Influence of planting date and sulfur coating in seed coating solution (SCS) on cotton (*Gossypium hirsutum* L.) seeds: physiological traits

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Abstract

In order to investigate the influence of sulfur coated seeds in SCS (a moisture absorbent polymer layer on seeds) on physiological traits of cotton, a field experiment was conducted in completely randomized design with factorial arrangement with 3 replications. Three planting date included early (E), optimum (O), and delayed (D) planting time. Seed coating priming treatments consisted of F (fuzzy seeds, control), FS (fuzzy seeds with SCS), FSS (fuzzy seeds with sulfur coating and SCS), D (delinted seeds), DS (delinted seeds with SCS), DSS (delinted seeds with sulfur coating and SCS). Findings showed that the effects of planting date and seed coating treatments on chlorophyll, anthocyanin, proline, and glycine betaine content were significant. Interaction of treatments of seed coating and planting date treatments showed that treatments of seeds with ED, EDS, ODS and DDSS, had the highest content of chlorophyll. EFSS treated seedlings had the highest content of anthocyanin (0.58 mg g^{-1}Fw) which increased with treatments application while anthocyanin content decreased in EDS and EDSS treatments, in comparison with ED. The highest (0.76 mg g^{-1}Fw) and lowest (0.21 mg g^{-1}Fw) concentrations of anthocyanins were recorded when F seeds were planted at optimum date (OF) and delayed (DF) planting dates, respectively. In three planting date treatments of ED (8.82 µg g^{-1}Fw), OFS (8.83 µg g^{-1}Fw), and DDS (9.37 µg g^{-1}Fw), the highest proline content was obtained. In early planting treatments, glycine betaine content decreased in all coating treatments. ED and EDS treatments had lowest glycine betaine content. Treatments of seed coating in optimum and delayed dates of planting resulted in identical amounts of glycine betaine production.

Keywords: cotton; planting date; sulfur coating; seed coating solution

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Introduction

The successful establishment of crop mainly depends upon good quality seeds by help of newly developed technologies called "Seed Enhancement Techniques". Two important enhancement technologies are seed coating and seed priming that have been employed successfully for many crops (Sangamnathrao, 2009). Seed coating technology has developed rapidly during the past two decades and provides an economical approach to seed enhancement (Sangamnathrao, 2009). Cotton planting area of the world was 32,150,000 ha in 2003 – 2004 and approximately 45-50% of the planting seeds in the world are delinted (Zeybek, et al., 2010).

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Today, coating fuzzy cotton seeds is proposed as an alternative to delintation, which makes fuzzy cotton seeds more suitable for germination and growth and unlike delintation, sulfuric acid is not used for coating and this eliminates the problems associated with its usage such as seed loss, pollution and threats to human health (Zeybek, et al., 2010). Hence, seed coating as an alternative to delintation has the potential to expand organic cultivation of cotton (Zeybek, et al., 2010). Seed coating, not only incorporates natural fungicide, but also eases planting and stops the seeds from sticking together (Elzakker, 1999).

Studies show that cultivation of coated cotton seeds has no disadvantage regarding the agronomic and technological characteristics of cotton seed; coating may even improve the characteristics of the seeds, but no significant difference exists in terms of yield (Zeybek, et al., 2010). In addition, oxidation of S in soils is affected by several factors such as pH and microbial activity such as *Thiobacillus* (Heydarnezhad et al., 2011; Wainwright, 1984). Sulphate deficiency in young plants has an effect on CO_2 assimilation rates and on Rubisco enzyme activity and protein abundance (Gilbert et al., 1997). This is a result of decreased synthesis of new protein under S-limiting conditions. Additionally, some degradation has been observed in response to S-limitation in the older leaves (Hawkesford, 2000). Since the amount of sulfur in soils is usually very low, in addition to SCS, a second coating treatment with sulfur may be advisable for seed enhancement procedures.

Date of planting is the most important factor affecting germination characteristics, seedling establishment, level of green fields, and yield. Among various agronomic factors in cotton, planting time is considered as the key management component in any cropping system to harvest a profitable seed yield. Studies show the highest direct effect of reproductive/vegetative ratio on cotton seed yield under early planting dates (Hakoomat, et al., 2009). Previous studies showed that early planting produced 10% more flowers, 23% more open bolls, and 18% more seed cotton yield in comparison with late planting date (Arshad et al., 2007). It was also argued that earliest planting provided for more favorable environmental conditions which allowed the plant to gain more plant height and number of bolls (Hakoomat, et al., 2009). This means that early planting date achieves higher growth rate during earlier plant growth phases.

A suitable special organic natural coat, the SCS reduces pH and increases microbial activity and in turn influences the persistence of the S in the soil and its availability to the germinated seeds. SCS treatment results in more acidic soil environment around the seedling roots and thereby increases nutrient uptake. Therefore, SCS improves plant nutritional conditions and protects seedlings from damage of pathogens at different times of planting.

This study investigates the planting of cotton seeds in different times and coating by the sulfur in SCS as a supplement to fuzzy and delinted seeds, which is a common practice in cotton farms. Also, it was aimed to determine the effects of coating treatments on cotton yield and yield components.

Material and Methods

This research was performed in crop year 2012, in Cotton Research Station of Karkandeh (Gorgan). Field experiment was conducted in randomized completely block design (RCBD) with four replications. The date of planting in three levels of early (E: 10 May), Optimum or on time (O: 15 May) and delayed (D: 20 May) sowing and seed coating priming treatments supplied by application in six levels of F: fuzzy seeds (as control or untreated seeds), FS: fuzzy seed with SCS coat, FSS: fuzzy seed with sulfur coat in SCS, D: delinted seeds, DS: delinted seed with SCS, and DSS: delinted seed with sulfur coat in SCS. Optimum date of planting was determined based on the optimum soil temperature at depth of 10 cm (15 °C for cotton). Full leaf lamina collected from 8-12 foliar plants (age of 40-45 days) for laboratory assays.

Chlorophyll assay

Chlorophyll content was determined in 1 g leaves of a plant (g), homogenized in 10 ml of acetone 80% and the amount of chlorophyll a and b were measured in wavelengths of 645 and

663 nm by the spectrophotometer (Jensen, 1987).

Anthocyanin assay

The method used by Mancinelli (1990) was applied for the determination of anthocyanin content. After being thoroughly extracted in 3 mL methanol-HCl (1% HCl, v/v), the samples were left at 4 °C in the refrigerator for 2 days. Later on, the extract was vortexed, filtered, and the total anthocyanin content was measured by an UV-visible spectrophotometer as the difference between the absorbance at 530 and 657 nm.

Proline assay

Quantitative determination of free proline content was performed according to Bates et al (1973). The optical density of the solution was read on a spectrophotometer at the wavelength of 528 nm, and the proline concentration was determined using a calibration curve obtained by plotting the rate of intensity increase as a function of L-proline concentration (0-5 µg/ml).

Glycine betaine assay

GB extraction and quantification

Estimation of Endo-GB was done on dried leaf powder. Powdered plant material (0.5 g) was mechanically shaken with 20 ml of de-ionized water for 24 h at 25 °C. The samples were filtered and then extracts were diluted as 1:1 with 2N sulfuric acid. Aliquot (0.5 ml) was measured in test tube and cooled in ice water for 1 h. Cold potassium iodideiodine reagent (0.2 ml) was added and the mixture was gently mixed with vortex mixture then centrifuged at 10000 g for 15 min at 8 °C. The crystals were dissolved in 9 ml of 1,2-dichloro ethane and after 2.0-2.5 h the absorbance was measured at 365 nm with UV-visible spectrophotometer (Sairam et al., 2000).

Statistical analysis

All data were analysed using SPSS software for Windows: Release 19.0-standard version. When analysis of variance showed significant difference between means, Duncan's multiple range test was applied to compare the means at p<0.5 and the mean differences of four replicates were compared by the Lowest Standard Error of Means test (SE).

Results

Chlorophyll

Effect of planting date and seed coating treatments on chlorophyll content was significant. The chlorophyll content in seedlings of ED and OD seeds was higher than in seedlings germinated from LD seeds. In ED and EDS seeds, the maximum chlorophyll content (0.04 mg g^{-1} Fw) was measured. The lowest amounts of chlorophyll (0.029 mg g-1 Fw) were observed in the treatments of LF, LFM, LD, and LDS (Table 1). Chlorophyll content in EFS and EFSS treatments increased compared to EF. Perhaps, lint remnants on seeds caused delay in water absorption and physicochemical processes of seed germination by decreasing the S uptake. EF seeds absorbed water at first in a higher rate, but later more slowly, than EDS seeds (Marani and Amirav, 1970).

In ODS (0.38 mg g^{-1} Fw) and OFSS (0.30 mg g^{-1} Fw), the highest and the lowest chlorophyll contents were recorded, respectively. The DDSS treatment had the highest concentration (0.029 mg g^{-1} Fw) of chlorophyll (Table 1). Interaction between treatments of seed coating and planting date showed that treatments of seeds with ES, ODS, and ODS had the highest content of chlorophyll.

Anthocyanin

Anthocyanin concentration was affected by planting date and seed coating treatments (Table 1). The highest concentration of anthocyanins (0.65 mg g^{-1} Fw) was obtained in OFSS and ODSS treatments while the lowest concentration (0.21 mg g^{-1} Fw) was recorded in OD seeds (Table 1). EFSS, OFSS, ODSS, and DDSS treated seedlings had high content of anthocyanin that increased with treatment applications. Statistical differences in anthocyanins were observed between plants in

Table 1
Influence of seed planting date and seed coating treatments on cotton physiological characteristics

Treatments		Chlorophyll a (mg g^{-1} FW)	Anthocyanin (mg g^{-1} FW)	Proline (µg g^{-1} FW)	Glycine betaine (µg g^{-1} DW)
Planting Date	Seed Coating				
Early(E)	EF	0.029 i	0.20 h	42.8 i	371.5339 d
	EFS	0.034 e	0.26 g	36.3 l	371.5239 g
	EFSS	0.032 f	0.58 b	73.9 d	371.5298 f
	ED	0.040 a	0.47 cd	88.2 b	371.5134 h
	EDS	0.040 a	0.36 de	67.9 f	371.5137 h
	EDSS	0.035 d	0.39 d	53.2 j	371.5262 g
Optimum (O)	OF	0.035 d	0.23 g	40.9 k	371.5368 b
	OFS	0.031 g	0.24 g	39.9 k	371.5337 d
	OFSS	0.030 h	0.65 a	58.2 g	371.5327 e
	OD	0.036 c	0.21 h	88.3 b	371.5381 ab
	ODS	0.038 b	0.31 f	73.2 d	371.5337 d
	ODSS	0.031 g	0.65 a	56.0 h	371.5371 b
Delayed(D)	DF	0.021 k	0.26 g	84.9 c	371.5378 ab
	DFS	0.021 k	0.44 d	69.1 e	371.5387 a
	DFSS	0.026 j	0.48 cd	57.9 g	371.5346 c
	DD	0.020 l	0.49 c	87.7 b	371.5381 ab
	DDS	0.019 m	0.40 d	93.7 a	371.5369 b
	DDSS	0.029 i	0.58 b	58.6 g	371.5370 b

E: Early planting date, O: Optimum planting date, D: Delayed planting date, F: fuzzy seeds (as control or untreated seeds), FS: fuzzy seed with SCS, FSS: fuzzy seed with Sulfur coat and SCS, D: delinted seeds, DS: delinted seed with SCS, DSS: delinted seed with Sulfur coat and SCS. Values in the table are mean ± SE (n = 3) and ($P < 0.05$).

delayed planting for DF seedlings as lowest (0.26 mg g^{-1} Fw) and DDSS seedlings as highest (0.58 mg g^{-1}Fw) (Table 1). Sulfur treatment in the study seemed to have an advantage for more anthocyanins production, at least through adjustment of pH. It was determined that anthocyanin production and concentration can be affected by environmental factors such as S and the planting time.

Proline

Early and late planting date treatments of ED and DDS produced the highest content of proline, 8.82 µg g^{-1} Fw and 9.37 µg g^{-1} Fw, respectively (Table 1). This may be due to pollution resulted from delintation process. EDSS, ODSS, DFSS, and DDSS treated seedlings had low content of proline which decreased with treatment applications. Since, proline as an osmolyte helps plants against stress, this results in increased resistance by sulfur treatments and reduced stress and subsequently contributes to the reduction of proline production.

Glycine betaine

The effect of seed coating treatments and planting dates on the accumulation of glycine betaine was significant ($P<0.01$). With early planting, glycine betaine content decreased in all treatments in comparison with control. ED and EDS treatments had the lowest glycine betaine content and appeared to be partially effective, preventing the reduction of glycine betaine accumulation by sulfur treatment in early planting date. Treatments of seed coating in optimum and delayed dates of planting had identical amounts of glycine betaine production (Table 1). The lowest glycine betaine content was obtained in OFSS and DFSS whereas the highest was recorded in DF, DFS and DD treatments.

Discussion

Effect of planting date and seed coating treatments on chlorophyll content was significant. Chlorophyll accumulation in the study proved to be time dependent, because of higher content in ED and OD than in the LD seeds and the maximum chlorophyll content in EDS.

Compared to EF, chlorophyll content in EFS and EFSS treatments increased, perhaps due to lint remnants on seeds which caused delay in water absorption. Physicochemical processes by decreasing the S uptake and EF seeds absorb water at first at a higher rate, but later more slowly than EDS seeds (Marani and Amirav, 1970). The DDSS had the highest concentrations of chlorophyll (Table 1). Interaction between treatments of seed coating and planting date showed that treatments of seeds with EDS and ODS, resulted in the highest chlorophyll content. Hongfu, et al. (2008) reported that chlorophyll content as a biochemical indicator in the rice seeds coated by seed coating fertilizer, were superior. Researchers were unable to find any reports on simultaneous effects of date of planting and seed coating on the amount of chlorophyll and its physiological basis; only in a study by Karas et al. (1999) higher chlorophyll content was recorded for late sowing in comparison with early sowing treatments.

Anthocyanin concentration was affected by planting date and seed coating treatments. The highest content of anyhocyanin was observed in OFMS and ODMS and the high level of anyhocyanin in EFSS, OFSS, ODSS, and DDSS shows S-dependent nature of anthocyanin production. Production of more anthocyanins in this study seems to be an advantage for sulfur treatment, at least through adjustment of pH. It has been established that anthocyanin production and concentration can be affected by environmental factors such as pH and the time of year (Phillips, 2006). The pH of the internal plant environment appears to play a significant role in the concentrations of each type of anthocyanin (Mancinelli, 1990; Mohr and Schopfer, 1995). The type, amount, and intensity of anthocyanin levels vary greatly in response to numerous external factors. Plant responses to these factors create considerable difficulty in distinguishing the primary effects of anthocyanin production (Phillips, 2006). Parks et al. (1972) were able to show that environment had a major role in the concentration and the ratio of each anthocyanin type relative to one another in individual plant species. In another study anthocyanin concentration was reported to have a positive correlation with increasing sampling date and a negative correlation with planting date confirming that anthocyanin levels increase with crop maturity (Phillips, 2006). Anthocyanin pigments play an important role in improving plant tolerance to stress and possibly sulfur treatments may play a role in attracting and building construction elements of the pigment and are stored similar to the way natural antioxidants are stored (Bagchi et al., 2004). Also, anthocyanin concentrations were significantly greater after late summer versus early summer (Gazula et al., 2007). Our data suggest that anthocyanin concentration in cotton is a function of annual or seasonal temperature patterns, sulfur treatments, and planting date.

Pollution resulted from delintation process was due to high proline production in early and late planting date treatments of ED and DDS due to delintation process. Badr et al.(2004) stated that the free proline content of plant leaves was negatively correlated with number of days from sowing date to the first flower anthesis, leaf water loss ratio, and leaf area. Seed treatment with $CaCl_2$ resulted in significantly higher proline content at 60 and 90 days after sowing than seed treatment with water in the absence of $CaCl_2$ treatment (Shivamutthy, 2005). Seedling growth and the indicators of biochemistry such as proline content and chlorophyll content (14.6%) of the rice seeds coated by seed coating fertilizer were superior (Hongfu, et al., 2008).

Seedlings treatments EDSS, ODSS, DFSS, DDSS, had low content of proline which decreased with treatments applications. Proline as an osmolyte helped plant to resist against stress, resulting in increased resistance by sulfur treatments and reduced stress which subsequently contributed to the reduction of proline production. Blum and Ebercon (1976) reported that free proline accumulation in sorghum associated positively with ‘recovery resistance’ possibly by serving as a source of respiratory energy to recovering plant.

Effect of seed coating treatments and planting dates on the accumulation of glycine betaine was significant. Reduction of glycine betaine accumulation occurred by sulfur treatment in early (E) planting date and identical amounts of glycine betaine production were obtained in seed coating treatments of optimum (O) and delayed (D) dates of planting. Glycine betaine accumulates abundantly in chloroplasts where it plays a key role in chloroplast adjustment and protection of thylakoid membrane, thereby maintaining photosynthetic efficiency (Robinson and Jones, 1986; Papageorgiou and Murata, 1995; Yang and Lu, 2005). A strong evidence to this effect can be observed in a number of studies that showed the improvement of photosynthesis by GB plants strongly correlates with enhanced PSII photochemical performance (Hayashi et al., 1997; Sakamoto et al., 1998; Holmström et al., 2000). It is concluded that chlorophyll, proline, anthocyanin and glycine betaine content may vary with planting date, developmental stage, and environmental conditions under which plants are grown and delintation with S treatments and SCS improves the growth, stability and physiological processes in the cotton plant.

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