, 1996; Omidi-Tabrizi, 2000; Bagheri *et al.*, 2001; Camas and Esendal, 2006). However, Chaudhary (1990) showed that number of seed/capitulum, number of capitula/plant and 1000-seed weight could be used for selection of high seed yielding varieties as primary selection criteria in an investigation with 50 safflower lines. In a research with three safflower cultivars, Camas and Esendal (2006) estimated the heritability for capitulum diameter, number of seed per capitulum and 1000-seed weight were 21, 69 and 81%, respectively and reported that 1000-seed weight could be used to succeed in selection in early generation.

Yield increase can be achieved either by increasing biomass production or Harvest Index (HI) or both (Ying *et al.*, 1998). Biomass production is, in turn, associated with leaf area expansion and duration. Close associations between leaf area and seed yield have been reported for many species (Evans, 1993); however, information on these associations in safflower is not available. Ghorpade *et al.* (1993) reported that safflower plant accumulated 64 to 72% of the total dry matter at harvest during vegetative phase. At flowering plant accumulated 41, 28, 18 and 13% dry matter in stem, leaves, bracts and capitula, respectively; while at harvest it was 30, 12, 13 and 45% in stem, leaves, bracts and capitula, respectively.

Pigment contents in the leaves play an important role in the crop photosynthesis (Schoefs, 2002). Morrison *et al.* (1999) showed a positive association between seed yield and chlorophyll a+b concentration in soybean. In their research no significant correlation was found between photosynthesis and leaf chlorophyll.

On the other hand, Daynard *et al.* (1971) have stated that the yield of a grain crop might be defined as the product of average rate of grain production and duration of grain formation. The relationships between these seed filling rate and duration and seed yield have been investigated in many crops like wheat (Bruckner and Froberg, 1987), rice (Jones *et al.*, 1979), barley (Voltas *et al.*, 1999), triticale (Santiveri *et al.*, 2002) and rapeseed (Soufizadeh, 2005). Nevertheless, the literature is inconsistent regarding the relative importance of the rate and duration of seed filling in determining final seed yield.

To the best of our knowledge, information regarding the relationships between yield components, dry matter, Leaf Area Index (LAI), Leaf Area Index Duration (LAID), chlorophylls and carotenoid content, patterns of seed filling and seed yield in safflower is not available or is very little. The purpose of this study was to assess the genotypic variation among yield components, the dynamics of dry matter accumulation, LAI and LAID, chlorophylls and carotenoid contents, patterns of seed filling and their relationships with safflower seed yield.

MATERIALS AND METHODS

Due to the fact that in different years and environmental conditions yields stability of genotypes used in present experiment were demonstrated in Iran (e.g., Omidi-Tabrizi, 2006), this study was conducted during 2003-2004 growing season at Abooreihan Campus, University of Tehran, Pakdasht (35° 28' N, 51° 44' E and 1280 masl). The research field had a loam soil. This location is an arid area (according to the De Martonne climate classification) characterized by warm and dry summers, moderate winters and 170 mm annual rainfall.

Six winter safflower genotypes which were improved by selection (mass selection and pure-line selection) among different landraces and lines in Iran were used in the experiment (Mokhtassi Bidgoli *et al.*, 2006). These genotypes included LRK-262, LRV.51.51, Aghkand-e-Miyaneh, Zarghan-279, IL.111 and Varamin-295. The experiment was arranged in a randomized complete block design with four replications. Plots were 5 m long and consisted of seven rows, 0.5 m apart. Prior to seeding 30 kg urea ha⁻¹ and 90 kg ammonium phosphate ha⁻¹ were broadcasted and incorporated into the soil. Plots were top-dressed at stem elongation stage with 75 kg urea ha⁻¹. Seeds were hand-planted on 29 Oct. 2003 at the rate of 12 seeds/m of row and then were thinned at rosette stage to achieve a density of approximately 133333 ha⁻¹. The field was frequently irrigated to avoid visible symptoms of drought stress. Weeds and insects were effectively controlled.

Sampling started just prior to the stem elongation stage. Plant samples were taken approximately every 8 days. At each sampling date, four randomly selected plants per replication and genotype were cut at ground level and depends on phenological stage were separated into leaves (including bracts), stems (including branches), capitula and finally seeds. All samples were dried at 75°C to constant weight and then weighed. Before drying, green leaf area was measured using leaf area meter (Model LI-3100, LI-COR Inc., Lincoln, NE). Total biomass was calculated by summing the dry weights of all plant tissue types. Final harvest consisted of 3 m of center row of each plot. Harvest Index (HI) at maturity was calculated by dividing seed weight by total biomass dry weight and multiplying by 100. The following yield components were measured: capitulum diameter, seed weight/capitulum, number of seeds/m², number of capitula/plant, number of seeds/capitulum and 1000-seed weight. The leaf area index duration (LAID, GDD) was determined by integrating the area under the LAI vs. GDD curve across the complete growing season according to the formula

$$\text{LAID} = \sum_{i=1}^n [(LAI_i + LAI_{i-1})/2](GDD_i - GDD_{i-1}),$$

where n is the number of harvest dates, LAI_i is the leaf area index at each harvest date and GDD_i-GDD_{i-1} is the GDD duration.

The chlorophyll and carotenoid pigments were extracted in 80% acetone by macerating the green leaves (which were plucked at flowering stage) with a mortar and pestle. The absorption of the extracts at wavelengths of 480, 645 and 663 nm were measured with a spectrophotometer (Model 6/20, Perkin-Elmer). The concentrations (mg g⁻¹ fresh leaf mass) of chlorophyll a, chlorophyll b and total carotenoids were then calculated using the equations of Jayaraman (1988).

The time from sowing to emergence, the beginning of stem elongation, the beginning of branching, the beginning of flowering, 50% flowering, full flowering and physiological maturity were recorded and were expressed in thermal time (GDD). A temperature index, GDD_i, rather than calendar time was used for measuring the rate and duration of seed filling. Accumulated GDD_i were calculated as $GDD_i = \sum[(T_{max} + T_{min})/2] - T_b$, where GDD_i is growing degree-days value for the ith day, T_{max} is the maximum daily air temperature (with an upper limit of 30°C), T_{min} is the minimum daily air temperature (with a lower limit of 5°C) and T_b is safflower base temperature considered 5°C. Linear regression was fitted by using all the seed dry weight between 5 and 95% of the final yield on accumulated growing degree days and the regression coefficient of the equation was considered as the rate of

seed filling (Johnson and Tanner, 1975). Effective seed filling duration was obtained by dividing maximum dry weight by the respective rate of growth.

All statistical analyses were performed using SAS software (SAS Institute, 1996). The homogeneity of variances was checked. Mean separation test was performed using Duncan's multiple range test at p≤0.05 (Little and Hills, 1978). Standard statistical procedures were used for calculating simple correlation coefficients and relationship between seed yield and LAID was performed by linear regression using Excel software.

RESULTS AND DISCUSSION

Among the genotypes, Varamin-295 exhibited the highest chlorophyll a, chlorophyll b and total carotenoids contents (Table 1). In study of Gadallah (2000), chlorophylls a and b contents ranged from 0.35 to 1.47 and from 0.26 to 0.78 mg g⁻¹, respectively. In a research on 18 genotypes of rice, Ali *et al.* (2004) found that chlorophyll a, chlorophyll b, chlorophyll a+b contents and chlorophyll a/chlorophyll b ranged from 0.447 to 0.602, from 0.273 to 0.365, from 0.499 to 0.939 mg g⁻¹ and from 1.637 to 2.055, respectively. Also, Tejada and Gonzalez (2006) found in control treatment values of chlorophyll a, chlorophyll b and carotenoids contents of rice varied from 2.678 to 2.725, from 1.899 to 1.948 and from 0.891 to 0.931 mg g⁻¹, respectively. Cui *et al.* (2001), in a survey of 72 Chinese and North American (NA) soybean cultivars, reported that chlorophyll a/b ratio varied from 3.1 to 3.4 and Jiang *et al.* (2006) indicated that the increase magnitude of chlorophyll a was much higher than that of chlorophyll b during leaf development. Also, Stancheva *et al.* (2004) in a study of effects of different nitrogen fertilizer sources in bean, stated the chlorophyll a content is higher in the flowering stage at the maximal rate of photosynthesis, chlorophyll b increased during the vegetation period and the carotenoid content did not changed on dependence of the leaf senescence. In their study, no significant difference regarding leaf pigment content among the variants was observed. Netondo *et al.* (2004) reported means of chlorophyll a, chlorophyll b concentrations and ratio of chlorophyll a/b in control treatment ranged from 1.6 to 2.3, 0.8 to 1.2 mg g⁻¹ and from 1.8 to 2.3, respectively between two sorghum varieties Serena and Seredo. Dong *et al.* (2006) showed contents of chlorophyll a, chlorophyll b, chlorophyll a+b and chlorophyll a/b ratio varied from 1.4 to 2.5, from 0.4 to 1.3, from 1.7 to 3.7 mg g⁻¹ and 1.4 to 5.2, respectively and these contents were higher at flowering stage in cotton. Daughtry *et al.* (2000) and Morrison *et al.* (1999) reported ranges of 1.32 to 2.31 for chlorophyll a/chlorophyll b in corn and 7.0 to 9.5 mg g⁻¹ for chlorophyll a+b concentration in soybean, respectively.

Table 1: Means comparison for measured traits in safflower genotypes

Genotype	LRK-262	LRV.51.51	Aghkand-e-Miyaneh	Zarghan-279	IL.111	Varamin-295
Chlorophyll a (mg g ⁻¹)	1.02a	0.98a	0.84b	0.78b	0.99a	1.10a
Chlorophyll b (mg g ⁻¹)	0.69ab	0.62bc	0.54c	0.59c	0.59c	0.71a
Chlorophyll a+b (mg g ⁻¹)	1.71a	1.61a	1.38b	1.37b	1.51ab	1.71a
Total carotenoids (mg g ⁻¹)	0.12ab	0.11bc	0.10c	0.09c	0.12ab	0.13a
Chlorophyll a/chlorophyll b	1.48c	1.57abc	1.60ab	1.33d	1.68a	1.56bc
(Chlorophyll a+b)/(total carotenoids)	14.22b	14.35ab	14.25b	14.82a	13.52c	14.46ab
Effective seed filling period (GDD)	637.01b	728.91b	905.14a	635.75b	848.95a	689.76b
Rate of seed filling (g/plant GDD)	0.0296abc	0.0242c	0.0259bc	0.0372a	0.0343ab	0.0228c
Capitulum diameter (mm)	24.93c	24.71c	26.42b	26.40b	28.91a	24.08c
Seed weight/capitulum (g)	1.26c	1.20c	1.46b	1.46b	2.04a	1.18c
Number of seeds/m ²	13165.4a	9373.7c	8704.5c	11909.3b	9315.7c	11095.1b
Number of capitula/plant	23.27a	21.27a	16.38b	22.70a	17.93b	21.20a
Number of seeds/capitulum	40.20a	35.65b	41.90a	39.43a	40.08a	38.33ab
1000-seed weight (g)	31.10c	34.20b	34.21b	37.03b	50.60a	29.94c
Seed yield (kg ha ⁻¹)	3024.50b	2670.20b	2852.60b	3897.20a	3878.60a	2452.60b
Harvest index (%)	21.83c	23.15c	27.03b	26.03b	29.62a	25.59b

* In each row, means followed by same letter(s) are not significantly different at p≤0.05

Table 2: Simple correlation coefficients of some variables in safflower genotypes (n = 24)

Traits	(2)	(3)	(4)	(5)	(6)	(7)	(8)	Seed yield
Chlorophyll a (1)	0.78**	0.97**	0.97**	0.38	-0.43*	-0.18	-0.26	-0.59**
Chlorophyll b (2)		0.90**	0.76**	-0.09	-0.08	-0.32	-0.20	-0.57**
Chlorophyll a+b (3)			0.95**	0.26	-0.40	-0.27	-0.44*	-0.61**
Total carotenoids (4)				0.29	-0.42*	-0.24	-0.14	-0.46*
Chlorophyll a/chlorophyll b (5)					-0.76**	0.83**	-0.35	-0.26
(Chlorophyll a+b)/(total carotenoids) (6)						-0.42*	0.26	0.10
Effective seed filling period (7)							-0.23	-0.03
Rate of seed filling (8)								0.87**

*Significant at p≤0.05. **Significant at p≤0.01

Negative relationships existed between seed yield and pigment contents (Table 2). But results of some other experiments have shown positive relationships between seed yield, photosynthesis rate and chlorophyll content (e.g., Khalil and Manan, 1992; Morrison *et al.*, 1999; Wells, 2001) which are contrary to our results. Also, In experiment of Schittenhelm *et al.* (2004) on potato, did not observe significant correlations between dry matter production or yield, photosynthesis rate and total chlorophyll (chlorophyll a+b) concentration. Kumudi (2002) reported also that neither selection for maximum leaf Carbon Exchange Rate (CER) nor maintenance of leaf CER during the seed filling period has proved to consistently improve yield. In fact, Present findings about the relationships between pigment contents in safflower and seed yield may be due to other reasons like differences in sink capacity, carbon and light uses efficiency, green leaf area duration, seed filling rate and duration but not chlorophyll contents. On the other hand, correlations between chlorophyll a, chlorophyll b, total carotenoids and chlorophyll a+b were positive and significant (Table 2). This is consistent with findings of Datt (1998). Also, it seems that, safflower genotype having less seed yield, owing to greater pigment especially carotenoids have the potential of forage for feeding livestock under arid conditions of Iran, particularly at the flowering stage.

The primary yield components of safflower are number capitula per plant, number of seed per capitulum and seed weight (Gonzalez *et al.*, 1994). Also, seed yield

can be given as the product between total biomass and harvest index (Liu *et al.*, 2005). There were significant yield differences among genotypes (Table 1). IL.111 had the highest capitulum diameter, seed weight/capitulum and 1000-seed weight. The correlation coefficients of seed weight/capitulum, 1000-seed weight and capitulum diameter with seed yield were 0.79**, 0.67** and 0.77**, respectively. Number of capitula/plant, number of seeds/capitulum and number of seeds/m² were not significantly correlated with seed yield (r = 0.15, p>0.05; r = 0.27, p>0.05 and r = 0.33, p>0.05, respectively). In former studies with safflower, 100-seed weight, number of capitula/plant (Omidi-Tabrizi, 2000), seed weight/capitulum, capitulum diameter, number of seeds/capitulum, number of capitula/plant (Bagheri *et al.*, 2001) exhibited strong positive correlations with seed yield. Genotypes differed in HI (Table 1) and the HI values ranged from 21.83% (LRK-262) to 29.62% (IL.111). The correlation coefficient of HI with seed yield was 0.64**. Koutroubas *et al.* (2004) also reported differences among safflower genotypes for HI. They reported seed yield and HI of winter safflower genotypes ranged from 2310 to 4600 kg ha⁻¹ and from 0.29 to 0.35, respectively. Thus, plant breeding may increase the safflower seed yield by developing varieties having higher HI. The trends of dry matter accumulation among the six genotypes were nearly similar in this study (Fig. 1). The highest dry matter was observed in the IL.111 between stem elongation stage and 75% flowering. In the studied set of 6 safflower

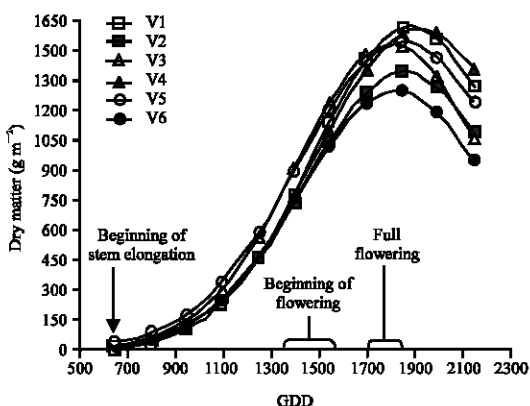


Fig. 1: Dynamics of dry matter of safflower genotypes during the growing season in 2003-2004 (V1, LRK-262; V2, LRV.51.51; V3, Aghkand-e-Miyaneh; V4, Zarghan-279; V5, IL.111; V6, Varamin-295)

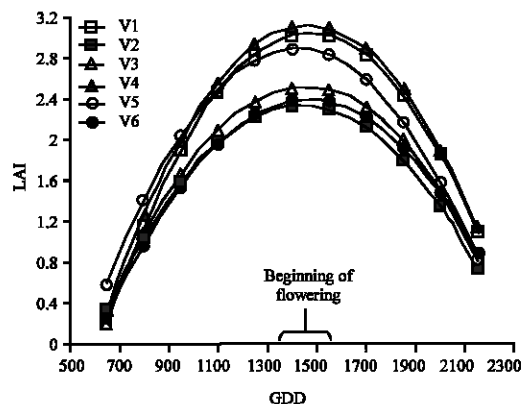


Fig. 2: Dynamics of LAI (leaf area index) of safflower genotypes during the growing season in 2003-2004 (V1, LRK-262; V2, LRV.51.51; V3, Aghkand-e-Miyaneh; V4, Zarghan-279; V5, IL.111; V6, Varamin-295)

genotypes, total biomass peaked around after full flowering and then a reduction was observed to maturity (Fig. 1). The reduction ranged from 177.93 (Zarghan-279) to 466.04 g m⁻² (Aghkand-e-Miyaneh). Genotypes differed in dry matter at maturity and it was especially determined by stem yield and size and number of capitula per plant. The highest and lowest aboveground dry matters at maturity were observed in the Zarghan-279 and Varamin-295, respectively and it was significantly correlated with seed yield ($r = 0.88^{**}$). The variation in dry matter among genotypes reflected mainly the differences in crop growth rate, which is consistent with findings of Koutroubas *et al.* (2004). In accord with the above mentions, total biomass than HI was more correlated with seed yield. Kumudi (2002) reported that total dry matter accumulation appears to be a more important contributor to yield improvement than harvest index. Also, Ying *et al.* (1998) reported further improvement in rice yield potential may depend more on the ability to increase biomass production than on increases in HI. Koutroubas *et al.* (2004) found that selection for high dry matter at anthesis may improve yield of safflower.

The dynamics of LAI and particularly the green leaf area duration have been reported to be important in determining growth and yield (Evans, 1993). IL.111 genotype had the highest LAI between end rosette and branching stage (Fig. 2). One of the most important traits may lead maximal crop yield is rapid expansion of leaf area after rosette stage to intercept radiation more efficiently (Evans, 1993). Zarghan-279 indicated the greatest LAI from 1100 GDD onward (Fig. 2). The increase in LAI in Zarghan-279 and IL.111 was the result of larger leaf area along with less number of smaller bracts, whereas a greater number of leaf (including bract) in whole plant accounted for the increase in LAI in LRK-262 (Data not

shown). In the studied set of 6 safflower genotypes, LAI was highest at the beginning of flowering (Fig. 2). Kumudi (2002) reported that LAI increases during vegetative development and then begins to decline around the beginning of the seed filling period. Zarghan-279 and LRV.51.51 showed the greatest and the lowest LAID, respectively (Fig. 3). Zarghan-279 had 25% longer LAID than LRV.51.51 too. Also, there was a positive and high correlation between seed yield and LAID (Fig. 4). Generally, more formation of seed yield as a result of longer LAID is due to greater interception of photosynthetically active radiation. This is consistent with findings of Kumudini *et al.* (2001), de Jesus Junior *et al.* (2001), Bassanezi *et al.* (2001), Schittenhelm *et al.* (2004) and Liu *et al.* (2005). In study of Ruiz and Maddonni (2006) on sunflower, LAID ranged from 913 to 3130 GDD and they found seed weight was positively correlated with LAID ($r^2 = 0.42$). Dolciotti *et al.* (1998) indicated that enhanced dry matter accumulation capacity of sweet sorghum could be the result of higher leaf thickness and leaf area duration.

Differences among genotypes for rate of seed filling and effective seed filling duration (period) were significant (Table 1). The highest belonged to Zarghan-279 significantly different from others except IL.111 in both traits and LRK-262 just in case of rate of seed filling. Since in our experiment effective seed filling duration had no significant relationship with seed yield (Table 2); differences in seed yield could be attributed to differences in the rate of seed filling. In contrast, in their experiment, Zope *et al.* (1994) showed that seed filling duration controls yield of safflower. Also, Santiveri *et al.* (2002) indicated the effects of seed filling rate and seed filling duration on a crop yield are generally affected by

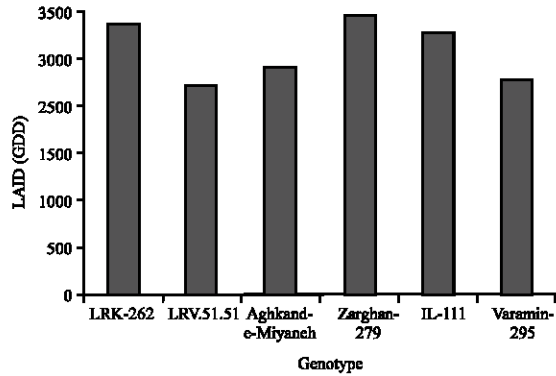


Fig. 3: Total LAID (leaf area index duration) of safflower genotypes in the period from stem elongation stage (650 GDD) to physiological maturity (2150 GDD) in 2003-2004

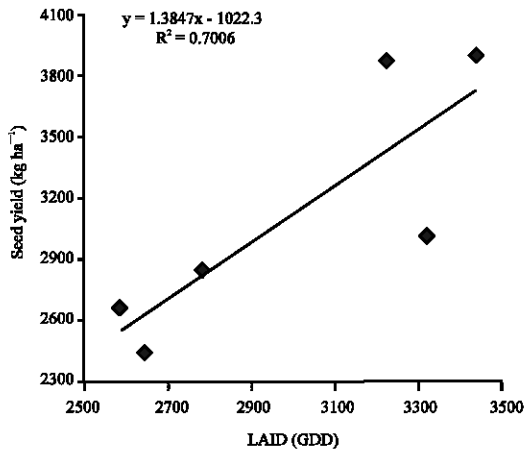


Fig. 4: Relationship between seed yield and total LAID (leaf area index duration) of safflower genotypes in 2003-2004

environment and genetics. They reported that maximum seed filling rate depended mainly on the genotype, whereas seed filling duration was mainly controlled by environmental conditions. In this study, effective seed filling period was mainly determined by the beginning of flowering ($r^2 = -0.70$). Santiveri *et al.* (2002) showed seed filling duration is determined by anthesis date and to optimize seed yield under terminal stress-prone areas, an early anthesis date is convenient in order to avoid limiting environmental conditions during seed filling. In the present study, a negative correlation has been found between seed filling rate and effective seed filling period (Table 2), suggesting a compensation between these two traits. Some studies have also reported a negative phenotypic correlation between seed filling rate and duration in wheat and triticale (Bruckner and Frohberg, 1987; Santiveri *et al.*, 2002).

CONCLUSIONS

In conclusion, results presented here show that physiological parameters including rate of seed filling, rapid leaf formation and expansion and delayed plant senescence are the characteristics of high-yielding safflower. Higher dry matter accumulation, HI, seed weight/capitulum, 1000-seed weight and capitulum diameter were found to be closely related to high-yield genotypes. In this experiment there is the possibility that genotypes with greater pigment contents may have worse photo-assimilate storage and translocation mechanisms the result of reduced sink demand. It may also have been due to we determined pigment concentrations in flowering stage, whereas pigment contents may be differ at alternative growth stages. Thus, there is need for other experiments in these cases in the future.

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