

## ORIGINAL ARTICLES

### Influence of water stress on photosynthetic pigments of some Fenugreek Varieties

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#### ABSTRACT

A pot experiment was conducted in during the two successive season of 2011 and 2012 in the greenhouse of the National Research Center, Dokki, Cairo, Egypt, to study the effect of missing of irrigation on growth and photosynthetic pigments of some fenugreek varieties (*Trigonella foenum-graecum* L.). Varieties used: Giza 1, Giza 6 and Giza 30 and irrigation treatments: missing of 2<sup>nd</sup> (D1) and 4<sup>th</sup> (D2) irrigation more than regular irrigation (RI) as a control. The main results were: V3 came first in plant height, while V2 came the 2<sup>nd</sup> and V1 came later. The reverse was true for dry mass of stem. The highest dry mass of pods was shown by Giza 6 followed by Giza 30 and the lesser was by Giza 1. The opposite was true for leaves and also for number of green leaves. Concerning the fresh and dry yield of Giza 30 ranked 1<sup>st</sup> and Giza 6 ranked 2<sup>nd</sup> and Giza 1 ranked 3<sup>rd</sup>. Subjected fenugreek plants to D2 markedly decreased fresh as well as dry weight. Missing of 4<sup>th</sup> irrigation decreased fresh and dry weight by 52.96 and 47.36 % in comparable with control plants (IR). Non significant differences between varieties were detected in the values of all pigments. The highest concentrations of chl.a, chl.b, total chlorophyll and total carotenoids were shown in plants of Giza 6 variety. The chl.a: Chl.b ratio showed its high value with Giza 30 followed by Giza 6 and Giza 1. The reverse was obtained with the ratio of chl.a+chl.b: carotenoids. Increased chl.a and chl.b however, carotenoides decreased by D1 and slightly increased by D2. chl.a: chl.b : carotenoids ratio quietly increased by D1 and tended to decrease by D2 but still more than the control. Furthermore, the opposite was true for chl.a+ chl.b; carotenoides ratio.

**Key words:** Fenugreek (*Trigonella foenum-graecum* L.)-Varieties-Irrigation intervals-Growth- yield-Chlorophyll- Carotenoids.

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#### Introduction

Environmental factors such as water, temperature and nutritional status affect the biochemical responses of plants to stress. Plants have genetically controlled mechanisms that allow them to live and grow under stress (Boyer, 1982). One of the most important environmental factors is the availability of water.

Salinity or other stressors cause a similar physiological stress response and thus drought is a general stress equivalent (Bergmann, *et al.* 1999 and Mascher, 2002). Sufficient water supply is the most important abiotic factor that land plants depend on growth (Bergmann, *et al.* 1999). Currently, drought is one of the most serious environmental problems and in arid and semi-arid regions, drought is a major constraint imposed on plant survival and growth.

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop belonging to the legume family. This crop is native to an area extending from Iran to northern India, but is now widely cultivated in China, north and east Africa, Ukraine and Greece (Petroponlas, 2002). In parts of Asia, the young plants are used as potherbs and the seeds as a spice or as herbal medicine (Lust, 1986 and Petroponlas, 2002). Fenugreek plant is susceptible to water stress during the vegetative growth stages, since a soil matric potential lower than -0.3 MPa causes substantial reduction in growth parameters such as height, weight and total leaf area (Alhadi, *et al.* 1999). Fenugreek is a dry land crop but responds well to minimum application of irrigation (kumar, *et al.* 2000 and Acharya, *et al.* 2006).

Varietal differences were studied by many authors: Chhibba, *et al.* (2000); Acharya, *et al.* (2007). Improving tolerance or selecting tolerant varieties, considered one of the better ways to successful cultivation of crops in the dry lands or areas could subjected to water deficit (Basu, *et al.* 2009; Painawadee, *et al.* 2009 and Ahari, *et al.* 2009). The use of drought-resistant varieties is an important strategy to combat the drought problem. These varieties should be able to provide higher yield under drought conditions. Genetic variability for drought resistance has been reported in peanut (Painawadee, *et al.* 2009).

Therefore, the current research aimed to study the effect of irrigation missing on growth, yield and photosynthetic pigments of fenugreek varieties.

## Materials and Methods

A pot experiment was conducted during the two successive season of 2011 and 2012 in the greenhouse of the National Research Center, Dokki, Cairo, Egypt, to study the effect of missing of irrigation on growth, yield and photosynthetic pigments of some fenugreek varieties. The treatments were as follows:

**Varieties:** Giza 1, Giza 6 and Giza 30.

**Irrigation:** Missing of 2<sup>nd</sup>, 4<sup>th</sup> irrigation and regular irrigation as a control.

The experiment included nine treatments, 3 varieties and 3 irrigation treatments. The experimental design was split plot in 6 replicates.

Seeds of fenugreek (*Trigonella foenum-graecum* L) varieties were sown in the 1<sup>st</sup> of Dec., (2011 and 2012 seasons) in plastic pots 45 cm in diameter and contained soil. Seeds were inoculated by the suitable ryzopium. Calcium superphosphate (15.5 % P<sub>2</sub>O<sub>5</sub>) and Potassium sulfate (48.5 % K<sub>2</sub>O) were added before sowing in the rate 3 and 6 g /pot, respectively.

Photosynthetic pigments i.e chl.a, chl.b and total carotenoids were determined according to the methods described by Von Wettstein (1957).

**Table 1:** Physical and chemical analysis of used soil.

A:Physical analysis and		Silt 20-2 μ %	Clay < 2 μ %	Soil Texture
Course >200 μ %	Fine 200-20μ %			
7.20	14.25	30.22	48.33	clay

**B:** Soil chemical analysis

pH 1:2.5	EC dSm <sup>-1</sup> 1:5	CaCO <sub>3</sub> %	CEC C mole Kg <sup>-1</sup>	OM %	Soluble cations and anions meq/100 g soil								
					Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	CO <sup>3-</sup>	HCO <sup>3-</sup>	Cl <sup>-</sup>	SO <sup>2-</sup>	
7.15	1.3	2.53	33.5	1.3	1.82	0.23	2.38	1.27	0.0	0.91	1.9	1.89	
Available macro-nutrients %					Available micro-nutrients ppm								
N		P		K		Zn		Fe		Mn		Cu	
0.47		0.25		0.95		3.1		4.8		7.3		5.2	

Data were subjected to the proper statistical analysis according to the methods described by Steel and Torrie, (1997)

## Results and Discussion

### Varietal differences:

#### a- Growth and yield:

Result of the experiment showed that V3 came first in plant height, while V2 came the 2<sup>nd</sup> and V1 came later. The reverse was true for dry mass of stem. The highest dry mass of pods was shown by Giza 6 followed by Giza 3 and the lesser was by Giza 1. The opposite was true for leaves and also for number of green leaves (Table 2). Concerning the fresh and dry yield of Giza 30 ranked 1<sup>st</sup> and Giza 6 ranked 2<sup>nd</sup> and Giza 1 ranked 3<sup>rd</sup> (Table 3).

Acharya, *et al.* (2006) observed considerable variability among fenugreek genotypes. They differ in morphology, growth habit, biomass and seed production capability. Chemical constituents of the seed, e.g. saponins, fibre, protein, amino acids and fatty acids contents also differ markedly. This variability is most often overlooked or underestimated in clinical trials. Malik and Tehlan (2009) mentioned that sixteen fenugreek genotypes were evaluated during 2005-06 and 2007-08 in Hisar, Haryana, India. Observations were recorded for plant height, branches per plant, pods per plant, length of pod, seeds per pod and seed yield. Significant variation was observed for all characters. Ahmed, *et al.* (2010) noticed that Giza-3 cultivar significantly surpassed Giza-2 cultivar in plant height, number of leaves/m<sup>2</sup>, number of branches/m<sup>2</sup>, total dry weight /m<sup>2</sup>, leaf area and specific leaf area at 70 and 100 days after sowing and also yield and yield attributes, i.e. plant height, number of pods/plant, number of pods/m<sup>2</sup>, weight of pods/plant, weight of pods/m<sup>2</sup>, weight of grains/m<sup>2</sup>, weight of 100 grains, straw yield and biological yield (Kg/fed). Hussein, *et al.* (2012) reported the varietal differences between Egyptian clover. In addition, these studies indicate that improvement through phenotypic selection for forage and seed yield is possible but, will require use of multiple locations and years. Improvement in seed yield can be achieved through selection for less variable seed size and/or early maturity (Basu, *et al.* 2009). They also added that, western Canada were mainly focused on development of early maturing and high seed yielding genotypes for the spice market (Slinkard *et al.*, 2008). Recent studies indicate that fenugreek developed in western Canada can also be used as a forage crop since the plant maintains high

nutritional quality irrespective of its maturity and the forage does not cause bloat in ruminants (Mir, *et al.* 1999). In addition, Tourian *et al.* (2013) reported the differences between agropyron cultivars in chl. a, chl.b and total carotenoids.

**Table 2:** Effect of irrigation missing on growth of fenugreek Varieties (Average of 2011 and 2012 growing seasons).

Varieties	Drought	Plant Height (cm)	No of leaves	Dry weight % (from total dry weight/plant)		
				Stem	Pods	Leaves
Giza 1 (V1)	RI.	27.70	8.30	39.66	42.36	17.98
	D1	28.00	6.70	32.63	45.87	21.50
	D2	27.00	6.30	29.21	46.36	24.43
Mean		27.57	7.10	33.83	44.86	21.30
Giza 6 (V2)	RI.	32.30	8.00	33.45	47.90	18.65
	D1	30.30	5.70	32.45	34.46	24.09
	D2	28.30	6.70	33.74	53.31	12.95
Mean		30.30	6.80	33.21	45.22	18.57
Giza 30 (V3)	RI.	33.30	9.30	34.99	51.47	13.54
	D1	33.00	8.30	32.72	40.47	26.81
	D2	31.70	8.70	31.52	44.74	23.74
Mean		32.67	8.77	33.08	45.56	21.36
Mean values of drought	RI.	31.10	8.53	36.03	47.24	16.72
	D1	30.43	6.90	32.60	40.27	23.63
	D2	29.00	7.23	31.49	48.14	20.37
LSD at 5%	Var.	N.S	N.S	.....	.....	.....
	D.	N.S	N.S	.....	.....	.....
	Var.xD.	N.S	N.S	.....	.....	.....

RI=Regular irrigation V1=Giza 1  
 D1= Missing of the 2<sup>nd</sup> irrigation V2=Giza 6  
 D2=Missing of 4<sup>th</sup> irrigation V3=Giza 30

#### Chlorophyll and carotenoids:

Non significant differences between varieties were detected in the values of the three pigments where highest concentrations of the total chlorophyll was shown by Giza 6 followed by Giza 1 and the lower concentration was in leaves of Giza 30. The highest concentration of all pigments were shown in plants of Giza 6 variety. The chl.a:Chl.b ratio showed its high value with Giza 3 followed by Giza 6 and Giza 1. The reverse was obtained with the ratio of chl.a+chl.b: carotenoids (Table 4).

On other crops, Researchers showed the varietal differences in photosynthetic pigments such as Munir, *et al* (2013) on radish (*Raphanus sativus L.*) Yildrin *et al.* (2008) on pea (*Pisum sativum L.*), Hussein, *et al.* (2011) on barley (*Hordeum vulgare L.*) and Hussein *et al.* (2012) on Egyptian Clover (*Trifolium alexandrinum L.*). Moreover, Rad, *et al.* (2012) in a breeding program for high chlorophyll varieties, found that the line Irena/Babax/Bastor transmitted high chlorophyll contents than the other used lines of wheat. Talebi *et al.* (2013) showed the different concentrations of chlorophyll and carotenoids in different genotypes of chickpea. Furthermore, Alsady *et al.* (2012) showed slight variation in chlorophyll on 8 Omani fenugreek genotypes.

#### Drought:

##### a- Growth:

Subjected fenugreek to D2 markedly decreased fresh as well as dry weight. Missing of 4<sup>th</sup> irrigation decreased fresh and dry weight by 52.96 and 47.36 % in comparable with plants irrigated regularly as a control (Tables 2&3). As sessile organisms, plants have to cope with drought stress at least at some point in their life cycle. They have however evolved mechanisms that allow them to adapt and survive periods of water deficit, if not at the whole plant level, at some level or form of plant structure. According to the type of strategy adopted, plants are said to escape, avoid or tolerate drought stress, although these are not mutually exclusive. The plant drought response will depend on the species inherent "strategy" but also on the duration and severity of the drought period. If prolonged over to a certain extent drought stress will inevitably result in oxidative damage due to the over production of reactive oxygen species (Carvalho, 2008). On fenugreek plants, Spyropoulos (1986) found that growth and dry weight were lower in fenugreek plants stressed than in non-stressed controls. The reduction in dry weight indicated a lower uptake of solutes from the endosperm and the decrease in water content and osmotic potential. However, Alhadi (1997) related the growth reduction in seedlings of fenugreek plants to the disturbance in carbohydrate and protein metabolism.

**Table 3:** Effect of irrigation intervals on yield of Fenugreek varieties (Average of 2011 and 2012 growing seasons).

Varieties	Drought	Fresh weight /g.	Dry weight/ g.	Dry matter %
Giza 1 (V1)	RI	147.80	26.95	18.23
	D1	75.50	13.89	18.40
	D2	76.30	17.47	22.90
Mean		99.87	19.44	19.84
Giza 6 (V2)	RI.	184.0	42.10	22.88
	D1	93.70	23.63	25.22
	D2	54.70	11.83	21.63
Mean		110.80	25.85	23.24
Giza 30 (V3)	RI	172.00	32.68	19.00
	D1	143.30	31.62	23.03
	D2	106.00	24.26	22.07
Mean		140.43	29.52	21.37
Mean values of drought	RI.	167.93	33.91	20.04
	D1	104.17	23.05	22.22
	D2	79.00	17.85	22.20
LSD at 5%	Var.	32.58	5.80	.....
	D.	28.66	12.15	.....
	Var. xD.	N.S	N.S	....

RI=Regular irrigation V1=Giza 1  
D1= Missing of the 2<sup>nd</sup> irrigation V2=Giza 6  
D2=Missing of 4<sup>th</sup> irrigation V3=Giza 30

Spyropoulos and Reid (1988) stated that when water stress was applied to fenugreek seeds after germination but before the beginning of galactomannan hydrolysis and galactomannan breakdown. It is argued: 1) that water stress after germination but before the beginning of galactomannan hydrolysis inhibits the production of hydrolytic enzymes in the endosperm, probably via decreased removal of lowered water content of diffusible inhibitory substances; and 2) that water stress after the beginning of galactomannan hydrolysis decreases the rate of galactomannan breakdown in vivo principally via decreased diffusion at lowered water content of enzymes from the aleurone layer through the storage tissue of the endosperm development of the hydrolytic enzyme.

The reverse responses of plant to water stress may be due to the alteration effect on photosynthesis and carbohydrate (Alhadi, *et al.* 1999) which water stress affected on stomatal closure (Ben Ahmed, *et al.* 2009) and Co<sub>2</sub> exchange. Water stress affected the osmotic condition in plant tissues. Chaves, *et al.* (2003) added that osmotic adjustment has been considered as an important physiological adaptation for plant resistance drought stress. ; Protein building (Alhadi, *et al.* 1999); Drought stress usually leads to oxidative stress due to stomatal closure which causes the over-reduction of photosynthetic electron (Ben Ahmed *et al.*, 2009).

#### a)-Chlorophyll and carotenoids:

Several responses were detected in chl.a, chl.b and total carotenoids and ratios of chl.a:chlb and chl.a+chl.b : carotenoid were reported in Table (4). Increased chl.a and chl.b however, carotenoides decreased by D1 and slightly increased by D2. Chl.a:chl.b ratio quietly increased byD1 and tended to decrease by D2 but still more than the control. Furthermore, the opposite was true for chl.a+chl.b: carotenoides ratio. Alhadi, *et al.* (1999) mentioned that Photosynthetic pigments (Chlorophyll a and b, and carotenoids) in the leaves diminished. Biomass, protein, and chlorophyll content of barley plants were affected by drought stress. The biomass produced under drought stress conditions, fell significantly in shoots and roots compared to well-watered plants (Mascher *et al.*, 2005). Surander *et al.* (2013) revealed that the total chlorophyll was decreased during drought stress. The reduction in chlorophyll under moisture stress may be due to the reduction in formation or/and destructure in the formatted pigments (Misra *et al.*, 1997).

#### Varietal differences x Drought:

##### a)- Growth and yield:

The interactive effects of varietals differences and drought on growth were illustrated in Table (2&3). Generally (except few cases) the delaying of irrigation missing affected negatively plant height and number of green leaves. Either fresh or dry yields pronouncedly lowered by delaying the missing of irrigation. Moreover, the decrement caused by D2 (in most cases) exceeded those caused byD1. Missing of 2<sup>nd</sup> irrigation decreased fresh weight by 48.92, 49.08 and 16.69 % and dry yield by 48.46, 43.87 and 3.24 % meanwhile withholding the 4<sup>th</sup> irrigation decreased fresh yield by 48.38, 70.27 and 38.37 % and dry yield by 35.18, 71.90 and 25.76 % , for V1, V2 and V3, respectively compared to that plants irrigated regularly. This means that the highest percentage

of decrements in fresh and dry yields (70.27 and 71.90%) were in V2 when 4<sup>th</sup> irrigation was missed and the lowest (16.69 and 3.24%) were obtained with V3 plants. This Data led to concluded that fenugreek Giza 30 variety (V3) the more tolerant to drought (missing of irrigation) and the lesser was Giza 6 variety, in varieties under study.

In this regard, Painawadee *et al.* (2009) stated that the use of drought-resistant varieties is an important strategy to combat the drought problem. These varieties would be able to provide higher yield under drought conditions. Genetic variability for drought resistance has been reported in peanut. The adverse effect of increased in osmotic pressure of soil solution in the root media caused by salinity may be due to the effect of salts in the soil properties (Crescimanno *et al.* (1995); Crescimanno and Santis (2004); Hussein *et al.* (2008) and Hussein *et al.* (2010). Growth is known to be affective by various environmental and genetic factors to an extent which depends on species, varieties as well as on growing plants conditions (Tourian *et al.*, 2013). These varieties would be able to provide higher yield under drought conditions. Genetic variability for drought resistance has been reported in peanut (Painawadee *et al.*, 2009). Drought resistance defined by Hall *et al.* (1993) as the relative yield of a genotype compared with other genotype subjected to the same drought stress. Drought susceptibility of genotype is often measured as a function of the reduction in yield under drought stress (Blum, 1988). Ahari *et al.* (2009) found varietal differences in drought resistant of fenugreek. The highest phenotypic and genotypic variances are found for grain yield under rain fed conditions and plant type and growth habit in both rain fed and irrigated conditions. The highest heritabilities were shown by thousand kernel weights, days to flowering and plant type under both rain fed and irrigated conditions. Cluster analysis allowed to classify the landraces into similar four groups for rainfed and irrigated conditions.

#### b)-Chlorophyll and carotenoids:

The different photosynthetic pigments response of the fenugreek varieties to drought stress were presented in Table (4). Data in this Table showed that chl.a and chl.b and total chlorophyll concentrations in V1 by missing of 4<sup>th</sup> irrigation, however, in V2 this parameters decreased by 2<sup>nd</sup> irrigation missing and increased with 4<sup>th</sup> irrigation missing treatment to be more than the control while for the case of V3 the reverse of V2 was true in chl.a as well as chl.b. Carotenoids concentration in V1 and V2 showed the same response of chl.a and b to irrigation missing but in the case of V3 it decreased by missing of 2<sup>nd</sup> irrigation and tended to increase but still less than the control concentration by delaying drought stress.

Furthermore, delaying missing of irrigation decreased the ratios chl.a:chl.b as well as chl.a+chl.b: carotenoids in V1 where gave the same values of increases in chl.a:chl.b either missing of 2<sup>nd</sup> or 4<sup>th</sup> irrigation missing were done in V2. In V3 leaves, the ratio of chl.a:chl.b lowered with D1 and D2 but the decrement less than that caused by D2. In this variety also chl.a+chl.b: carotenoids ratio showed the opposite response. Concerning these criteria,

**Table 4:** Effect of irrigation intervals On photosynthetic pigments of fenugreek varieties (Average of 2011 and 2012 growing seasons).

Varieties	Drought	Chl. a	Chl. b	Carotenoids	Chl.a+Chl.b	Chl.a:Chl.b	Chl.a+Chl.b :carotenoids
Giza 1 (V1)	RI.	2.28	1.40	0.55	3.67	1.63	6.74
	D1	3.31	2.20	0.77	5.50	1.51	7.17
	D2	3.85	3.49	2.01	7.34	1.10	3.65
	Mean	3.15	2.36	1.11	5.50	1.41	5.85
Giza 6 (V2)	RI.	3.20	2.72	2.39	5.93	1.18	2.48
	D1	2.77	1.65	0.91	4.42	1.68	4.84
	D2	4.79	2.87	1.90	7.65	1.67	4.02
	Mean	3.59	2.41	1.73	6.00	1.51	3.78
Giza 3 (V3)	RI.	2.52	0.84	1.76	3.56	2.99	1.90
	D1	2.82	1.47	1.02	4.29	1.93	4.22
	D2	2.05	1.03	1.20	3.08	2.00	2.57
	Mean	2.46	1.11	1.33	3.64	2.31	2.90
Mean values of drought	RI.	2.67	1.65	1.57	4.39	1.93	3.71
	D1	2.97	1.77	0.90	4.74	1.71	5.41
	D2	3.56	2.46	1.70	6.02	1.59	3.41
LSD at 5%	Var.	N.S	N.S	N.S	N.S	.....	.....
	D.	N.S	N.S	N.S	1.38	.....	.....
	Var.xD.	N.S	N.S	N.S	1.96	.....	.....

RI=Regular irrigation V1=Giza 1  
D1= Missing of the 2<sup>nd</sup> irrigation V2=Giza 6  
D2=Missing of 4<sup>th</sup> irrigation V3=Giza 3

Loggini *et al.* (1999) subjected two wheat varieties to drought and found that after exposure to drought, cv Adamello showed a larger reduction in the actual photosystem II photochemical efficiency and a higher increase in nonradiative energy dissipation than cv Ofanto. Tourian *et al.* (2013) noticed the differences in chl.a, chl.b

and total carotenoids between agropyron cultivars under different treatments of water stress. Surandar *et al.* (2013) found that Karpuravalli, Karpuravalli x Pisang Jajee, Saba, and Sannachenkathali was identified as tolerant to water deficit with least reduction in total chlorophyll content, whereas, Matti, Pisang Jajee x Matti, Matti x Anaikomban and Anaikomban x Pisang Jajee were notified as sensitive cultivars and hybrids with highest reduction compare to the control.

Talebi *et al.* (2013) indicated that drought stress can alter the tissues concentration of chlorophyll. This finding supported by Jaleel *et al.* (2008). This phenomenon may be due to and/or the restructure in chlorophyll under moisture stress in plant tissues (Misra *et al.*, 1997). The total chlorophyll significantly decreased in all tested genotypes of chickpea under drought stress but the reduction not as great in tolerant genotypes. On the opposite side, the increase in chlorophyll and carotenoids content may be related to a decrease in leaf area, it also can be a defensive response to reduce the harmful effects of drought stress (Farooq *et al.*, 2009). In addition, the high level of carotenoids in drought tolerance of genotypes has also been reported by Deng *et al.* (2003).

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