

## Effect of Drought Stress and Seed Pretreatment with CCC on Yield and Yield Components of Maize Varieties

Mısır Varyetelerinde Kuraklık Stresi ve Tohumlarına Cycocel Uygulamasının Verim ve Verim Componentleri Üzerine Etkisi

Amir SOLTANBEİĞİ<sup>1</sup>

### Abstract

In order to study the effects of drought stress and seed pretreatment of two maize varieties with Cycocel an experiment was carried out with the aim to reduce the stress on the yield of this crop during their critical growth stages. Field trials were arranged in split-split plot design and randomized complete block design with three replications. For statistical evaluation, irrigation as the main factor at four levels (complete irrigation as the control and eliminating one stage of irrigation at the beginning of stalking, tasseling and dough stages), maize var. KSC704 and KSC666 as the sub factor at two levels and application of Cycocel as the sub-sub factor at three levels (0, 0.4, and 0.8 g L<sup>-1</sup> concentration) were created. Eliminating one stage of irrigation in stalking and tasseling stages affected the ear length, ear diameter, grain number per ear, grain number per row and ultimately the grain yield, while the drought stress at dough stage had no significant difference in the results of the experiment compared to the control group. Apart from enhancing 1000-grain weight, the use of Cycocel pretreatment affected the test results slightly and comparison of these findings with similar studies, which applied Cycocel as foliar, indicates the higher ability of foliar than the pretreatment.

**Keywords:** Chlormequat Chloride, Drought, Irrigation, Maize, PGRs

### Öz

Bu deneme iki farklı mısır varyetesinde, bitkinin en hassas büyüme ve gelişme dönemlerinde kuraklık stresi ve tohumlara bitki büyüme düzenleyici olarak Cycocel muamelesinin verim üzerine etkilerinin en aza indirmek amacıyla kurulmuştur. Deneme Split-split Plot Deneme Desenine göre 3 tekrarlamalı olarak yürütülmüştür. İstatistiksel değerlendirmede, sulama ana parselleri dört düzeyde (düzenli sulama kontrol ve sapa kalkma dönemi, çiçeklenme dönemi ve danelerin hamurlaşma döneminin başında bir nöbet sulama kesintisi), mısır varyetesi alt parselleri iki düzeyde (KSC704 ve KSC666) ve Cycocel uygulaması alt-alt parsellerini üç düzeyde (0, 0.4, and 0.8 g L<sup>-1</sup> concentration) oluşturmuştur. Kontrol bitkilerle karşılaştırıldığında sapa kalkma ve çiçeklenme dönemlerinin başında bir nöbet su kesimi koçan uzunluğu, koçan çapı, koçanda dane sayısı, her sırada dane sayısı ve dane verimini olumsuz etkileyerek, dane hamurlaşma dönemi kuraklık stresinden etkilenmemiştir. Mısır tohumlarında Cycocel uygulaması 1000 dane ağırlığı yükselişi hariç, deneme sonuçlarını çok az etkilemiştir. Bu bulgular diğer benzeri araştırma sonuçlarıyla karşılaştırıldığında yapraktan uygulama Cycocel yöntemi, tohum uygulamasından daha etkilidir.

**Anahtar Kelimeler:** Chlormequat Chloride, Kuraklık, Mısır, PGRs, Sulama

<sup>1</sup>**Corresponding Author:** Amir Soltanbeği, Food Control Research and Application Center, Afyon Kocatepe University, Afyonkarahisar, Turkey. E-mail: [soltanbeği@aku.edu.tr](mailto:soltanbeği@aku.edu.tr)  ORCID: 0000-0002-8791-0482

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Drought is one of major abiotic stresses which cause heavy crop production losses around the world. Furthermore, the climatic-change models predict that crop losses due to increased aridity in most regions of the world will be enhanced more in future (Athar and Ashraf, 2005). Drought is a permanent constraint on agricultural production in most of developing countries and an occasional cause of agricultural production losses in developed ones (Ceccarelli and Grando, 1996). Essentially, moisture stress plays significant role on numerous plant processes such as photosynthesis, cell development, and division and accumulation of nutrients' transfer (Boyer and McPherson, 1998). Iran, with an annual rainfall equal to 240 mm, is classified as a dry region of the world and drought is one of the most devastating environmental stresses which has affected Iran (Jajarmi, 2009).

Maize (*Zea mays* L.) is one of the most developing dynamic cereal crops around the world. Maize production has been increased approximately 70% around the world in the last fifteen years (Sandor, 2010). Soil drought stress, especially along with too high temperatures is the most common constraint on yield throughout the world (Beck et al., 1996). Maize is sensitive to drought stress in some stages of growth and the yield of this plant has a potential close relationship with access to water (Koliaei et al., 2011). Drought stress has a particular effect on the ability of maize plant to produce seed at three critical stages of plant growth: at the beginning of growing season (when plant has sufficient growth), at flowering stage, and during the middle to the end of grain filling (Guelloubi et al., 2005).

Adverse effects of water stress on maize growth and yield depend on the time of stress, growth stage, plant genotypes, varieties, cultivation methods, soil quality, deficiency level and environmental conditions during the drought period (Allen and Musick, 1993). Maize grain yield reduction, caused by drought, ranges from 10 to 76% depending on the severity and stage of occurrence (Bolaos et al., 1993). Drought stress reduces the yield and some of its components (Ilkai et al., 2010; Golbashy et al., 2010; Shiri et al., 2010; Khodarahmpour and Hamidi, 2012). Hashemzadeh (2009), indicated the significant effects of different irrigation treatments at different growth stages of maize on some traits such as ear length, ear diameter, 1000-grain weight, and grain yield. Irrigation after 70 mm evaporation was associated with improved grain yield properties.

Nowadays, different methods have been considered to get over the effects of drought stress (Royo et al., 2004). One of these methods is to apply the chemical plant growth regulators such as Cycocel (Gallagher and Biscoe, 1978). Plant growth regulators (PGRs) consist of a large group of endogenous and exogenous chemical compounds which can regulate the plant growth in numerous ways (Rajala, 2003). There are some further evidence under which the applied PGRs as a seed treatment at early growth stages may improve the tolerance of cereals to abiotic stresses. Seed treatment along with PGRs improves the drought, heat and waterlogging tolerance (Webb and Fletcher, 1996; Gilley and Fletcher, 1997). Foliar and soil application of PGRs have significant disadvantages such as waste and accumulation of these chemicals (Barrett and Nell, 1992) while seed priming with PGRs solutions is an alternative method to control plant growth that has more benefits such as cost reduction, accumulation of active substances reduction and also is ease of use (Pasian and Bennett, 2001).

Chlormequat chloride or Cycocel is a plant growth regulator (PGR) which belongs to the quaternary ammonium class of chemicals. Cycocel inhibits the plant hormone biosynthesis, which is useful in regulating the growth properties in most of the plants (US Environmental Protection Agency, 2007). It can make the plant shorter but stronger (Shekoofa and Emam, 2008), make the leaves darker and thicker and increase the ability to resist the collapse, drought and cold stresses (Emam and Moaied, 2000). Cycocel plays the role in thickening the stems and this enhances the plant stability in water stress tolerance (Farooq and Bano, 2006). Applying different concentration of Cycocel in different periods of plant growth has a significant effect on growth and yield of plant components and it enhances the plant yield through increasing the number of florets (Moniruzzaman, 2000). Cycocel application, which depends on the concentration and climatic conditions, increased the cereal products from 0-13 percent. Seed treatment with Cycocel increased the number of maize in spatial unit and consequently increased the rate of ultimate yield (Peltonen-Sainio and Rajala, 2001). Cycocel treatment caused a significant increase in maize grain yield (Ilkai et al., 2010). Furthermore, application of Cycocel in maize increased the stem diameter, ear length, ear diameter, cob weight, 1000-grain weight, biological yield, grain yield and harvest index (Hashemzadeh, 2009).

### **Material and Methods**

This study was conducted at the field of Agriculture Research Station and Natural Resources in Khoy city in 2009. This area is located at latitude 38°35' North and longitude 44° 57' East at an altitude of 1103 meters. According to Köppen climate classification, this area has semi-arid climate with dry summers (FAO, 2014). Some meteorological data of experiment region during crop growth is shown in Table 1. Results of soil analysis at the depth of 30 and 60 cm indicate that the soil of farm was loamy-clay with pH equal to 7.4 with electrical conductivity equal to 0.64 dsm<sup>-1</sup> (Table 2). N, P and Zn fertilizers were applied according to recommendations of soil testing in form urea, triple superphosphate and zinc sulphate, respectively. Considering the results of soil analysis, 310 kg ha<sup>-1</sup>

of pure urea (1/3 during planting, 1/3 in staking stage and 1/3 in flowering stage), 180 kg ha<sup>-1</sup> phosphorus (during planting) and 25 kg ha<sup>-1</sup> of zinc (during planting) were applied.

**Table 1: Some meteorological data of experiment region during crop growth (2009)**

Month	Temperature (°C)			Rain (mm)	Relative humidity (%)
	Minimum	Maximum	Mean		
June	14.3	28.6	21.4	65.1	52
July	17.7	32.6	25.1	8.8	50
August	15.8	30.7	23.2	2.9	51
September	12.3	25.4	18.8	69.1	61

Khoy city meteorological service

This experiment was done as split-split plots in randomized complete block design with three replications. In this test, irrigation was chosen as the main factor at four levels, maize varieties as the sub factors at two levels, and different concentration of Cycocel as sub-sub factor at three levels. Choosing the stages of eliminating an irrigation step was done based on the critical stages of maize growth and there regular irrigation continued after stress. The first level (I<sub>0</sub>) was considered as the stage without eliminating the irrigation until the end of plant growth stage after each 70±5 evaporation determined using daily evaporation rate of class A evaporation pan (121 cm diameter and 25.4 depth with an anemometer) as the control. The other levels (I<sub>2</sub>, I<sub>3</sub> & I<sub>4</sub>) were considered as the stage for eliminating one step of irrigation at the beginning of staking, tasseling and dough stages, respectively, and regular irrigation continued after 140±5 mm evaporation from class A evaporation pan. All calculations of evaporation were performed by climatology experts from Khoy weather station. Also, from planting to staking stage and first moisture stress (I<sub>2</sub>), sprinkler irrigation method was used and after that continued surface irrigation through the furrows. Seeds, applied in experiment were the var. KSC704 and KSC666. For Cycocel treatment, the seeds were put in distilled water (control) and prepared solutions from 0.4 and 0.8 g L<sup>-1</sup> growth regulator obtained from Cycocel crystal soluble in distilled water and ethanol as second and third levels, respectively, based on the type of treatment for 4 hours. After drying, the seeds were planted into the holes on ridges as stack or two or three seeds in each hole on June 7th. Plots had 5×2 dimensions with cultivating lines spacing equal to 60 cm and plants spacing on cultivating strip equal to 20 cm. Farm fertilizer was prepared according to the results of soil analysis (Table 2) and it was applied on the strip and 5 cm lower than these seeds planted at recommended stages. When the whole farm became green and reached 4-6 leaf stage, the extra bushes were thinned and after a while the weeds were pulled with hand method of weeding and later the soil was added to the plant's foot. Traits were measured after removing the margins through randomized selection of eight plants from each plot and then obtained data was statistically analyzed by MSTAT-C software.

**Table 2: Physical and chemical properties of soil**

Fe	Ma	Zn	Cu	K (p.p.m)	P (p.p.m)	O. C (%)	T.N.V (%)	pH	EC (ds m <sup>-1</sup> )	S. P.	Depth (cm)
5.40	6.30	1.04	2.60	217	5.13	0.76	13.0	7.4	0.64	45	30
8.66	7.48	1.12	3.02	107	1.55	0.67	12.0	6.54	0.52	50	60

## Results and Discussion

### Ear length

According to analysis of variance, the effect of different levels of irrigation on ear length was statistically significant at the 1% level (Table 3). Mean comparison indicated that the shortest ear length was related to the moisture stress treatment at staking stage and also moisture stress treatment at tasseling stage (Table 4). It seems that the longer intervals between irrigations will lead to the reduced ear length. Sadeghi et al. (2007), Hashemzadeh (2009) and Khodarahmpour and Hamidi (2012), also support the idea of different irrigation types on the ear length. Effect of variety on the ear length was statistically significant at 1% level (Table 3). Given the mean comparison, it was identified that the variety 666 produced long ear compared to the variety 704 (Table 4). Interaction of irrigation and variety on ear length was also significant at 5% level (Table 3). Thus, the maximum length of ear was consequence of interaction between the control treatment and moisture stress at dough stage and variety 666 (Figure 1). This can be justified as the variety 666 has higher ear length and also because the plant vegetative growth is not affected by the drought stress in treatments of control irrigation and moisture stress at dough stage.

**Table 3: Analysis of variance on some traits associated with yield and its components**

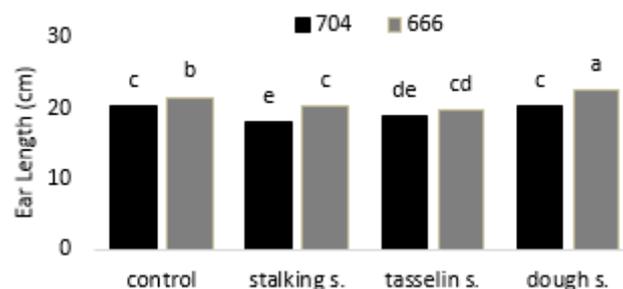
Source of variance	D.F.	M. S.			
		Ear length	Ear diameter	Row number per ear	Grain number per row
Replication	2	7.819	11.885	0.097	30.597
Irrigation	3	23.328**	12.958*	1.532	307.458**
Error 1	6	2.310	1.478	0.606	14.208
Variety	1	47.450**	17.781**	2.347*	165.014**
Irrigation×Variety	3	2.622*	3.106*	0.236	30.755
Error 2	8	0.628	0.580	0.264	9.611
CCC	2	1.502	5.354	1.097	13.181
Irrigation×CCC	6	2.068	2.294	1.671	12.514
Variety×CCC	2	5.590	1.890	0.181	11.931
Irrigation×Variety×CCC	6	1.128	0.911	0.347	8.782
Error 3	32	2.127	2.407	0.563	14.792
CV		7.23	3.44	4.89	8.67

\*\* and \*, Significant at 0.05 and 0.01 probability levels, respectively

**Table 4: Mean comparison on some traits associated with yield and its components**

Treatment	Ear length (cm)	Ear diameter (cm)	Row number per ear	Grain number per row
<i>Irrigation</i>				
I <sub>1</sub>	20.92a	45.49a	15.50	46.94a
I <sub>2</sub>	19.14b	45.21a	15.33	42.78b
I <sub>3</sub>	19.27b	43.92b	14.94	39.28c
I <sub>4</sub>	21.38a	45.87a	15.61	48.39a
<i>Variety</i>				
704	19.37b	45.62a	15.53a	45.86a
666	20.99a	44.63b	15.17b	42.83b
<i>CCC</i>				
control	19.89	44.62	15.17	43.50
0.4 (g L <sup>-1</sup> )	20.32	45.55	15.58	44.67
0.8 (g L <sup>-1</sup> )	20.32	45.20	15.29	44.87

The means which have no letters are statistically non-significant at 5% probability level



**Figure 1: Mean comparison interaction of the irrigation and variety on ear length**

### Eardiameter

Effect of different irrigation treatments on the ear diameter was significant at 5% level (Table 3). Accordingly, the results of mean comparison indicate that the moisture stress treatment at tasseling stage had lower ear diameter

compared to other treatments (Table 4) and other treatments were classified in a statistical group. Results of this study on the effect of drought stress on ear diameter were consistent with the results of test by Hashemzadeh (2006) and Dağdelen et al. (2008), who studied the effect of different irrigation types on ear. Investigating the results of analysis of variance table indicates that the effect of variety on ear diameter is significant at 1% level (Table 3). The results of mean comparison suggest that the maize variety 704 had higher diameter than the variety 666. Since the variety 704 is a serotinous variety compared to 666, it seems that it has long period of growth and this enhances the cell division and dimensions of this variety and finally the ear diameter through continuing process of photosynthesis. Moisture stress at tasseling stage led to the disruption in the growth and ultimate development of tasseling, thus the plant became incapable to produce perfect pollen during pollination, so tasseling is among the most critical steps in supply of water for plants, and the lack of plant's access to water during the above-mentioned period will lead to the irreparable damages at maize plant. Interaction of irrigation and variety became significant at 5% level (Table 3). Thus Variety of 704 in all irrigation treatments except eliminating irrigation at tasseling stage produced high Ear diameter and were put in the same statistical group (Figure 2).

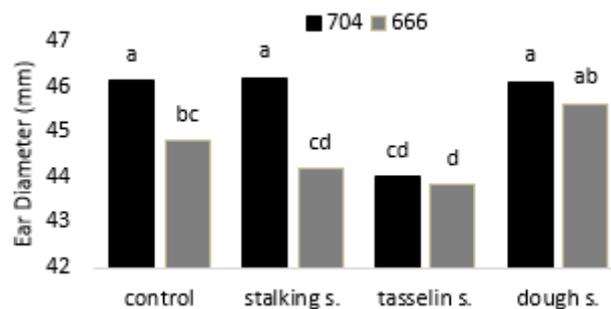


Figure 2: Mean comparison interaction of the irrigation and variety on ear diameter

#### Row number per ear

Impact of variety on the number of rows in ear was statistically significant at 5% level (Table 3). Corresponding mean comparison indicated that the variety 704 had a larger number of rows than variety 666 (Table 4). This is due to the genetic characteristics of plant and is less affected by the environmental factors (Wu et al., 2003). Other treatments showed no significant effect (Table 3).

#### Grain number per row

Impact of different irrigation types on the grain number per row was statistically significant at 1% level (Table 3). Mean comparison in Table 4 showed the lowest grain number in moisture stress treatment at tasseling stage and stalking stage, respectively. Occurrence of stress at the early vegetative stages and before differentiation of male reproductive organ is less effective than any stresses in the ear differentiation and growth as well as pollination. This was clearly observed in moisture stress treatments at tasseling stage, so that most of produced ears in this treatment had heterogeneous appearance and irregular rows. Results of this test are consistent with the results by Karimian et al. (2005) and Khodarahmpouret al. (2012). According to the analysis of variance in Table 3, the effect of variety on the number of grains per row was significant at 1% level. Conducted mean comparison in Table 4 indicates that the variety 704 has more grains per row compared to variety 666 and this is probably due to the genetic characteristics of plant.

#### Grain number per ear

Impact of different irrigation types on the number of grain per row was statistically significant at 1% level (Table 5). Mean comparison showed the lowest grain number in stress treatment at tasseling stage and stalking stage, respectively (Table 6). Results of this study are consistent with findings of tests by Dağdelen et al. (2008) and Hashemzadeh (2009), based on the production of less number of grain in ear as the result of drought stress. As previously explained, the grain production deficiency at tasseling stage is due to the damage to the system of production and eventually the plant pollination. According to the results of analysis of variance in table 3, the effect of variety was significant on the number of grains per ear at 1% level and the variety 704 had greater potential for grain production than the variety 666 (Table 6).

**Table 5: Analysis of variance on some traits associated with yield and its components**

Source of variance	D.F.	M. S.		
		Grain number per ear	1000 grain weight	Grain yield
Replication	2	9354.389	2097.775	6939431.136
Irrigation	3	91916.704**	1417.925	42749564.511*
Error 1	6	5286.093	609.816	4959332.016
Variety	1	64320.889**	15332.921**	438344.511
Irrigation×Variety	3	2726.407	924.850*	4703359.002
Error 2	8	1860.028	184.710	1473526.576
CCC	2	9129.264	859.582*	3759989.761
Irrigation×CCC	6	6333.301	843.366*	2961082.208
Variety×CCC	2	2734.847	986.596*	12132271.121*
Irrigation×Variety×CCC	6	1438.032	323.781	1454284.875
Error 3	32	4033.285	266.920	2315351.955
CV		9.30	5.42	11.98

\*\* and \*, Significant at 0.05 and 0.01 probability levels, respectively

**Table 6: Mean comparison of for some traits associated with yield and its components**

Treatment	Grain number per ear	1000 grain weight (gr)	Grain yield (kg ha <sup>-1</sup> )
<i>Irrigation</i>			
I <sub>1</sub>	723.78a	307.17	13678.11a
I <sub>2</sub>	663.94b	288.83	11802.04b
I <sub>3</sub>	590.67c	308.04	11031.24b
I <sub>4</sub>	752.06a	302.51	14305.38a
<i>Variety</i>			
704	712.50a	287.04b	12626.17
666	652.72b	316.23a	12782.22
<i>CCC</i>			
control	661.50	295.11b	12427.77
0.4 (g L <sup>-1</sup> )	699.96	306.87a	13157.62
0.8 (g L <sup>-1</sup> )	686.37	302.93ab	12527.19

The means which have no letters are statistically non-significant at 5% probability level

### 1000-Grain weight

Effect of experimental varieties on 1000-grain weight was significant at 1% level (Table 5). Results of mean comparison indicated that the variety 666 had higher 1000-grain weight compared to variety 704 (Table 6). The above-mentioned trait is influenced by various environmental factors in addition to the genetic characteristics of the plant. This is probably due to the high yield of variety 666 in transmitting more photosynthetic material to grains during grain filling stage under which the heavier grains are produced. Despite the fact that the early-mature varieties have less number of grain and also the number of grains have the negative relationship with its weight, hybrid 666 as an early-mature variety has higher 1000-grain weight. Effect of Cycocel on 1000-grain weight was significant at 5% level according to the results of Table 5. Consumption of 0.4 g L<sup>-1</sup> of Cycocel had the greatest effect on the increase of 1000-grain weight. Consumption of 0.8 g L<sup>-1</sup> of Cycocel also was put in the next rank with production of 1000-grain weight and ultimately the lack of Cycocel consumption was put in the final rank (Table 4). Cycocel probably increases the 1000-grain weight through affecting the grain size; It also seems that the anti-transpiration materials play a role in filling the grains and increasing the weight of grains through transferring sufficient photosynthetic material to grains. Hashemzadeh (2006) and Kazempour and Tajbakhsh (2002), also reported similar results about the effect of Cycocel on grain weight. According to the results of analysis of variance in

Table 5, the interaction of irrigation and variety, irrigation and Cycocel and variety and Cycocel was significant at 5% level. With evaluation of mean comparisons in Figure 3., 4. and 5. on the one side and revisal the results in Table 6. about 1000-grain weight on the other side, we can explain the interaction effects of experimental factors. This result indicates the effectiveness of Cycocel in mitigating the effects of drought stress and also vulnerable of staling and tasseling stages in maize.

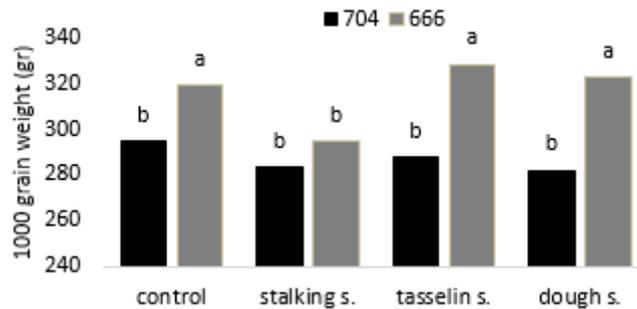


Figure 3: Mean comparison interaction of the irrigation and variety on 1000 grain weight

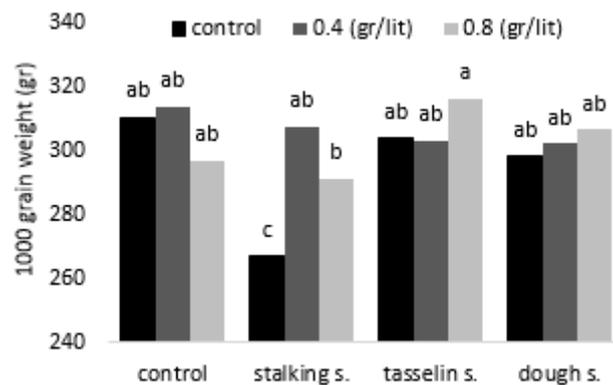


Figure 4: Mean comparison interaction of the irrigation and CCC on 1000 grain weight

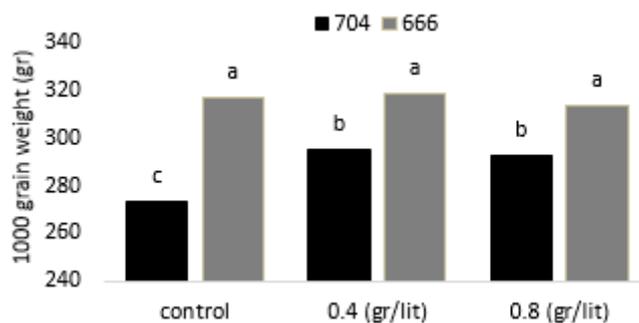


Figure 5: Mean comparison interaction of the variety and CCC on 1000 grain weight

### Grain yield

The effect of different irrigation types on grain yield was significant at 5% level based on analysis of variance in Table 5. According to the results of mean comparing (Table 6), the moisture stress treatment at dough stage of grain and control irrigation were put in the same experimental group with highest grain yield. Moisture stress treatments during tasseling and staling also had the lowest yield per unit area and were statistically put in the same group (Table 6). The grain yield increased due to enhanced consumed water through increased ear length and diameter, number of grains per ear, number of grain per row, number of grain per ear, and 1000-grain weight (Hashemzadeh, 2006). Numerous tests also reported the grain yield increase through the rate of consumed water and this is consistent with the findings of this experiment (Patrick et al., 2004; Nadvar et al., 2006). Since the reproductive parts are created at staling stage and also the tasseling stage is the closest time to the emergence of maize and grain, plant has the highest influence by stress at these two stages. Interaction of variety and Cycocel was

also significant at 1% level (Table 5). Thus, variety 666 led to the highest grain yield through applying Cycocel concentration equal to  $0.4\text{gL}^{-1}$  (Figure 6).

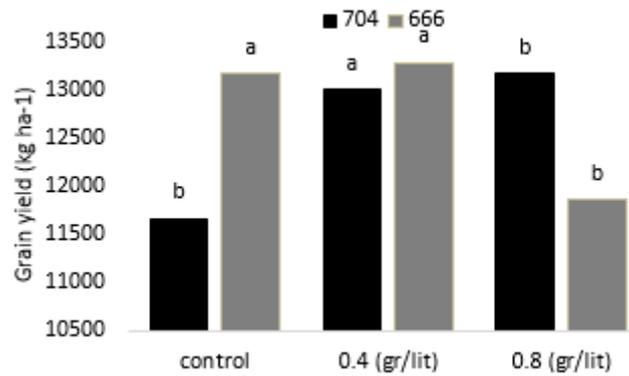


Figure 6: Mean comparison  $\text{kg ha}^{-1}$  of the variety and CCC on grain yield

### Conclusion

The obtained results in the current study indicate that the highest damage in moisture stress is made during tasseling and staking stages and this refers to the sensitivity of these two phases in plant growth process. Results of numerous studies by agricultural researchers in the field of drought stress indicate that the normal procedure of irrigation and not damaging to the plants due to moisture stress during the vegetative and reproductive stages will lead to the maximal and normal yield of plant. Not damaging at moisture stress treatment at dough stage is also because of reaching the later stages of plant growth and completion of plant vegetative and reproductive growth phase under which the plant enters the feedback phase (movement of stored materials to the grain and accumulation in grains). Despite the fact that the impact of seed pretreatment with Cycocel plant growth regulator was observed on various traits, investigating different resources similar to this experiment indicate that the foliar application of Cycocel was more efficient and led to better results.

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