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Response of Oil and Protein Content to Seed Size in Cotton

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ABSTRACT

This study was designed to identify the response of oil and protein content to non-heritable variation of seed size in cotton (*Gossypium hirsutum* L.). Six classes of seed size were <800, 801–930, 931–990, 991–1050, 1051–1120, >1121 mg. The results showed that germination and emergence increased linearly with seed size and R^2 of these relationships were 92 and 89%, respectively. This means that larger seed had higher potential of germination and emergence. Also, there was a strong linear relationship between seed weight and oil content. Seed weight provided a better indication of oil content (R^2=0.78) than protein content (R^2=0.43). There are no considerable relationship between seed size and protein content of seed. There was a positive and significant correlation between seed weight and oil content (r=0.88**), germination percent (r=0.95**), germination index (r=0.84*), emergence percent (r=0.94**) and emergence index (r=0.88**). This results suggest that oil content, germination and emergence of cotton seed was largely affected by seed size.

Key Words: Cotton; Oil; Seed; Emergence; Germination

INTRODUCTION

Cotton is the leading fiber crop, which is grown in over 88 countries with a combined production of 54 million metric tons of cottonseed (Zhu *et al.,* 2005). About two-third of the harvested crop comprises cottonseed, which is a potential source of oil and protein for human and animal consumption. Cottonseed contains 20-40% of oil, 20-30% protein and this level reaches as high as 45-50% in cotton seed cake after oil extraction. Cottonseed oil commands a higher price in the market than regular vegetable oils, because of its higher stability than oils like soybean and canola, because it has a very low content of triunsaturated fatty acids, which have a higher tendency to oxidize (Smith & Cothern, 1999). Also, information on the factors influencing content of oil and protein in the cottonseed is of both interest and value to breeders for formulation an appropriate breeding scheme.

The content of seed oil and protein is associated with the seed size that it has a considerable contribution to yield of the crop in cotton (Poehlman, 1987) and other crops like soybean and mungbean (Marega *et al*., 2001; Afzal *et al.,* 2003). In addition, seed size plays a major role in germination and establishment of vigorous seedlings that is essential to achieving high yield. Some researchers showed that size had great influence on the seed germination in cotton seed (Tupper *et al.,* 1971; Bartee & Krieg, 1972; Tupper & Kunz, 1981). They also noted a positive linear relationship between seed weight and emergence in the field. In pea (*Pisum sativum* L.) it was showed that cultivars with low 100 seed weight had higher germination percentage than larger seed ones (PekÕen *et al.*, 2004). Baalbaki and Copeland (1997) reported that in wheat, seed size not only influence emergence and establishment but also affected yield components and ultimately grain yield. Therefore, understanding of effects of seed size on oil and protein content, seed germination and emergence of seedlings help cotton researchers to improve the effectiveness of breeding methods.

Cotton is generally considered to be a self-pollinating crop, but it is often cross-pollinated and out-crossing rates are affected by activity of pollinators. Also, the majority of the cotton cultivars are a mixture of closely related genotypes that show plant to plant variation for the all traits especially for seed and seedling growth characteristics (Poehlman, 1987; Smith & Cothern, 1999). On the other hand, indeterminate nature of cotton and extended bloom period, which exposes different seed set to various environmental conditions and field weathering durations, result in different seed qualities and constituents within the same plant. Typically, seed from late-season bolls are usually smaller and less vigorous than seed from earlier bolls (Jenkins *et al.,* 1990a & b). Turner *et al*. (1979) showed that in cotton as the season progressed weight of seed was more dependent upon environmental factors and fruit load than genetic factors. As a single seed view point, variability of the all limiting factors during development of the seeds, such as nitrogen fertility, water deficit, physiological stresses and excessive plant populations can considerably impact its final quality in relation to the other seeds. These within-plant variations along with between-plant variation due to cross pollination causes seeds of a particular cultivar had considerable variation in seed traits, especially for seed size (Fasoula & Boerma, 2007).

Although within-cultivar variation of seed size is common; however, the extent to which this variability is effective on seed quality and germination remains un-clear in cotton. Also, in previous work, impact of seed size on quality and germination were evaluated by use of different genotypes, therefore, the pure effects of seed size usually confounded with genotypic effects. So, it is hypothesized that the larger seeds, have grater oil and protein content and would increase seed germination and emergence in relation to smaller seeds. The present study was designed to investigate the pure effect of seed size on oil and protein content of seeds and to asses the influence of seed size on the germination and emergence in cotton.

MATERIALS AND METHODS

This study was conducted at the Seed Laboratory, Faculty of Agronomy Sciences, Gorgan University of Agricultural Sciences, Gorgan Iran in 2005. Seeds of cotton (*Gossypium hirsutum* L.) cultivar 'Sahel' obtained from Iranian Cotton Research Institute (ICRI), which were grown in 2004 at Research farm of ICRI located at Gorgan, Iran. After ginning with electric gin, delinted seeds were obtained by acid treating with concentrated (commercial) sulphuric acid according to Cross (1962). The acid-delinted seeds were air-dried at room temperature (25±1ºC) on bench tops. The seeds were cleaned manually to remove all foreign matter and stored for 2 months before the start of the experiment. For better understanding of pure effects of size, seeds were separated one by one by hand for weight into size classes of <800, 801–930, 931–990, 991–1050, 1051–1120, >1121 mg. The mean single seed weight of un-graded sample was 986.7 mg and for the size classes were 740.7, 910.6, 960.6, 1018.0, 1081.2 and 1209.6 mg, respectively (Table I). We used from SW1 to SW6 abbreviations for showing 6 seed weight classes in our study.

In spring of 2005, two experiments were carried out. One was conducted in the field for emergence and other in the laboratory for germination. The experiment carried out in the field included six seed weight classes (SW1, SW2, SW3, SW4, SW5 & SW6). Each plot was made up of four 3.5 m rows with 0.4 m between them and 0.1 m spacing between seeding. One hundred seeds per each m of rows were used. Treatments were arranged in a randomized complete block design, with three replications. Emerging seedlings at the soil surface were counted every day for seven days after planting and terminated 21 days later in the middle two rows of each plot to determine field emergences percentage. Field emergence percentage (EP) was calculated as the number of emerging seedlings at the last day to the number of planted seeds. Also, emergence index (EI), a measure of the speed of emergence, was calculated by means of data on daily emerging according to the formula developed by Steiner (1990). In the lab experiment, germination test for six weight classes were performed according to ISTA rules. Each Petri dish comprised 50 delinted seeds with 4 replications. Similar to field experiment seed germination were recorded daily and germination percentage (GP) and germination index (GI), a measure of the speed of germination, were calculated. Similar to EI, germination index (GI) was calculated by means of data on daily germination according to the formula developed by Steiner (1990).

The oil and protein content of seeds in the each class were determined by Soxhlet and Kjeldahl methods, respectively. The samples for oil and protein content were analyzed in triplicate and their means were considered in statistical analysis. Nitrogen percentage was multiplied by a constant factor (6.25) for calculating protein content. The oil and protein content were determined according to standard methods of oil and fat analysis (Kaufmann, 1958).

The weight of single seed (SW) and single embryo (EW) and also hull content for all classes was individually determined. Also, dry weight of single embryos was calculated. Since, there was no significant difference between dry weight and fresh weight of embryos, the latter was considered for final statistical analysis. To measure the moisture content (MC) on the basis of dry weight the equation $MC\,(\%) = \left[\frac{b-a}{a}\right] \times 100$ were used for three replications of 100-seeds sample, where *a* was weight (g) of the same ample after drying in oven at 130±5ºC for 2 h and *b* was fresh weight (g) of seed sample. To determine the percentage water absorption by the seeds, 3 replications of 100-seed sample were soaked in distilled water at 5±2ºC during a 24 h period. They were extracted from the water and surface dried. Water absorption rate (WAR) was calculated using the equation $WAR\,(\%) = \left[\frac{c-b}{b}\right] \times 100$

where *c* was weight (g) of sample after soaking in distilled water for 24 h.

Linear regression was calculated to determine the relationship between seed weight and others including EW, HC, MC, WAR, GP, GI, EP, EI, oil and protein content (Zar, 1974). Also, Pearson coefficient of correlation among EW, HC, MC, WAR, GP, GI, EP, EI, oil and protein content was determined. All statistical analyses were performed by using SAS procedures: PROC MEANS, PROC REG and PROC CORR (SAS Institute, 1989).

RESULTS AND DISCUSSION

The seed weight of cotton varied within population by a factor about 6, from 216 to 1453 mg per seed (data not shown). The mean and standard deviation of single seed weight of this population was 986.8 and 160.7 mg, respectively. This within-cultivar variation was predictable, because the indeterminate nature of growth in cotton caused great differences among seeds that appear on different

Table I. Mean values ± standard error of eleven characteristics for six seed weight classes

Class	SW† (mg)	EW (mg)	Hull %	Oil (%)	Protein (%)	MC (%)
SW1	740.7 ± 8.58	466.8 ± 20.16	36.5 ±1.62	17.3 ± 0.87	23.6 ± 0.50	9.4 ± 0.43
SW2	910.6 ± 0.93	614.3 ± 6.21	32.5 ± 0.69	20.9 ± 0.23	26.5 ± 1.16	9.3 ± 0.63
SW3	960.6 ± 0.92	636.1 ± 5.70	33.5 ± 0.53	21.2 ± 0.43	28.0 ± 0.87	7.6 ± 0.74
SW4	1018.0 ± 0.89	678.5 ± 3.60	33.1 ± 0.37	21.6 ± 0.46	28.9 ± 0.50	6.6 ± 0.32
SW5	1081.2 ± 1.25	727.9 ± 4.66	32.6 ± 0.45	21.6 ± 0.24	28.9 ± 0.00	6.8 ± 0.20
SW6	1209.6 ± 4.11	803.9 ± 11.23	33.8 ± 0.47	22.1 ± 0.17	26.8 ± 0.58	7.9 ± 0.98
Average	986.8	654.6	33.7	20.8	27.1	7.9
	WAR (%)	**GP (%)**	**GI (%)**	**EP (%)**	**EI (%)**	
SW1	95.0 ± 1.21	42.5 ± 5.06	14.5 ± 2.32	42.6 ± 8.11	17.1 ± 3.21	
SW2	84.5 ± 2.20	63.5 ± 4.64	18.5 ± 3.29	69.3 ± 1.76	28.6 ± 1.67	
SW3	84.4 ± 0.37	68.0 ± 5.03	22.4 ± 3.56	68.7 ± 9.26	29.1 ±4.09	
SW4	81.4 ± 0.32	75.5 ± 5.31	28.9 ± 3.60	69.3 ± 3.52	28.3 ± 0.84	
SW5	79.3 ± 1.40	75.0 ± 3.69	24.6 ± 4.42	75.3 ± 1.76	27.5 ± 2.60	
SW6	79.9 ± 1.67	82.0 ± 3.16	26.7 ± 4.03	83.3 ± 8.97	34.3 ± 3.69	
Average	84.1	67.7	22.6	68.1	27.5	

SW: single seed weight, EW: single embryo weight, MC: moisture content, WAR: water absorption rate, GP: germination percentage, GI: germination index, EP: emergence percentage, EI: emergence index.

Table II. Phenotypic correlations among the eleven seed related traits in Cotton cv. *Sahel* (n=6)

Characteristics	EW (mg)	Hull (%)	MC (%)	WAR (%)	Oil (%)	Protein (%)	GP (%)	GI (%)	EP (%)	EI (%)
SW(mg)	0.99**	-0.58	-0.64	-0.91**	0.88**	0.65	0.95**	0.84*	0.94**	0.88**
EW (mg)	1	-0.65	-0.64	-0.94**	0.91**	0.69	0.97**	0.84*	0.94**	0.88**
Hull (%)		1	0.52	0.85*	-0.86*	-0.83*	-0.74	-0.59	-0.79*	-0.73
MC (%)			1	0.74	-0.67	-0.89**	-0.74	-0.87*	-0.56	-0.46
WAR (%)				1	-0.97**	-0.87*	-0.97**	-0.86*	-0.96**	-0.87*
Oil (%)					1	0.87*	0.97**	0.85*	0.97**	0.94**
Protein (%)						1	0.81*	0.82*	0.72	0.65
GP (%)							1	0.92**	0.96**	0.92**
GI (%)								1	0.78	0.71
EP (%)									1	0.96**
EI (%)										1

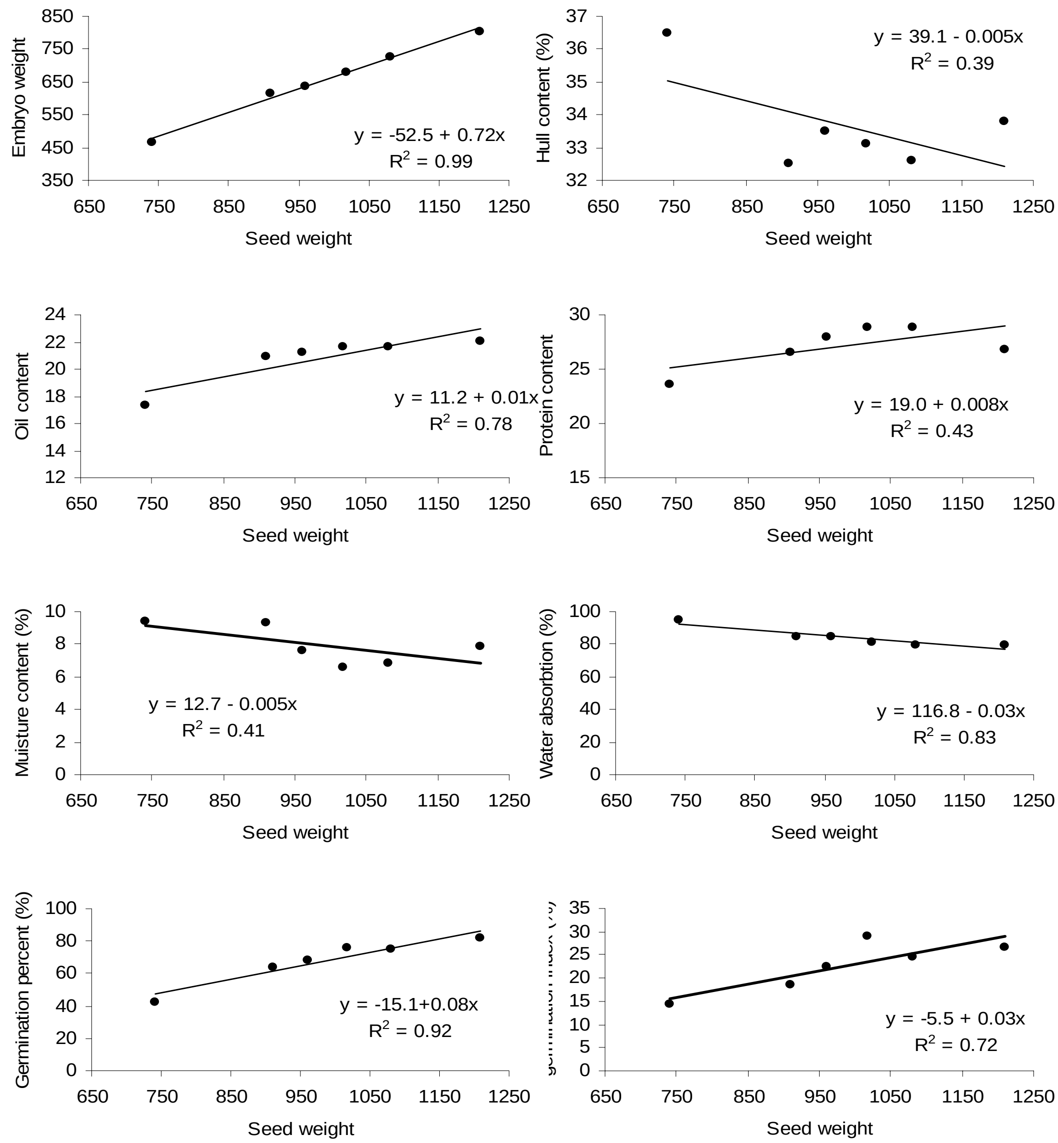
* and ** significant at 5 and 1%, respectively.
SW: single seed weight, EW: single embryo weight, MC: moisture content, WAR: water absorption rate, GP: germination percentage, GI: germination index, EP: emergence percentage, EI: emergence index.

flowers in each plant or even on different plans (Smith & Cothern, 1999). As shown in Table I, there was considerable variability among six seed weight classes for weight of single embryo (EW), hull, oil and protein content, moisture content (MC), water absorption rate (WAR), germination percentage (GP), germination index (GI), emergence percentage (EP) and emergence index (EI). The highest and lowest values for EW, oil content, GP, EP and EI were belonged to SW6 and SW1, respectively. In average, the classes had 654.6 mg EW, 20.8% oil, 67.7% GP, 68.1% EP and these values could be consider as mean of cotton cultivar Sahel (Table I). The lowest values were belonged to SW2 for hull, protein and GI, SW4 for MC and SW5 for WAR and the highest value of these traits was occurred in SW2, SW5, SW4, SW5 and SW4, respectively. The average value of hull, protein, MC, WAR and GI in all classes was 33.7%, 27.1%, 7.9%, 84.1 and 22.6%, respectively (Table I). The mean of oil and protein content that observed in this study were similar to those reported by others in cotton (Lukonge *et al.*, 2006). The content of oil in cotton seeds increased from 17.3 in SW1 (seed size class) to 22.1% in SW6. A similar trend was observed for seed growth related parameters including GP, GI, EP and EI and improvement order was SW1 < SW2 < SW3 < SW4 < SW5 < SW6 (Table I). A similar observation was made by Cornish and Hindmarsh (1988), while working with two cultivar of wheat, *Triticum aestivum* L. and they concluded that this was attributed to the larger food reserves in the larger seeds.

Embryo weight increased as the seed weight increased ($R^2=0.99$). There was a strong linear relationship between seed weight and oil content (Fig. 1). Regression analysis indicated that a 1.0 unit increase in the seed weight improved oil content 0.01 (Fig. 1). Also, seed weight provided a better indication of oil content ($R^2=0.78$) than protein content ($R^2=0.43$). Marega *et al.* (2001) showed that at the field experiment, the combined analysis showed a high significant relationship between weight of a hundred seed and oil content. Similar results were obtained by Chaudhy and Ikram (2001) in maize where seed size had no significant effect on protein content. The results of regression analysis also showed that increasing of seed weight decreased moisture content ($R^2=0.41$) and water absorption ($R^2=0.83$) over six seed weight classes.

Seeds with a lower weight (SW1 & SW2) had higher

Fig. 1. The relationship between the seed weight and embryo weight, oil content, protein content, hull content, moisture content, water absorption, germination percent, germination index, emergence percent and emergence index in cotton cv. *Sahel*



moisture content; whereas those of higher seed weight (SW4 & SW5) had lower moisture content (Fig. 1). Seed moisture can have the largest effect on seed quality throughout the production, ginning, storage, delinting and conditioning processes. Ideally, seed should be dry and harvesting should only occur when seed moisture content is less than 12%.

Germination percent (GP), germination index (GI), emergence percent (EP) and emergence index (EI) increased linearity with seed size and coefficient of determination (R^2) of theses relationship were 0.92, 0.72, 0.89 and 0.78, respectively (Fig. 1). Tupper *et al.* (1971) and Tupper and Kunz (1981) reported that seed size is the physical characteristics that had greatest influence on the germination of cotton. Bartee and Krieg (1972) also reported that seed size was directly correlated to percent germination. Furthermore, they noted in the field a positive linear relationship between seed size and emergence. A similar observation was made by Arunachalam *et al.* (2003), while working with the tree species, and this was attributed to the larger food reserves in the larger seeds. These results also are in conformity with Singh and Singh (2003) in wheat. Also, these results indicated that seed size had greater effect on percent than index of germination and emergence (Fig. 1).

As shown in Table II, positive and significant correlation was found between seed weight with oil content (r=0.88**), germination percent (r=0.95**), germination index (*r*=0.84*), emergence percent (*r*=0.94**) and emergence index (*r*=0.88**) (Table II). This means that larger seed had higher potential of germination and emergence as described by Smart and Moser (1999) in switchgrass and Hopper *et al.* (1979) in soybean. Amico *et al*., (1994) also concluded that higher vigor that occurred in larger seed is due to the larger food reserves in these seeds. Association between seed size and oil content previously reported in soybean (Marega *et al.*, 2001).

As expected, significant and negative correlation was observed between water absorption rate with GP (*r*=-0.97**), GI (*r*=-0.86*), EP (*r*=-0.96**) and EI (*r*=-0.87*) (Table II). In the other word, larger seed had higher ability of water absorption in relation to smaller them. These results are in conformity with Borji *et al*. (2007). Positive and significant association was observed between GP with GI (*r*=0.82*) and also between EP and EI (*r*=0.96**) (Table 2). These results indicated that total germination/emergence of a seed lot is related to the speed with which seeds germinate/emerge, respectively. Thus a seed lot exhibiting uniform and rapid germination/mergence should also have a high total germination/emergence. Such positive correlations have been reported in cotton (Pahlavani, 1999) and other species, including sunflower (Ahmad, 2001) and soybean (Adkins *et al.*, 1996).

CONCLUSION

The effects of seed size are pure effects of size and are not confounded by other effects such as genotypic factors, which is common in these studies that previously performed. Overall, results of this study showed that oil content, germination and emergence of cotton seed was largely affected by size of seeds. This findings helps cotton breeders that commonly, does not look for the genetic improvement of isolated traits, but for the genetic improvement of a set of traits, since, it is interesting for the breeder to know the intervention in one trait can cause alteration in others.

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