



## Investigation of correlation between germination and growth components in cotton under waterlogging and temperature treatments

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### Abstract

Waterlogging is one of the problems in cotton fields which affects germination and growth of cotton. Following rainfall is declined soil and environment temperature and can cause considerable damages to the crop. To investigate the correlation between components of the germination and growth in cotton under different levels of waterlogging and temperature, an experiment was carried out in laboratory and green house at Gorgan University of Agricultural Sciences and Natural Resources in Iran during the 2010-2011 cropping seasons. Experimental treatments were the waterlogging period duration (0, 24, 48 and 96 h) and temperature (16, 20 and 25 °C). The results showed that increasing the waterlogging period duration from 0 to 96 h and decreasing the temperature from 25 to 16 °C led to decrease in germination rate, seedling dry weight and normal seedling percentage in cotton. Also, the results showed with increasing the waterlogging period duration and decreasing the temperature decreased leaf area, plant height and dry weight, chlorophyll and Fv/Fm ratio. However, the interaction of waterlogging period duration and temperature was significant only on leaf area, plant height and dry weight. Also the results showed that there was high correlation between germination and growth components in cotton, so that these results can be used for evaluation of germination and growth of this plant under different waterlogging and temperature treatments.

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## Introduction

Cotton is one of the sensitive plants to waterlogging. This problem is considered as one of the main limitations of cotton production in some regions. The rainfall and low temperatures in early spring affect planting of this crop. Germination and growth of plants in the early stages is influenced by soil temperature and moisture (Seefeldt *et al.*, 2002; Soltani *et al.*, 2006; Soltani *et al.*, 2008). In low oxygen stress conditions due to lack of oxygen required for respiration, germination occurs more slowly and is produced weak seedling. In addition to after the waterlogging condition the crust that is created causes problems in growth of the new established plant. The amount of damage to plant during germination and under waterlogging conditions depends on the type of plant. Good quality of cotton seeds will be important in their tolerance to cold stress. Bange *et al.* (2004) reported that early planting if is not accompanied with cold stress can increase the yield of cotton. In waterlogged soils, plant seeds lose their viability and germination. Wuebker *et al.* (2001) by studying the effects of flooding and temperature on soybean seeds reported that in flooding conditions decreases seed germination percentage. Also they concluded that in flooding seed germination percentage at low temperatures decreases more than higher temperatures. Ismail *et al.* (2009) reported that flooding slows seed germination, imposes fatalities and delays seedling establishment in rice. Flooding stress is reduced leaf area, rate of photosynthesis and dry matter production in plant tissues (Galeshi *et al.*, 2000). Also in waterlogging reduction of leaf area is accompanied with reduction of photosynthetic capacity and light use efficiency and this affect adversely on production and transportation of carbohydrates to the roots. Christianson *et al.* (2010) reported waterlogging caused significant reduction in stem elongation, shoot mass and root mass in cotton. Bange *et al.* (2004) showed that leaf area is reduced in waterlogging. Also carbohydrate production and energy that is required for stem elongation is decreased in this condition (kafi *et al.*, 2009). In anaerobic conditions deficiency of nitrogen and other

nutrients and the inhibitory effect of ethylene prevent shoot growth and stem elongation (Galeshi *et al.*, 2009). Reduction of leaf extension, stomatal closure and decreasing the quantum efficiency of PSII in flooding reported by Else *et al.* (2009). In flooding genes of responsible for carbohydrate metabolism are changed in cotton leaves. Genetic variation in response to waterlogged conditions and reduction of leaf photosynthetic rates in cotton reported by Conaty *et al.* (2008). Smethurst and Shabala (2003) reported that in waterlogging chlorophyll content and maximal quantum efficiency of PSII decreased significantly. Also leaf chlorosis increased during waterlogging. Reduction of photosynthesis in cotton at 24 hours after waterlogging reported by Thongbai *et al.* (2001). Fv/Fm (ratio of variable fluorescence to maximum fluorescence), which represents the maximum quantum efficiency of photosystem II for conversion of absorbed light to chemical energy is used for show the effects of environmental stresses on plants (Maxwell and Johnson, 2000). Stomatal closure and decreasing the quantum efficiency of PSII in flooding reported by Else *et al.* (2009). Reduction of temperature decreases the quantum efficiency of PSII due to reduction of electron transport in photosynthesis. Netto *et al.* (2005) reported diminishing of electron flow and Fv/Fm ratio due to PSII impairment in environmental stresses. The effective quantum yield of PS II decreased in flooded plants (Janowiak *et al.*, 2002; Ahmed *et al.*, 2006). Also temperature reduction decreases the PSII efficiency and Fv/Fm ratio (Baker and Rosenqvist, 2004).

One of the main problems in some areas under cotton cultivation is rainfall in early spring, so that in farms without proper drainage occurs waterlogging. Also, rainfall decreases soil and environment temperatures that affect adversely the germination and seedling growth. Due to the high sensitivity of cotton and reduction the germination and growth of this plant in waterlogging, this research was carried out to study the effects of waterlogging and temperature on seed germination and seedling growth of cotton in the laboratory and greenhouse conditions.

## Materials and methods

### Study site and experimental treatments

This experiment was carried out in laboratory and green house at Gorgan University of Agricultural Sciences and Natural Resources in Iran during the 2010-2011 cropping seasons. Experimental treatments were the waterlogging period duration (0, 24, 48 and 96 h) and temperature (16, 20 and 25 °C). For this experiment, cotton seeds were prepared from the cotton research institute. The seeds were placed in petri dishes and were added distilled water to them. So that 2 cm above the seeds were covered with water. Then they were placed in incubator under different waterlogging and temperature treatments.

### Germination and seedling growth tests

At the end of experiment period, the seeds were taken out of water and were planted on paper towels. Then the germination rate, seedling growth rate and cool germination tests were carried out on them. Germinated seeds were counted twice a day for 7 days. Then germination rate, seedling dry weight and normal seedling percentage were measured. To calculate the percentage and rate of germination was used Germin program. To calculate these parameters was interpolated from the germination curve versus time (Soltani and Maddah Yazdi, 2010).

### Measurement of growth factors in green house

To perform experiment in the greenhouse was used perlite and soil (in the ratio 1:2). The soil included 28% clay, 62% silt and 10% sand. Also the saturation percentage 49%, electrical conductivity 0.8 ds/m and soil bulk density 1.7 g/cm<sup>3</sup> were determined. Then 10 cotton seeds were planted in

pots with 25 cm diameter and 17 cm height. After plants reached the 4 true leaves stage, the pots were placed in growth chamber under waterlogging period duration and temperature treatments. Then the pots were out and chlorophyll index was measured with chlorophyll meter (model SPAD 502). Measurement of chlorophyll fluorescence parameters carried out by chlorophyll fluorometer (model OS-30) and Fv/Fm was calculated using the following equation:

$$Fv / Fm = (Fm - Fo) / Fm$$

Where Fo is initial fluorescence, Fm is maximum fluorescence and Fv is variable fluorescence.

Also at the end of experiment leaf area, plant height and dry weight were measured.

### Statistical analysis

The data were analyzed using the SAS statistical software. Means were compared using the least significant difference test (LSD) at the 5% level.

## Results and discussion

### Effect of waterlogging and temperature on germination components

Analysis of variance (Table 1) showed that the effect of waterlogging period duration on percentage and rate of germination, seedling dry weight and normal seedling percentage was significant at the 1% level. Also the effect of temperature on germination rate, seedling dry weight and normal seedling percentage was significant at the 5 and 1% level. Interaction of waterlogging period duration and temperature on the other traits except germination percentage was significant at the 5% level.

**Table 1.** Analysis of variance for Germination Percentage (GP), Germination Rate (GR), Seedling Dry Weight (SDW) and Normal Seedling Percentage (NSP) under Waterlogging (W) and Temperature (T) treatments.

S.o.v.	Df	Mean Square				
		GP	GR	SDW		NSP
T	2	159.28 ns	0.00096**	0.00018*	0.00006 ns	4628.51**
E <sub>1</sub>	9	227.46 ns	0.00010 ns			ns 347.54
W	3	2373.60**	0.0017**	0.00031**	0.00004*	3320.83**
W×T	6	380.25 ns	0.00042*			645.92*
E <sub>2</sub>	27	169.86	0.00014	0.00003	3.8	473.51
Cv		1.4	1.4			5.9

ns= no significant; \* and \*\* = Significant (p< 0.05 and p< 0.01, respectively).

Means comparison (Table 2) showed that at 16°C, the most of germination rate was observed in control (0.092 per hr) but statistically significant difference was not shown to 24 and 48 hours waterlogging. Also the least of germination rate in this temperature was observed in 96 hours waterlogging (0.055 per hr). At 20°C, the most of germination rate was observed in control (0.092 per hr) but statistically significant difference was not shown to 24 and 48 hours. Also the least of germination rate in this temperature was observed in 96 hours waterlogging (0.070 per hr). Between the waterlogging treatments at 25°C was not observed significant difference in germination rate. Means comparison showed that at 16°C, the most of seedling dry weight was observed in control (0.021 g) but significant difference was not shown to 24 and 48 hours waterlogging. Also the least of seedling dry weight in this temperature was observed in 96 hours waterlogging (0.008 g) but significant difference was not shown to other waterlogging treatments. At 20°C, the most of seedling dry weight was observed in control (0.022 g) but statistically significant difference was not shown to 24 hours waterlogging. Also the least of seedling dry weight in this temperature was observed in 96 hours waterlogging (0.004 g) but significant difference was not shown to 48 hours waterlogging. Between the waterlogging

treatments at 25°C was not observed significant difference. Means comparison of normal seedling percentage showed that at 16°C, the most of normal seedling percentage was observed in control (19.99%) but significant difference was not shown to 24 hours waterlogging. The least of normal seedling percentage in this temperature was observed in 48 and 96 hours waterlogging (1.66%). At 20°C, the most of normal seedling percentage was observed in control (33.33%) but between the waterlogging treatments was not observed significant difference. At 25°C, the most of normal seedling percentage was observed in control (66.66%) but significant difference was not shown to 24 hours waterlogging. Also the least of normal seedling percentage in this temperature was observed in 96 hours waterlogging (4.99%). The results showed that seeds exposed to low temperatures were more sensitive to waterlogging compared with high temperatures. Finally it can be concluded that decreasing the temperature adversely affects on germination and seedling growth and under waterlogging conditions when the temperature is reduced germination and seedling growth in cotton is decreased more than when the temperature is increased. This can be explained due to damages of cold and water absorption at low temperatures (Wuebker *et al.*, 2001).

**Table 2.** Mean comparison for Germination Rate (GR), Seedling Dry Weight (SDW) and Normal Seedling Percentage (NSP) under Waterlogging (W) and Temperature (T) treatments.

T(°C) W(h)	GR (hr)			SDW (g)			NSP (%)		
	16	20	25	16	20	25	16	20	25
0	0.092 a	0.092 a	0.095 a	0.021 a	0.022 a	0.023 a	19.99 a	33.33 a	66.66 a
24	0.090 a	0.092 a	0.092 a	0.016 ab	0.014 ab	0.022 a	16.66 a	16.66 b	63.33 a
48	0.085 a	0.090 a	0.092 a	0.017 ab	0.009 bc	0.015 a	1.66 b	9.99 b	36.66 b
96	0.055 b	0.070 b	0.080 a	0.008 b	0.004 c	0.016 a	1.66 b	1.69 b	4.99 c

Means followed by same letter do not differ through LSD test at 5% probability.

#### *Correlation between germination components*

To determine the relationship between the studied traits in cotton were calculated simple correlation coefficients (Table 3). The results showed that there was significant positive correlation between germination percentage to germination rate and normal seedling percentage. Also there was significant positive correlation between germination

rate to germination percentage and seedling dry weight. The correlation between normal seedling percentage to all factors was significant positive except germination uniformity. Also the results showed that there was a negative correlation between germination uniformity to other traits. This means that with increasing the waterlogging period duration and decreasing the temperature increased

germination uniformity and decreased percentage and rate of germination, seedling dry weight and normal seedling percentage in cotton. Because with increasing the germination uniformity and

prolongation the time between start and end of germination was decreased seed function and seedling growth.

**Table 3.** Correlation coefficients between germination components (GP = Germination Percentage, GR = Germination Rate, GU = Germination Uniformity, SDW = Seedling Dry Weight, NSP = Normal Seedling Percentage) in cotton under Waterlogging and Temperature treatments.

Trait	GP	GR	GU	SDW	NSP
GP	1				
GR	0.38**	1			
GU	-0.10	-0.61*	1		
SDW	0.10	0.51**	-0.27	1	
NSP	0.33*	0.36*	-0.24	0.37**	1

\* and \*\* = Significant ( $p < 0.05$  and  $p < 0.01$ , respectively).

#### *Effects of waterlogging and temperature on growth components*

Analysis of variance (Table 4) showed that the effect of waterlogging period duration and temperature on leaf area, plant height and dry weight, chlorophyll and

Fv/Fm ratio was significant at the 5 and 1% level but the interaction of waterlogging period duration and temperature only on leaf area, plant height and dry weight was significant at the 1% level.

**Table 4.** Analysis of variance for Leaf Area (LA), Plant Height (PH), Plant Dry Weight (PDW), Chlorophyll (C) and Fv/Fm ratio under Waterlogging (W) and Temperature (T) treatments.

S.o.v.	Df	Mean square				
		LA	PH	PDW	C	Fv/Fm
T	2	3371.42**	174.48**	0.2393**	9.99*	0.00076**
E <sub>1</sub>	9	1.79 ns	0.02 ns	0.00005ns	2.49ns	0.00009*
W	3	110.46**	23.92**	0.0228**	10.45**	0.00092**
W×T	6	45.09**	3.99**	0.0058**	1.01ns	0.00002ns
E <sub>2</sub>	27	4.47	0.03	0.00004	1.90	0.00003
Cv		1.45	0.64	0.52	4.28	0.79

ns= no significant; \* and \*\* = Significant ( $p < 0.05$  and  $p < 0.01$ , respectively).

Means comparison (Table 5) showed that at 16°C, with increasing the waterlogging period duration was not observed significant difference in cotton leaf area. At 20°C, with increasing the waterlogging period duration decreased the cotton leaf area. So that the most of leaf area was observed in control (78.76 cm<sup>2</sup>) but statistically significant difference was not shown to 24 hours waterlogging. Also the least of leaf area was observed in 48 and 96 hours waterlogging. At 25°C, the most of leaf area and the least of that

showed in control and 96 hours waterlogging respectively (90.03 and 74.80 cm<sup>2</sup>). These results correspond with results of Bange et.al (2004). Means comparison showed that with increasing the waterlogging period duration from 0 to 96 hours decreased plant height and this reduction was significant in each three temperatures. So that at 16°C, the most of plant height and the least of that was shown in control (27.75 cm) and 96 hours (24.03 cm) waterlogging respectively. At 20°C, the most of

plant height was observed in control (32.73 cm) but statistically significant difference was not shown to 24 hours waterlogging. Also the least of plant height was observed in 96 hours (27.72 cm). At 25°C, the most of plant height was shown in control (32.87 cm) but significant difference was not shown to 24 hours waterlogging. Also the least of that was observed in 96 hours (30.01 cm). Galeshi *et al.* (2009) reported that in anaerobic conditions deficiency of nitrogen and other nutrients and the inhibitory effect of ethylene prevent shoot growth and stem elongation. The results showed that with increasing the waterlogging

period duration and decreasing the temperature decreased plant dry weight. So that at 16°C, the most of plant dry weight and the least of that was observed in control and 96 hours waterlogging respectively (1.171 and 0.993 g). Also at 20°C, the most of plant dry weight was shown in control (1.34 g) and the least of that was observed in 96 hours (1.29 g). At 25°C, the control and 96 hours waterlogging was shown the most and the least of plant dry weight respectively (1.38 and 1.03 g). These results correspond with results of Christianson *et al.* (2010).

**Table 5.** Mean comparison for Leaf Area (LA), Plant Height (PH) and Plant Dry Weight (PDW) under Waterlogging (W) and Temperature (T) treatments.

T(°C)	LA (cm <sup>2</sup> )			PH (cm)			PDW (g)		
	16	20	25	16	20	25	16	20	25
W(h)									
0	57.42 a	78.76 a	90.03 a	27.75 a	32.73 a	32.87 a	1.171 a	1.34 a	1.38 a
24	56.74 a	78.59 a	87.99 b	26.27 b	32.72 a	32.85 a	1.157 b	1.33 b	1.33 b
48	55.96 a	75.8 b	84.84 c	26.07 b	30.72 b	32.6 b	1.153 b	1.30 c	1.33 b
96	55.89 a	74.78 b	74.8 d	24.03 c	27.72 c	30.01 c	0.993 c	1.29 d	1.03 c

Means followed by same letter do not differ through LSD test at 5% probability.

**Table 6.** Correlation coefficients between growth components (LA = Leaf Area, PH = Plant Height, PDW = Plant Dry Weight, C = Chlorophyll) in cotton under Waterlogging and Temperature treatments.

Traits	LA	PH	PDW	C	Fv/Fm
LA	1				
PH	0.9**	1			
PDW	0.9**	0.92**	1		
C	0.42**	0.49**	0.5**	1	
Fv/Fm	0.56**	0.62**	0.6**	0.52**	1

\* and \*\* = Significant ( $p < 0.05$  and  $p < 0.01$ , respectively).

#### Correlation between growth components

To determine the relationship between the studied traits in cotton were calculated simple correlation coefficients (Table 6). The results showed that there was significant positive correlation between leaf area to plant height and dry weight, chlorophyll and Fv/Fm ratio. Galeshi *et al.* (2000) reported that flooding stress is reduced leaf area, rate of photosynthesis and dry matter production in plant tissues. Also between plant height and dry weight to

other traits was observed significant positive correlation. Reduction of leaf area, carbohydrate production and energy that is required for stem elongation in waterlogging reported by (Bange *et al.*, 2004; kafi *et al.*, 2009). Thus it can be concluded that reduction of plant height caused by decreasing in photosynthetic capacity and reduction of shoot development in waterlogging and finally reduced vegetative growth and plant dry weight. The results showed that there was significant positive correlation

between chlorophyll and Fv/Fm ratio. This shows that with decreasing the chlorophyll in waterlogging is decreased Fv/Fm ratio. Because in waterlogging conditions due to reduction the activity of photosynthetic enzymes and decreasing the net photosynthesis are reduced carbohydrates production and chlorophyll in plant and finally is decreased the maximum quantum efficiency of photosystem II.

### Conclusion

According to the results, it can be concluded that waterlogging and low temperature had negative effects on germination and growth components in cotton. So that, in waterlogging conditions with reduction the temperature germination and seedling growth in cotton is decreased more than when the temperature is increased. This can be explained due to damages of cold and water absorption at low temperatures. Also due to the cotton sensitivity to increasing the waterlogging period duration and reduction the temperature was decreased photosynthesis and growth of plant. The results showed that there was high correlation between the germination and growth components in cotton and these results can be used for evaluation of germination and growth of this plant under different treatments of waterlogging and temperature. Finally, we can say is better that the planting date of this crop be regulated based on the rainfall time and soil and environment temperatures to prevent the adverse effects of waterlogging and low temperatures on germination and growth of plant.

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