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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

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

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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^{-1} 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^{-1} 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 Cycosel 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

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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 theworld 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 (Boyerand 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 hasaffected Iran (Jajarmi, 2009).

Maize (*Zea mays* L.) is one of the most developing dynamic cereal crops around the world.Maize production has been increasedapproximately 70% aroundthe world in the last fifteen years (Sandor, 2010). Soil drought stress, especially along withtoo high temperatures is the most common constraint on yield throughout the world (Beck et al., 1996). Maizeis sensitive to drought stress in some stages of growth andtheyield 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 effectsof 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). Maizegrain yield reduction,caused by drought, ranges from 10 to 76% depending on the severity and stage of occurrence (Bolaoos et al., 1993). Drought stress reduces the yield and some of its components (Ilkaei et al., 2010;Golbashy et al., 2010; Shiri et al., 2010; Khodarahmpour and Hamidi, 2012). Hashemzadeh (2009), indicatedthe significant effects ofdifferent irrigationtreatmentsatdifferent growthstagesof maizeon some traitssuch asear length, ear diameter, 1000-grain weight,andgrain 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 this 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 propertiesin 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). Cycocelplays the role in thickening thestemsand this enhances the plant stability in water stress tolerance (Farooq and Bano, 2006). Applying different concentration of Cycocel in different periods of plant growthhas 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 maizein spatial unit and consequently increased the rate of ultimate yield (Peltonen-Sainio and Rajala, 2001). Cycocel treatmentcaused asignificant increaseinmaizegrain yield (Ilkaei et al., 2010). Furthermore, application of Cycocelin maize increasedthe stem diameter, ear length, ear diameter, cobweight, 1000-grainweight, biological yield, grainyieldandharvest index (Hashemzadeh, 2009).

Material and Methods

This study was conducted at the fieldofAgriculture Research Station and Natural Resources in Khoy city in 2009. This areais located atlatitude 38°35' North and longitude 44° 57' East at an altitude of 1103 meters. According toKöppen climate classification, this area hassemi-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 wasloamy-clay with pH equal to 7.4 with electrical conductivity equal to 0.64dsm^{-1} (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 $kgha^{-1}$

of pure urea (1/3 during planting, 1/3 in stalking stage and 1/3 in flowering stage), 180 kg ha^{-1} phosphorus (during planting) and 25 kg ha^{-1} 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 inrandomized complete block design with three replications. In this test, irrigation was chosen as the main factor at four levels, maizevarieties 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 maizegrowth and theregular irrigation continued after stress. The first level (I_0)was considered as the stage without eliminating the irrigation until the end of plant growth stage after each 70±5 evaporationdetermined using daily evaporation rate of class Aevaporation pan (121 cm diameter and 25.4 depth with an anemometer)as thecontrol. The other levels (I_2, I_3&I_4) were considered as the stage for eliminatingone step of irrigation at the beginning of stalking, tasseling and dough stages, respectively, and regularirrigation continued after 140±5 mm evaporation from class A evaporation pan.All calculations of evaporation were performed by climatology experts fromKhoyweather station. Also, from planting to stalking stage and first moisture stress (I_2),sprinkler irrigation method was used and after that continued surface irrigation trough the furrows.Seeds,applied in experiment were thevar. KSC704 and KSC666. For Cycocel treatment, the seeds were put in distilled water (control) andprepared solutionsfrom0.4 and 0.8 gL^{-1} growth regulator obtained from Cycocelcrystal soluble in distilled water and ethanol as second and third levels, respectively,based on the type of treatmentfor 4 hours.After drying,the seeds were planted into the holes onridgesas stackor 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 preparedaccording to the results of soil analysis (Table 2) and it was appliedon the strip and 5cm lower than theseeds 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 handymethod of weeding and later the soil was added to the plant's foot. Traits were measured afterremoving the margins through randomized selection of eight plants from each plot and then obtained data was statisticallyanalyzed 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^{-1})	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 theshortest ear lengthwas related to themoisture stress treatment at stalking stage and also moisture stress treatment at tasseling stage (Table 4). It seems that the longer intervals between irrigations will lead to the reducedear 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 varietyon the ear length was statistically significant at1% level (Table 3). Given themean comparison, it was identified that the variety 666 produced long earcompared 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 earwas consequence of interaction between the control treatment and moisture stress atdough stage and variety 666 (Figure 1). This can be justified as the variety 666 has higherear length and also because theplant vegetative growth is not affected by the drought stress in treatments of control irrigation and moisture stressat 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
Irrigation				
I_1	20.92a	45.49a	15.50	46.94a
I_2	19.14b	45.21a	15.33	42.78b
I_3	19.27b	43.92b	14.94	39.28c
I_4	21.38a	45.87a	15.61	48.39a
Variety				
704	19.37b	45.62a	15.53a	45.86a
666	20.99a	44.63b	15.17b	42.83b
CCC				
control	19.89	44.62	15.17	43.50
0.4 (g L^{-1})	20.32	45.55	15.58	44.67
0.8 (g L^{-1})	20.32	45.20	15.29	44.87

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

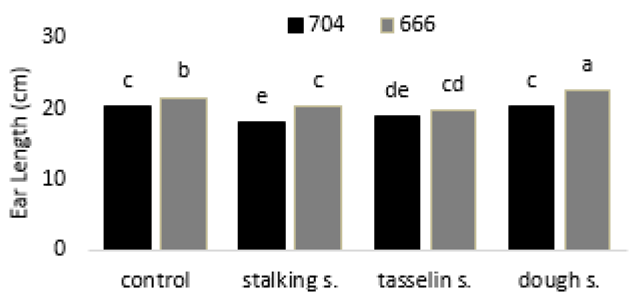

Figure 1: Mean comparison interaction of the irrigation and variety on ear length

Eardiameter

Effect of different irrigation treatments on the ear diameter was significant at5%level (Table 3). Accordingly, the results of meancomparison indicate that the moisture stresstreatment at tasseling stage had lowerear 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 diameterwere 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 indicatesthat the effect of variety on ear diameter is significant at 1% level (Table 3). The results of mean comparison suggest that themaizevariety 704 had higher diameter than the variety 666. Since the variety 704 is aserotinousvariety compared to 666, it seems that it has long period of growth and this enhances thecell division and dimensions of thisvariety and finally theear diameter through continuing process of photosynthesis. Moisture stress at tasseling stage led to thedisruption 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 stageproduced high Ear diameter and were put in the same statistical group (Figure 2).

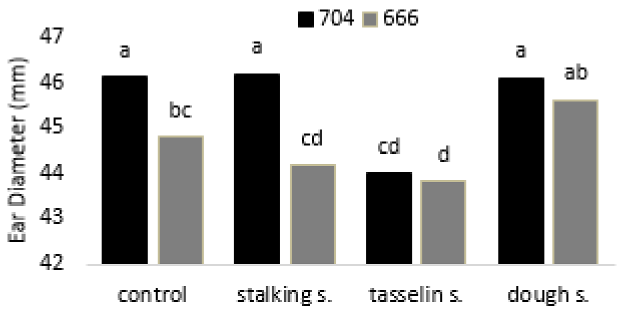

Figure 2: Mean comparison interaction of the irrigation and variety on ear diameter

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 thegrain number per row was statistically significant at 1% level (Table 3). Mean comparison in Table 4 showed the lowest grain numberinmoisture stress treatmentat tasseling stage and stalking stage, respectively. Occurrence of stressat the early vegetative stages and before differentiation ofmalereproductiveorgan is less effective than any stresses in the ear differentiation and growth as well aspollination. This was clearly observed inmoisture stress treatments at tasseling stage, so that most of produced earsin this treatment had heterogeneousappearance and irregular rows. Results of this test are consistent with the results by Karimian et al. (2005) and Khodarahmpouret al. (2012). According to theanalysis of variance in Table 3, the effect of variety on the number of grains per row was significant at 1% level. Conducted mean comparisonin Table 4 indicates that thevariety 704 has more grains per row compared tovariety 666 and this is probablydue to the genetic characteristics of plant.

Grain number per ear

Impact of different irrigation types on the number ofgrain 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 grainin ear as the result of drought stress. As previously explained, the grain production deficiencyat tasseling stage is due to the damage to the system of production and eventually the plant pollination. According to the results of analysis of variancein table 3, theeffect of variety wassignificant on the number of grains per ear at 1%level and thevariety 704 hadgreater 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^{-1})
Irrigation			
I_1	723.78a	307.17	13678.11a
I_2	663.94b	288.83	11802.04b
I_3	590.67c	308.04	11031.24b
I_4	752.06a	302.51	14305.38a
Variety			
704	712.50a	287.04b	12626.17
666	652.72b	316.23a	12782.22
CCC			
control	661.50	295.11b	12427.77
0.4 (g L^{-1})	699.96	306.87a	13157.62
0.8 (g L^{-1})	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-mentionedtrait is influenced by various environmental factors in addition to thegenetic characteristics of the plant. This is probably due to the high yield of variety 666 in transmitting morePhotosynthetic materialsto grains during grain filling stageunder which theheavier grains are produced.Despite the fact that theearly-mature varieties have less number of grainand also the number of grainshave thenegative relationship with itsweight,hybrid 666 as anearly-mature variety has higher 1000-grainweight. Effect of Cycocel on 1000-grain weight wassignificant at5% level according to the results of Table 5. Consumption of 0.4 g L^{-1}ofCycocelled to the greatest effect on the increase of 1000-grain weight. Consumption of 0.8 g L^{-1}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 probablyincreases the1000-grain weight through affecting the grain size; It also seems that the anti-transpiration materials play role in filling the grains and increasing the weight ofgrainsthrough transferringsufficient photosynthetic materialsto 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 Cycocelwas significant at 5% level. With evaluation of mean comparisonsin Figure3., 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 stalking and tasseling stages in maize.

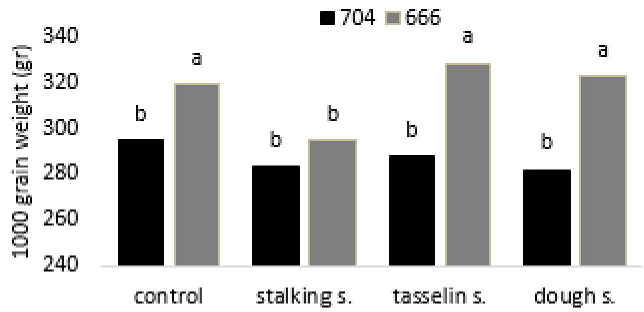

Figure 3: Mean comparison interaction of the irrigation and variety on 1000 grain weight

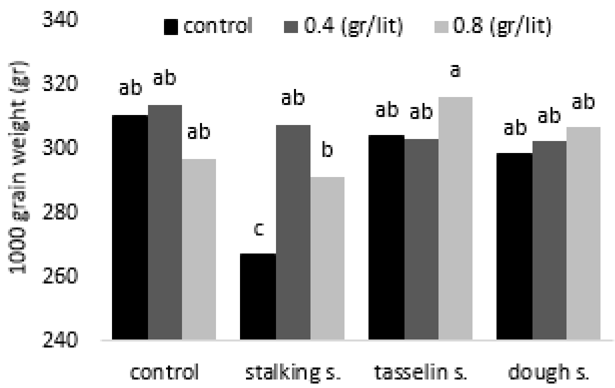

Figure 4: Mean comparison interaction of the irrigation and CCC on 1000 grain weight

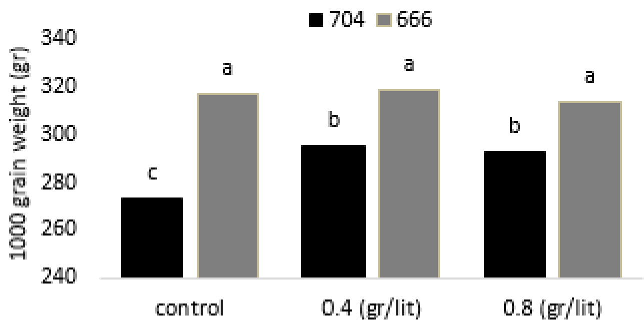

Figure 5: Mean comparison interaction of the variety and CCC on 1000 grain weight

Grainyield

The effect of different irrigation types on grainyield was significant at5% level based on analysis of variancein Table5. According to the results of mean comparing (Table 6), themoisture stress treatment atdough stage of grain and controlirrigation were put in the same experimental group withhighest grain yield. Moisture stress treatments during tasseling and stalkingalso had the lowest yield per unit area and were statistically put inthe same group (Table 6). The grain yieldis increased due to enhanced consumed water throughincreased 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 alsoreported 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 atstalking stage and also thetasseling stage is the closest time to the emergence of maize and grain, plant has thehighest influence by stressat 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 0.4gL^{-1} (Figure 6).

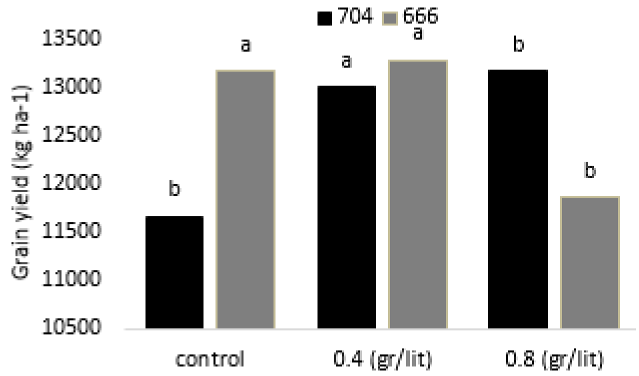

Figure 6: Mean comparison kg ha^{-1}of the variety and CCC on grain yield

Conclusion

The obtained results in the current studyindicate that the highest damage in moisture stress is made during tasseling and stalking stages and this refers to the sensitivity of these twophases in plant growth process. Results of numerousstudies byagricultural researchers in the field of drought stress indicate that the normal procedure of irrigation and not damaging to the plants due to themoisture stress during the vegetative and reproductive stages will lead to the maximal and normal yield of plant. Not damaging at themoisture stress treatmentat 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 grainand accumulation in grains). Despite the fact that the impact of seed pretreatment with Cycocel plant growth regulatorwas observed on various traits, investigating different resources similarto this experiment indicate that the foliar application of Cycocel was more efficient and led to better results.

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