



## Evaluation of seed cotton (*Gossypium hirsutum* L.) production and quality in relation to the different irrigation levels and two row spacings

D. Zaxos<sup>a</sup>, S. Kostoula<sup>a</sup>, E.M. Khah<sup>a,\*</sup>, A. Mavromatis<sup>a</sup>,  
D. Chachalis<sup>b</sup>, M. Sakellariou<sup>a</sup>

<sup>a</sup>University of Thessaly, School of Agricultural Sciences, Department of Agriculture, Crop Production and Agricultural Environment, Fytoko Street, 38446, N. Ionia, Magnesias, Volos, Greece.

<sup>b</sup>Demosthenis Chachalis, Benaki Phytopathological Institute, 8 S. Delta Str., Athens 14561, Greece.

\*Corresponding author. E-mail: ekhah@uth.gr

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### Abstract

The effect of row spacings and irrigation levels on the earliness of seed production of cotton was investigated under the Mediterranean environment of central Greece. This particular cotton growing area is exposed to risk of autumn rainfall that might cause low seed quality in fields allocated primarily for seed production. Two varieties of cotton, Celia and Hersi, were planted in two row spacings (93 and 75 cm) and two irrigation levels (normal and low levels 6160 and 3080 mm ha<sup>-1</sup>). No significant difference in the yield was found among the two varieties, nor between the two row spacings. The low irrigation level had significantly negative effects on the number of squares, bolls, total dry weight and seed cotton production when compared with irrigation level. However, the low irrigation level resulted in a harvest earlier by ten days, which contributed to avoiding autumn rainfall. Seed quality (measured by germination and Warm-Cold Vigour Index Test) was better in seeds from the low irrigation level than in those from the normal irrigation level. Although yield of both varieties was higher under normal irrigation, seed quality was lower compared to low irrigation level. This was evidenced by the higher Free Fatty Acid (FFA) in “normally” irrigated plants. The results of this study showed that low irrigation level offers substantial benefits for early harvesting and production of high seed quality. Moreover, the FFA could be successfully employed as a quick criterion for seed quality ranking.

**Keywords:** Cotton seed quality; Row spacings; Irrigation; Vigour test; Free fatty acid.

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## Introduction

Cotton (*Gossypium hirsutum* L) is one of the important crops in Greece (an area of about 400.000 ha) representing 12% of total cultivated area and 18% of arable land with a production of 1.2 million tones; it comprises of the 80% of the total European Union cotton production (Bartzialis and Galanopoulou, 2005). Cotton is an important crop creating the income of more than 80.000 agricultural households as well as contributing significantly to the National economy. Seed production is also well-developed in local industry that is related to cotton. One of the basic constraints for the production of high seed quality (with measurable high vigour level) is the wet and hot climate conditions occurring at the post seed maturation stage which is often induced to late earliness. Cotton seed production performed in central and northern areas of the country is frequently low and, although some varieties have been bred with high performance and adaptability to Greek environmental conditions, their seed quality is sometimes lower compared to imported seeds. It is worth mentioning that, although until 1995, 50%, of the country's need for seeds was produced within the country, in 2005, it fell to a only 10-12% (Efthimiadis, 2005). There are several factors which may affect seed quality, such as improper agronomic practices; improper row space between plants; inadequate irrigation systems; biotic and abiotic stress on sensitive varieties; timing of harvest and post-harvest conditions of delinting and storage (Griffin and McCaskill, 1964; Baskin, 1987; Khah and Passam, 1994; Buxton et al., 1979, Gürsoy et al., 2011). A proper space between plants and row spacing is a very important factor and remain the primary concern for many growers, in order to optimize the crop profit. Some researchers have introduced alternative cultivation systems such as a Narrow Row, (NR) or an Ultra Narrow Row, (UNR) system, from 19 to 75 cm, which has general operation expenses comparable to the Conventional Row system of 0.97 to 1.02 m (CR), (Atwell, 1996; Parvin et al., 2000; Jost and Cthern, 2000; Larson et al., 2004; Darawsheh et al., 2007; Bartzialis, 2004). An advantage of the NR or UNR production system is more canopy closure (Jost et al., 2001) which has led to better light interception, (Krieg, 1996; Heitholt et al., 1992), which in turn reduces weed competition (Snipes, 1996; Wright et al., 2004). Also, some researchers have reported that cotton grown in a narrow row system produced equal yield (Willcut et al., 2006; Nichols et al., 2004; Harrison et al., 2006), or even higher yield (Karnei, 2005; Wilson et al.,

2006; Buehring et al., 2006) than the conventional spacing system. For instance, Avgolas et al. (2005) found significant increase in yield up to 12.95% in 75 cm compared to 96 cm row distance under Greek environmental conditions. Also, Darawsheh et al. (2009a) and Darawsheh et al. (2009b) found in Greece that plant density in narrow row spacings strongly affected the total and vegetative dry mass and Leaf Area Index (LAI) compared to the 96cm row spacing treatment.

The effect of plant density on the earliness of the crop, especially for seed production, may be greater and of more economic importance than that of the yield *per se*. In a dense stand, most of the bolls are concentrated near the main stem (Conkerton et al., 1993). Further more, a dense cotton stand produces less vegetative growth, initiation of the bolls are at a greater height, and earlier and of a more uniform maturity (Saleem et al., 2009; Mert et al., 2006). In addition, irrigation management and water use efficiency (dry matter production per unit water used, i.e. Kg/m<sup>3</sup>) has positive effect on crop growth and on the final yield (Bierhuizen and Slatyer, 1965; Alishah and Ahamdikhah, 2009). Several reports exist relating to minimizing irrigation requirement due to water deficits and at the same time to increasing lint yield (Weir, 1996, Silvertooth et al., 1999, McCarty, 1992). Also, it is well documented that increased cotton irrigation frequency has a positive effect on the yield components and induces vegetative growth as well as retarding the generative phase, (Alishah and Ahamdikhah, 2009; Onder et al., 2009). However, under drought stress conditions a decrease in seed cotton yield, boll numbers, boll individual weight and also induction of earliness was measured (Neil, 1991; Krieg, 1996).

Howell et al. (1984) reported that imposition of crop water deficits through irrigation management of narrow row cotton reduced the crop growth rates the extent that moderate water stress prior to flowering can result in larger boll partitioning and an increased harvest index. Reddy et al. (2009), studying the narrow row system under an irrigated and a non-irrigated environment, found that cotton grown in narrow rows can close canopy earlier and induced earliness (more open bolls and higher yield) than did cotton grown in 102 cm rows, regardless of irrigation. Reduction in irrigation requirements are widely attributed to the narrow row cotton system, which might induce early harvesting. This could be particularly applied in a short-season production system under Mediterranean environments due to detrimental effects of autumn rainfall (Mert et al., 2006; Ozpinar and Isik, 2004).

In Greece, water shortage is a major problem and is considered an expensive input factor for crop cost. Therefore, reduction of the maturity period can result in a concomitant reduction of irrigation water required, pesticide applications, disease protection etc. Since earlier planting does not contribute to earliness in harvest and is associated with higher levels of climatic risk (Hearn, 1995; Constable and Shaw, 1988), the earliness that is particularly desired in seed production could be achieved by selection of the early cotton cultivars, by narrow spacing, by minimizing irrigation requirement or by a combination of these techniques.

The aim of the present study was to test the hypothesis that narrow row spacings and low irrigation levels induce earliness and produce high seed quality. Moreover, this study compared different seed quality tests, aiming to select the quickest and the most reliable one. Specific objectives included: (i). to compare two different commercial cotton cultivars under 2 row spacing (93 vs. 75 cm) and 2 irrigation levels (6160 vs. 3080 mm/ha), and (ii) to test whether the free fatty acid test (FFA) could separate seed vigour.

## **Material and Methods**

### *Plant material*

The experimental field was performed at the Velestino farm of the Agricultural School, University of Thessaly, which is located in Volos, Central Greece (latitude 39° 39' N, longitude 22° 75' E) during the 2008 summer. Seeds of two well known commercial varieties named Celia (V<sub>1</sub>) and Hersi (V<sub>2</sub>), with earliness maturation characteristics, were used in the experiment and were provided from Bayer Hellas, Athens and P. N. Gerolymatos SA, Athens, Greece respectively.

Soil was a sandy loam with 24% clay, 41% silt and 35% sand. Seedbed was prepared using a cultivator and, later disked four times and smoothed with a wooden harrow for a proper seedbed. All plots were fertilized with 400 kg ha<sup>-1</sup> of NPK (11-15-15) fertilizer, applied before planting. Additionally, 4 weeks after sowing, urea fertilizer N (46-0-0) was also applied at a rate of 90 kg ha<sup>-1</sup>. All other agronomic practices similar to local practices followed, were applied according to plant needs and were kept normal and uniform for all the treatments.

The experimental design was a split-plot where irrigation treatments were split in the main plot and row spacings were split in the subplots. Varieties were randomized within the plots.

Irrigation treatments were applied with two irrigation levels, one considered as a normal irrigation level (i.e.  $W_1=6160 \text{ mm ha}^{-1}$ ) based on historical data supplied by farmers for the local area (Velesino, Thessaly), and another as a semi drought stress condition (i.e.  $W_2=3080 \text{ mm ha}^{-1}$ ). Also, two row spacings were implemented with 93 cm ( $R_1$ ) and 75 cm ( $R_2$ ).

Seeds were sown, on May 13, 2008 when 25 seeds per meter long with a hand experimental machine, (Bassi, Co., Italy) on the plots each having 4 to 10 m length ( $40 \text{ m}^2$ ), in completely randomized design. Plant establishment was achieved by thinning emerged plants in order to obtain 60% of field emergence in each row (i.e.  $15 \text{ plant m}^{-1}$ ) with an approximate target population of  $R_1=161290 \text{ plant ha}^{-1}$  and  $R_2=200000 \text{ plant ha}^{-1}$ . A common first irrigation of  $1000 \text{ mm ha}^{-1}$  used a gun irrigation cannon, which was applied at approximately 2 weeks after field emergence to sustain early emergence and uniformed growth crop stand. Afterwards, the 2 levels of irrigation ( $6160 \text{ vs. } 3080 \text{ mm ha}^{-1}$ ) were applied by using common polyethylene sprinklers (rate of  $4 \text{ lit h}^{-1}$ ) with auto-regulative supply sprinklers, (Table 1). Growth analysis was done four successive times on plant samples that were harvested from the second row of each plot (1 m long) for the duration of 10 and 24 July, 4 August and 5 September i.e. 55, 65, 80 and 110 Days After Planting (DAP) respectively. To measure final yield the whole two middle rows (20 m length each) were manually harvested at three picking times on 10 and 20 October and 13 November, (i.e. at 147, 157 and 180 DAP respectively).

Table 1. Average daily climatological data of experimental area during the growth period for indicated months 2008.

Month (2008)	Irrigation By water cannon (mm)	Full sprinkler irrigation (mm) $W_1$	$\frac{1}{2}$ sprinkler irrigation (mm) $W_2$	Precipitation (mm)	Min. mean Temperature $^{\circ}\text{C}$	Max. mean Temperature $^{\circ}\text{C}$	Evaporation Pan (mm)
May	50	-	-	13.3	13.4	32.1	93.1
June	50	-	-	9.4	15.5	32.9	172.9
July	-	376	188	13.7	18.5	39.7	224.0
August	-	240	120	22.0	18.8	40.6	187.0
September	-	-	-	83.6	15.4	33.5	88.6
October*	-	-	-	46.7	10.7	26.0	48.3
November**	-	-	-	42.5	7.9	22.5	35.0
Total	100	616	308	231.2	-	-	848.9

Although the harvest of all the picks was calculated as a total final yield, only the harvest of the first pick (147 and 157 DAP of low and normal irrigation respectively) of fuzzy seeds was suitable and therefore converted to the delinted seeds and their weight and proportion percentage recorded. The second pick (180 DAP) from normal irrigation level due to the late bolls maturity which resulted in production of low quality seeds, not being suitable for sowing. Plant samples were measured as a number of nodes, squares, bolls, fresh and dry matters (dried in an oven at 80 °C at least 48 h). The leaves from each plant of 0.5 m<sup>2</sup> of each plot were removed and the total area of all the leaves was determined with a portable optical integrating leaf area meter LI-COR model LI 3000A. The Leaf Area Index (LAI) was computed from measured total leaf area and plant population.

#### *Seed vigour evaluation*

*The standard germination test* was carried out using 4 replicates of 50 seeds put in a rolled paper towel moistened with water at the proportion of 2.5 times the dry substratum weight and germinated at 25±2 °C. Seeds were counted at 4 and 12 days after seedling emerged as prescribed by the ISTA rules (ISTA, 1985). The results were expressed as percentage of normal seedlings for each lot.

*The cold test* was performed in the same way as the standard germination test at 18 °C.

*Cool-Warm Vigour Index* was determined when cool germination and standard germination numbers were added together; high quality seed will have a high vigour index (ranging from high to low vigour level, i.e. 160 to 120 respectively).

*The tetrazolium test* was carried out using four replicates of 50 seeds soaked in deionized water for 18 hours at room temperature. The seed coat and membranes of each seed were carefully removed and the naked seeds incubated at 40 °C in a 1% solution of 2, 3 triphenyl-tetrazolium chloride in a phosphate buffer (pH 6.5-7.0) for 1 hour. Seeds were recorded as sound, viable or unviable based on the degree of staining (Moor, 1985).

*Ginning* took place using a laboratory ginning machine (model SDL, Crown Royal Co., England) with 4 Kg h<sup>-1</sup>. Each plant sample from each plot was weighed and placed manually into the machine and was ginned and partitioned into lint and fuzzy seed, each was then weighed and recorded separately.

*The lint* on the seeds was removed by mixing seeds with commercial grade H<sub>2</sub>SO<sub>4</sub> in plastic containers with 1 liter acid per 10 Kg cotton seed. After delinting, the seeds were thoroughly rinsed with deionised water and dried under shade by air. The seed moisture was 9% (dry weight basis). Each sample's weight was recorded before and after the delinting process. The seeds were stored in sealed plastic bags and kept in a cold room until used.

#### *Free fatty Acid analysis*

Each sample of 200 g seeds was used for determination of FFA so that cottonseed oil was extracted by solvent extraction using diethyether as solvent accordingly. The obtained oil was filtered and used for the determination of Free Fatty Acid% and peroxide value (AOCS, 1990).

#### *Statistical analysis*

Data were subjected to analysis of variance (ANOVA) using the statistical programme of SPSS for Windows. Means were separated using the Duncan's multiple range tests at 5% probability level.

### **Results**

The climatic data and the amount of irrigation during the growing season are shown in Table 1. It is evident that in the pre-harvesting period, i.e. October and November of 2008, the amount of rainfall was 46.7 and 42.5 mm respectively, of the total 213.2 mm, which means that 41.8% of the total rainfall took place during the harvest period, i.e. 10 and 20 October and 13 November 2008, with maximum temperatures of 26 and 22 °C, respectively. This high rate of rainfall is likely to negatively influence the quality of the produced seeds. These data reflect the intimate association between seed moisture content, air temperature and seed longevity. This was recognized much earlier by Harrington (1972), who suggested the 'Rule of Thumb' which quantified this relationship and found that an increase of 1% of the seed moisture content (in a moisture range between 5 and 14%), as well as an increase of 5 °C of storage temperature decreased to half the life span of the seed. Therefore, the longer the cotton seeds remain in the field (maturing in mother plants), the higher the probability of being exposed to autumn rainfall, with obvious detrimental effects on their quality.

Measuring the final plant populations indicated that there were no differences in plant establishment between varieties at 65 and 110 DAP in irrigation and row spacings' treatments (Table 2). However, results from four harvested samples (Table 3) showed that there were differences in plant height at 65 and 110 DAP between the two varieties. This is also supported by the analysis of variance that reveals significant effects of irrigation and variety ( $P \leq 0.05$ ). The number of node per plant was higher in  $W_2$  comparing to  $W_1$  treatments, at all the growth measurements. Also, the effect of the irrigation and row spacing was significant ( $P \leq 0.05$ ) at the 80 and 110 DAP but not at 65 DAP (Table 4). Row spacing effect was significant at 65 DAP (Table 4). However,  $W_1$  positively affected the number of both squares and bolls per plant at both row densities particularly at the last measurements of 80 and 110 DAP respectively ( $P = 0.05$ ; Tables 5 and 6 respectively).

Table 2. The effect of irrigation and row spacing on plant establishment of two varieties at different growth periods.

Days After Planting		Plant No.1 m <sup>-1</sup> row							
		55		65		80		110	
Treatments		Mean	SD (±)	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
CELIA	75 cm	W <sub>1</sub>	10.0 ± 1.73	11.2 ± 0.58	12.6 ± 2.51	10.0 ± 1.00			
		W <sub>2</sub>	12.6 ± 1.55	11.2 ± 0.57	11.2 ± 0.57	10.6 ± 1.53			
	93 cm	W <sub>1</sub>	11.2 ± 2.08	10.0 ± 1.73	12.2 ± 1.53	11.2 ± 0.58			
		W <sub>2</sub>	13.2 ± 0.57	9.2 ± 0.57	10.6 ± 1.53	9.2 ± 0.58			
HERSI	75 cm	W <sub>1</sub>	13.2 ± 0.57	16.6 ± 0.47	16.0 ± 1.00	12.6 ± 0.58			
		W <sub>2</sub>	12.6 ± 1.52	14.6 ± 0.57	12.6 ± 0.57	13.2 ± 2.08			
	93 cm	W <sub>1</sub>	12.6 ± 2.08	13.2 ± 1.53	13.2 ± 0.58	12.0 ± 1.00			
		W <sub>2</sub>	15.2 ± 1.52	14.0 ± 1.00	12.6 ± 0.58	15.2 ± 0.58			
Variety		ns (0.196)	* (0.001)	ns (0.133)	* (0.005)				
Row spacing		ns (0.430)	ns (0.085)	ns (0.536)	ns (0.720)				
Irrigation		ns (0.196)	ns (0.624)	ns (0.076)	ns (0.476)				
Variety * Row spacing		ns (1.000)	ns (0.870)	ns (0.536)	ns (0.720)				
Variety * Irrigation		ns (0.597)	ns (0.870)	ns (1.000)	ns (0.163)				
Row spacing * Irrigation		ns (0.597)	ns (0.624)	ns (0.756)	ns (1.0000)				
Variety * Row spacing * Irrigation		ns (0.430)	ns (0.417)	ns (0.357)	ns (0.163)				

SD, Standard Deviation.

Table 3. The effect of irrigation and row spacing on plant height of two varieties at different growth periods.

Days After Planting			Mean Plant height (cm)							
			55		65		80		110	
Treatments			Mean	SD (±)	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
CELIA	75 cm	W <sub>1</sub>	36.20 ± 5.929		64.22 ± 0.536		78.81 ± 8.404		90.00 ± 2.56 <sup>a</sup>	
		W <sub>2</sub>	40.43 ± 2.052		64.41 ± 5.319		65.20 ± 8.711		67.14 ± 3.25 <sup>b</sup>	
	93 cm	W <sub>1</sub>	47.90 ± 5.718		74.77 ± 3.095		93.13 ± 3.477		99.32 ± 3.18 <sup>a</sup>	
		W <sub>2</sub>	38.38 ± 6.720		68.76 ± 6.888		78.57 ± 4.089		78.01 ± 3.99 <sup>b</sup>	
HERSI	75 cm	W <sub>1</sub>	46.98 ± 4.295		71.77 ± 4.248		77.65 ± 7.253		88.24 ± 4.55 <sup>a</sup>	
		W <sub>2</sub>	39.88 ± 3.259		55.92 ± 5.162		62.42 ± 1.339		66.43 ± 4.86 <sup>b</sup>	
	93 cm	W <sub>1</sub>	47.08 ± 0.764		66.77 ± 3.873		92.87 ± 7.532		95.01 ± 2.59 <sup>a</sup>	
		W <sub>2</sub>	32.64 ± 4.535		54.10 ± 3.303		60.88 ± 6.593		57.37 ± 6.51 <sup>b</sup>	
Variety			ns (0.629)		* (0.020)		ns (0.053)		* (0.01)	
Row spacing			ns (0.741)		ns (0.790)		* (0.001)		* (0.017)	
Irrigation			* (0.002)		* (0.004)		* (0.000)		* (0.000)	
Variety * Row spacing			* (0.039)		ns (0.094)		ns (0.200)		* (0.04)	
Variety * Irrigation			* (0.045)		ns (0.076)		ns (0.880)		* (0.038)	
Row spacing * Irrigation			* (0.012)		ns (0.227)		ns (0.111)		ns (0.051)	
Variety * Row spacing * Irrigation			ns (0.403)		ns (0.795)		ns (0.151)		* (0.020)	

SD, Standard Deviation.

Table 4. The effect of irrigation and row spacing on number of nods plant<sup>-1</sup> of two varieties at different growth periods.

Days After Planting			Number of nods plant <sup>-1</sup>					
			65		80		110	
Treatments			Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
CELIA	75 cm	W <sub>1</sub>	16.65 ± 01.00		16.71 ± 0.95		15.57 ± 0.23	
		W <sub>2</sub>	17.07 ± 0.81		18.89 ± 0.61		20.06 ± 0.51	
	93 cm	W <sub>1</sub>	14.56 ± 0.41		16.38 ± 0.66		18.46 ± 0.28	
		W <sub>2</sub>	16.18 ± 0.70		16.71 ± 0.89		19.27 ± 0.07	
HERSI	75 cm	W <sub>1</sub>	15.93 ± 0.20		15.80 ± 0.917		16.38 ± 0.98	
		W <sub>2</sub>	16.50 ± 0.87		17.28 ± 0.623		17.93 ± 0.79	
	93 cm	W <sub>1</sub>	14.09 ± 0.44		14.60 ± 0.432		14.45 ± 1.80	
		W <sub>2</sub>	14.37 ± 0.42		15.05 ± 0.250		17.61 ± 0.95	
Variety			* (0.000)		* (0.001)		* (0.000)	
Row spacing			ns (0.433)		* (0.009)		ns (0.056)	
Irrigation			ns (0.175)		* (0.001)		* (0.000)	
Variety * Row spacing			ns (0.420)		ns (0.122)		ns (0.476)	
Variety * Irrigation			ns (0.381)		ns (0.115)		* (0.036)	
Row spacing * Irrigation			ns (0.475)		ns (0.712)		ns (0.789)	
Variety * Row spacing * Irrigation			ns (0.196)		ns (0.874)		ns (0.258)	

SD, Standard Deviation.

Table 5. The effect of irrigation and row spacing on number of squares plant<sup>-1</sup> of two varieties at different growth periods.

			Mean No. squares/ m <sup>2</sup>					
Days After Planting			55		65		80	
Treatments			Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
CELIA	75 cm	W <sub>1</sub>	23.83 ± 5.01		50.87 ± 7.32		38.63 ± 8.72	
		W <sub>2</sub>	23.73 ± 2.90		49.91 ± 5.90		22.23 ± 5.36	
	93 cm	W <sub>1</sub>	25.37 ± 2.07		53.54 ± 2.64		35.17 ± 7.53	
		W <sub>2</sub>	17.42 ± 3.57		46.42 ± 4.72		33.65 ± 6.61	
HERSI	75 cm	W <sub>1</sub>	24.53 ± 3.12		36.55 ± 4.13		22.90 ± 0.65	
		W <sub>2</sub>	22.77 ± 3.40		26.82 ± 3.76		13.25 ± 2.31	
	93 cm	W <sub>1</sub>	20.02 ± 3.93		33.60 ± 3.31		29.41 ± 4.78	
		W <sub>2</sub>	12.06 ± 3.71		28.96 ± 4.36		16.727 ± 2.07	
Variety			ns (0.218)		* (0.000)		* (0.003)	
Row spacing			* (0.031)		ns (0.901)		ns (0.113)	
Irrigation			* (0.030)		ns (0.429)		ns (0.055)	
Variety * Row spacing			ns (0.165)		ns (0.999)		ns (0.701)	
Variety * Irrigation			ns (0.948)		ns (0.888)		ns (0.432)	
Row spacing * Irrigation			ns (0.115)		ns (0.675)		ns (0.218)	
Variety * Row spacing * Irrigation			ns (0.912)		ns (0.112)		ns (0.114)	

SD, Standard Deviation.

Table 6. The effect of irrigation and row spacings on number of boll of two varieties at different growth periods.

			Mean No. Boll /m <sup>2</sup>					
Days After Planting			65		80		110	
Treatments			Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
CELIA	75 cm	W <sub>1</sub>	3.72 ± 1.41		24.79 ± 3.39		39.72 ± 1.50	
		W <sub>2</sub>	5.60 ± 2.69		21.59 ± 3.21		24.79 ± 5.79	
	93 cm	W <sub>1</sub>	4.30 ± 1.90		24.51 ± 3.81		31.18 ± 3.88	
		W <sub>2</sub>	9.03 ± 5.14		22.36 ± 5.10		29.24 ± 5.51	
HERSI	75 cm	W <sub>1</sub>	3.33 ± 1.15		22.13 ± 3.36		22.13 ± 1.77	
		W <sub>2</sub>	4.27 ± 0.53		18.93 ± 5.36		16.80 ± 3.78	
	93 cm	W <sub>1</sub>	3.23 ± 1.42		22.36 ± 2.89		27.09 ± 4.94	
		W <sub>2</sub>	3.01 ± 0.18		15.91 ± 1.69		12.69 ± 2.78	
Variety			ns (0.082)		* (0.007)		* (0.001)	
Row spacing			ns (0.576)		ns (0.660)		ns (0.887)	
Irrigation			ns (0.142)		ns (0.631)		* (0.037)	
Variety * Row spacing			ns (0.272)		ns (0.166)		ns (0.999)	
Variety * Irrigation			ns (0.236)		ns (0.140)		ns (0.315)	
Row spacing * Irrigation			ns (0.710)		ns (0.147)		ns (0.279)	
Variety * Row spacing * Irrigation			ns (0.407)		ns (0.139)		* (0.019)	

SD, Standard Deviation.

LAI of sample plants was measured during the growth periods from 55 to 110 DAP and results showed that the maximum LAI was in  $W_1$  and  $R_1$  in both varieties at 80 and 110 DAP with the exception of Celia in  $W_1$  and  $R_2$  at 55 and 65 DAP. However, examination of the effects of irrigation and row spacings separately showed that both had significant effect on LAI, with ( $P \leq 0.05$ ) at the 80 and 110 DAP, while the effect of the variety was only on the latest period with  $P \leq 0.05$ , (Table 7).

Table 7. The effect of irrigation and row spacing on LAI of two varieties at different growth periods.

			LAI			
Days After Planting			55	65	80	110
Treatments			Mean	Mean	Mean	Mean
CELIA	75 cm	$W_1$	1.21	2.84	5.24	6.36
		$W_2$	2.44	3.34	3.73	3.08
	93 cm	$W_1$	1.98	3.12	5.70	6.21
		$W_2$	1.60	3.01	3.94	3.88
HERSI	75 cm	$W_1$	2.26	3.17	5.66	5.22
		$W_2$	1.4	2.62	3.65	2.84
	93 cm	$W_1$	2.28	3.12	4.95	6.34
		$W_2$	1.01	2.09	3.29	2.60
Variety			ns (0.574)	ns (0.127)	ns (0.460)	* (0.050)
Row spacing			ns (0.084)	* (0.047)	* (0.000)	* (0.000)
Irrigation			* (0.023)	ns (0.163)	* (0.000)	* (0.000)
Variety * Row spacing			ns (0.565)	ns (0.455)	ns (0.275)	ns (0.936)
Variety * Irrigation			* (0.000)	* (0.037)	ns (0.837)	ns (0.539)
Row spacing * Irrigation			* (0.002)	ns (0.176)	* (0.005)	ns (0.179)
Variety * Row spacing * Irrigation			ns (0.148)	ns (0.938)	ns (0.739)	ns (0.057)

SD, Standard Deviation.

The plant total dry weight during 80 to 110 DAP (Table 8) was positively affected by  $W_1$  and  $R_1$ . These differences were pronounced at the late growth period measurements (80 and 110 DAP) when both effects of irrigation and row densities were highly significant ( $P \leq 0.05$ ). It was also noted that in the same period the interaction between irrigation and row spacings was significant ( $P \leq 0.05$ ).

Cotton was harvested by hand at 147, 157 and 180 DAP. Two picks took place from  $W_1$  treatments due to late bolls maturation and gave (on average over all the treatments)  $531.4 \text{ Kg ha}^{-1}$  more yield than the one pick harvest, which was harvested from  $W_2$  treatment. Table 9 shows that although yield of Celia was significantly affected by irrigations ( $P \leq 0.05$ ), it was not affected by row spacings. Furthermore, it was found that the total yield of all the picks in Celia was significantly higher by 20.4% in  $W_1 R_1$ , compared to the  $W_2 R_1$  treatments ( $5405.6$  and  $4302.0 \text{ Kg ha}^{-1}$  respectively) and higher by 16.9 % in  $W_1 R_2$ , compared to the  $W_2 R_2$  treatments ( $4784.4$ . and  $3975.0 \text{ Kg h}^{-1}$  respectively). In the case of the Hersi, these differences were also highly significant, by 33.0% and 29.2% ( $5360.6$ ,  $3590.6$ ,  $5366.0$  and  $3797.7 \text{ Kg ha}^{-1}$  respectively). Analysing of variance on the total cotton seed yield (including all picks) revealed significant effects of irrigation ( $P \leq 0.05$ ). Neither row spacing nor the interaction between irrigation and row spacing were significant ( $P > 0.05$ ).

Table 8. The effect of irrigation and row spacing on plant total dry weight of two varieties at different growth periods.

Days After Planting		Mean total dry weight $\text{g}/\text{m}^2$							
		55		65		80		110	
Treatments		Mean	SD ( $\pm$ )	Mean	SD ( $\pm$ )	Mean	SD ( $\pm$ )	Mean	SD ( $\pm$ )
CELIA	75 cm	$W_1$	$111.71 \pm 12.50$	$429.49 \pm 22.10$	$779.01 \pm 88.46$	$1630.26 \pm 124.58$			
		$W_2$	$193.82 \pm 12.55$	$500.94 \pm 105.66$	$663.57 \pm 133.73$	$1245.15 \pm 119.75$			
	93 cm	$W_1$	$190.49 \pm 61.12$	$443.76 \pm 67.68$	$1016.52 \pm 69.68$	$1765.15 \pm 71.96$			
		$W_2$	$159.96 \pm 56.14$	$431.51 \pm 127.22$	$710.36 \pm 190.25$	$1355.58 \pm 91.16$			
HERSI	75 cm	$W_1$	$205.28 \pm 43.51$	$431.36 \pm 101.28$	$840.06 \pm 77.15$	$1295.41 \pm 120.48$			
		$W_2$	$171.42 \pm 19.68$	$443.62 \pm 60.78$	$713.42 \pm 84.65$	$1060.27 \pm 340.10$			
	93 cm	$W_1$	$173.94 \pm 15.22$	$430.0 \pm 47.19$	$860.65 \pm 200.83$	$1685.39 \pm 288.77$			
		$W_2$	$143.62 \pm 19.65$	$309.39 \pm 38.46$	$489.99 \pm 104.53$	$977.07 \pm 133.47$			
Variety		ns (0.711)	ns (0.158)	ns (0.222)	* (0.018)				
Row spacing		ns (0.482)	ns (0.159)	ns (0.701)	ns (0.157)				
Irrigation		ns (0.632)	ns (0.709)	* (0.000)	* (0.000)				
Variety * Row spacing		ns (0.055)	ns (0.543)	* (0.033)	ns (0.864)				
Variety * Irrigation		* (0.026)	ns (0.214)	ns (0.722)	ns (0.688)				
Row spacing * Irrigation		* (0.047)	ns (0.113)	* (0.050)	ns (0.195)				
Variety * Row spacing * Irrigation		ns (0.140)	ns (0.708)	ns (0.803)	ns (0.246)				

SD, Standard Deviation.

Table 9. The effect of irrigation and row spacing on total cotton yield of two varieties at three picking harvests.

Treatments			Final yield					
			First pick harvest		Second pick harvest		Total yield	
			Mean	SD(±)	Mean	SD(±)	Mean	SD(±)
CELIA	75 cm	W <sub>1</sub>	4226.6	95.04	557.7	282.4	4784.4	74.48
		W <sub>2</sub>	3975.5	56.03	-	-	3975.5	56.03
	93 cm	W <sub>1</sub>	4898.6	51.62	507.0	107.9	5405.6	41.06
		W <sub>2</sub>	4302.1	13.14	-	-	4302.0	13.14
HERSI	75 cm	W <sub>1</sub>	4884.4	69.46	482.2	298.7	5366.0	45.96
		W <sub>2</sub>	3797.7	67.61	-	-	3797.7	67.61
	93 cm	W <sub>1</sub>	4781.6	10.48	578.9	274.14	5360.6	17.76
		W <sub>2</sub>	3590.6	13.48	-	-	3590.6	13.48
Variety			* (0.003)		ns (0.990)		ns (0.653)	
Row spacing			ns (0.706)		ns (0.877)		ns (0.354)	
Irrigation			* (0.000)		* (0.000)		* (0.000)	
Variety * Row spacing			ns (0.627)		ns (0.620)		ns (0.150)	
Variety * Irrigation			ns (0.459)		ns (0.990)		ns (0.082)	
Row spacing * Irrigation			ns (0.135)		ns (0.877)		ns (0.528)	
Variety * Row spacing * Irrigation			ns (0.169)		ns (0.620)		ns (0.905)	

SD, Standard Deviation.

\*\* First Pick Harvest date 10, 20/10/08, i.e. 147 and 157 DAP for low and normal irrigation respectively.

\*\*\* Second pick Harvest date 13/11/08 i.e. 180 DAP for normal irrigation.

Analysis of the partition of cotton yield which was converted to the fuzzy seeds and lint production showed that this proportion, as an average of all the treatments, was 56.79% to 43.01% respectively. Lint quality (staple length mm and micronaire) as well as lint weight was not significantly varied between treatments and among varieties (Table 10).

In the process of the seed cleaning by gravity of fuzzy seeds belonging to the first and second pick, which were delinted, it was found that the obtained sound seeds from W<sub>1</sub> in both row densities had a proportion of sound seeds of (77.7%). On the other hand, 22.3% of the seeds were abnormal in their morphology i.e. with a light brown colour, light in weight, not physiologically matured and unsuitable for sowing. However, in the W<sub>2</sub> treatments these proportions were 87.1% and 12.9% respectively. Although the earliness effect of first pick harvest (for 10 days) in the W<sub>2</sub> treatments resulted in less yield of delinted seeds, these seeds were heavier and of better morphological quality than the delinted seeds from W<sub>1</sub> treatment of the second pick harvest. Moreover, the results of the obtained fuzzy and delinted seeds indicated that irrigation had a significant effects on the yield ( $P \leq 0.05$ ) whereas the effect of row spacing and varieties were not significant (Table 10).

Table 10. The effect of irrigation and row spacing treatments on two varieties of cotton on lint yield and their relation to the percentage lint proportion.

Treatments	Harvested cotton converted to lint from first and second pick harvest		Harvested cotton converted to fuzzy seeds from first and second pick		Harvested cotton converted to fuzzy seeds Kg ha <sup>-1</sup> from first and second pick		Percentage of Fuzzy to Delinted seeds %			
	%	SD(±)	Kg ha <sup>-1</sup>	%	SD(±)	Before cleaning Kg/ha	After cleaning Kg/ha	%	SD(±)	
CELIA	W <sub>1</sub>	42.89	0.510	2052.1	56.97	0.514	2407.9	1928.7	80.1	1.107
	W <sub>2</sub>	42.69	1.007	1697.1	57.19	1.002	2273.6	2182.6	97.0	1.094
	W <sub>1</sub>	43.05	0.095	2327.1	56.82	0.092	2783.4	2148.8	77.2	0.770
	W <sub>2</sub>	43.50	0.258	1871.4	56.37	0.248	2425.1	23576.5	97.0	0.666
HERSI	W <sub>1</sub>	42.96	0.358	2305.4	56.91	0.373	2779.7	2123.7	76.4	1.526
	W <sub>2</sub>	43.04	0.382	1634.5	56.84	0.392	2158.6	2119.7	98.2	0.281
	W <sub>1</sub>	43.11	0.139	2310.9	56.76	0.139	2714.0	2089.8	77.0	0.476
	W <sub>2</sub>	43.41	0.459	1558.7	56.48	0.459	2028.0	1871.8	92.3	0.231
Variety		ns (0.627)			ns (0.669)					ns (0.024)
Row spacing		ns (0.076)			ns (0.079)					ns (0.536)
Irrigation		ns (0.420)			ns (0.475)					* (0.003)
Variety * Row spacing		ns (0.599)			ns (0.570)					ns (0.665)
Variety * Irrigation		ns (0.866)			ns (0.865)					ns (0.327)
Row spacing * Irrigation		ns (0.280)			ns (0.279)					ns (0.649)
Variety * Row spacing * Irrigation		ns (0.588)			ns (0.576)					ns (0.810)

SD, Standard Deviation.

The quality of the obtained fuzzy seeds was additionally evaluated using different tests such as: Standard germination test, cold test, tetrazoulim test, and warm-cold vigour index test, before and after the seed delinting process found that in both varieties  $W_2$  had higher quality seeds than  $W_1$  regardless of row spacing. However, these differences in seed quality tests were reduced after the delinted process (Table 11). Analysis of variance reveal that irrigation was significantly affected ( $P=0.05$ ) the standard germination test and Warm-Cold Vigour Index Test in both fuzzy and delinted seeds (Table 12). However, the effect of irrigation was not significant on the cold and Tetrazoulim test. The results of the Free Fatty Acid content (Table 13) suggested that both varieties in  $W_2$  treatment had significant lower percentage in FFA compared to the  $W_1$  treatment, regardless of row densities. Seeds obtained in the  $W_2$  had almost half the value of FFA than  $W_1$  treatments (overall mean of FFA was in  $W_2$  and  $W_1$ , 45.7% and 89.2% respectively). It is well known that FFA is used extensively as a seed quality indicator and usually builds up under high temperatures and high seed moisture conditions. The lower the FFA value below 1%, the higher the seed quality (Boman and Hopper, 2005). In both fuzzy and delinted seeds the effect of irrigation on the level of FFA was highly significant ( $P=0.05$ ).

Table 11. The effect of irrigation and row spacing treatments on obtained seeds of two varieties of cotton on the standard germination test, Cold test and Tetrazoulim test.

Treatments			Standard Germination Test %				Cold Test %				Tetrazoulim test %	
			Fuzzy seeds		Delinted seeds		Fuzzy seeds		Delinted seeds		Delinted seed	
			Mean	SD ( $\pm$ )	Mean	SD ( $\pm$ )	Mean	SD ( $\pm$ )	Mean	SD ( $\pm$ )	Mean	SD ( $\pm$ )
CELIA	75cm	$W_1$	67.2 $\pm$ 2.629	84.3 $\pm$ 3.753	49.5 $\pm$ 3.969	49.83 $\pm$ 2.777	81.6 $\pm$ 3.386					
		$W_2$	82.2 $\pm$ 2.610	87.5 $\pm$ 1.500	57.7 $\pm$ 1.756	58.83 $\pm$ 0.289	85.0 $\pm$ 4.799					
	93 cm	$W_1$	74.5 $\pm$ 1.000	86.1 $\pm$ 1.528	50.8 $\pm$ 5.635	48.5 $\pm$ 3.053	70.0 $\pm$ 4.163					
		$W_2$	84.1 $\pm$ 1.377	88.0 $\pm$ 4.444	52.5 $\pm$ 3.607	51.1 $\pm$ 2.878	75.3 $\pm$ 5.620					
HERSI	75 cm	$W_1$	74.5 $\pm$ 2.962	84.6 $\pm$ 3.819	53.7 $\pm$ 5.393	48.6 $\pm$ 2.371	70.6 $\pm$ 2.309					
		$W_2$	83.8 $\pm$ 1.893	88.6 $\pm$ 2.082	56.0 $\pm$ 6.144	62.5 $\pm$ 3.279	87.3 $\pm$ 2.309					
	93 cm	$W_1$	81.5 $\pm$ 3.173	85.0 $\pm$ 7.550	54.0 $\pm$ 6.062	53.3 $\pm$ 4.041	58.6 $\pm$ 2.309					
		$W_2$	85.7 $\pm$ 3.014	88.6 $\pm$ 3.215	55.3 $\pm$ 5.808	57.5 $\pm$ 3.679	76.0 $\pm$ 1.436					
Variety			* (0.042)	ns (0.897)	ns (0.412)	ns (0.219)	ns (0.305)					
Row spacing			* (0.037)	ns (0.685)	ns (0.685)	ns (0.396)	ns (0.636)					
Irrigation			* (0.000)	ns (0.067)	ns (0.430)	* (0.014)	* (0.007)					
Variety * Row spacing			ns (0.298)	ns (0.760)	ns (0.733)	ns (0.429)	ns (0.636)					
Variety * Irrigation			ns (0.311)	ns (0.685)	ns (0.639)	ns (0.562)	ns (0.525)					
Row spacing * Irrigation			ns (0.370)	ns (0.799)	ns (0.222)	ns (0.154)	ns (0.220)					
Variety * Row spacing * Irrigation			ns (0.298)	ns (0.879)	ns (0.508)	ns (0.759)	ns (0.868)					

SD, Standard Deviation.

Table 12. The effect of irrigation and row spacing treatments on the obtained seeds of two varieties of cotton on the Warm-Cold Vigour Index test.

Warm-Cold Vigour Index Test					
Treatments		Fuzzy seeds		Delinted seeds	
		Mean	SD(±)	Mean	SD(±)
CELIA	75 cm	W <sub>1</sub>	116.7 ± 4.268	134.1 ± 7.189	
		W <sub>2</sub>	139.9 ± 1.127	146.3 ± 1.258	
	93 cm	W <sub>1</sub>	127.0 ± 6.500	134.6 ± 6.007	
		W <sub>2</sub>	134.9 ± 4.684	139.1 ± 6.563	
HERSI	75 cm	W <sub>1</sub>	128.2 ± 2.537	133.3 ± 4.713	
		W <sub>2</sub>	139.8 ± 5.508	151.1 ± 2.021	
	93 cm	W <sub>1</sub>	136.5 ± 6.715	138.3 ± 7.786	
		W <sub>2</sub>	140.0 ± 6.083	146.1 ± 7.848	
Variety		ns (0.086)		ns (0.305)	
Row spacing		ns (0.477)		ns (0.636)	
Irrigation		* (0.000)		* (0.007)	
Variety * Row spacing		ns (0.838)		ns (0.636)	
Variety * Irrigation		ns (0.363)		ns (0.525)	
Row spacing * Irrigation		ns (0.131)		ns (0.220)	
Variety * Row spacing * Irrigation		ns (0.275)		ns (0.868)	

Table 13. The effect of irrigation and row spacing treatments on the obtained seeds of two varieties of cotton on the Free Fatty Acid.

Free Fatty Acid %					
Treatments		Fuzzy seeds		Delinted seeds	
		Mean	SD(±)	Mean	SD(±)
CELIA	75 cm	W <sub>1</sub>	1.35 ± 0.161	0.83 ± 0.247	
		W <sub>2</sub>	0.48 ± 0.175	0.39 ± 0.083	
	93 cm	W <sub>1</sub>	1.47 ± 0.313	0.95 ± 0.133	
		W <sub>2</sub>	0.68 ± 0.133	0.52 ± 0.269	
HERSI	75 cm	W <sub>1</sub>	1.12 ± 0.173	0.97 ± 0.249	
		W <sub>2</sub>	0.45 ± 0.075	0.34 ± 0.040	
	93 cm	W <sub>1</sub>	0.94 ± 0.266	0.82 ± 0.280	
		W <sub>2</sub>	0.56 ± 0.118	0.58 ± 0.101	
Variety		* (0.010)		ns (0.951)	
Row spacing		ns (0.417)		ns (0.315)	
Irrigation		* (0.000)		* (0.000)	
Variety * Row spacing		ns (0.211)		ns (0.640)	
Variety * Irrigation		ns (0.067)		ns (1.000)	
Row spacing * Irrigation		ns (0.266)		ns (0.224)	
Variety * Row spacing * Irrigation		ns (0.531)		ns (0.246)	

SD, Standard Deviation.

## **Discussion**

The results of the present study indicate that the normal irrigation level ( $W_1$ ) increased total cotton seeds. However, its effect on plant growth rate was slower than in  $W_2$  treatments. Seed yield obtained from  $W_2$  in the first pick harvest was earlier by at least 10 days, which may help to obtain better quality seeds under less moist conditions. Our climatic data indicated that, between the first and the second harvest period, (i.e. 10 to 20 October) heavy precipitation prevailed (43.7 mm), which could negatively affect the quality of produced seeds. Also, the seed quality evaluation by gravity and the cleaning process showed that the obtained matured and heavy seeds from  $W_1$  treatment were 80% of total seed weight, whereas in the  $W_2$  this rate was as high as 95% i.e. 15% more sound seeds were obtained (Table 10). This study shows that in central Greece under a short growing season, with high risk of unfavourable climatic conditions (heavy rain, high air temperature and high atmospheric moisture content), it would be safer to implement factors which facilitate earliness in cotton seed harvesting. Another factor that contributes to earliness is lower row densities (Neil, 1991; Reddy et al., 2009). Although narrow spacing affected the height of plants when they were young, its effects were rather negligible later in the season when plants grow older. Contrary to the findings of others (Karnei, 2005; Wilson et al., 2006; Buehring et al., 2006; Avgoulas et al., 2007) ours clearly suggest that low row spacing affect neither the height of plants nor the overall yield.

In conclusion, although drought stress is one of the most important abiotic stresses, which induces low yield in cotton, its effect on promoting earliness due to the improper climatic condition during pre-harvest period could be beneficial for the production of high seed quality in a Mediterranean environment such as that of Greece. Therefore, “drought stress” may contribute to overcoming constraints encountered by seed companies in the region to produce high quality cotton seeds. Moreover, our results suggest that the FFA content could be used as a quick criterion for seed quality evaluation under such environments. Future studies should investigate the response of more cotton varieties in a wider range of drought stresses.

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