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# EFFECTS OF IRRIGATION REGIME, FOLIAR APPLICATION OF CHLORMEQUAT CHLORIDE ON GRAIN YIELD OF TWO GRAIN SORGHUM (Sorghum bicolor (L.) Moench) CULTIVARS

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ABSTRACT

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A field experiment was conducted at Agricultural Research and Natural Resources Station, Chabahar, Iran, to evaluate the effects of irrigation regime and foliar application of chlormequat chloride (CCC; 2-chloroethyl-trimethylammonium chloride) on grain yield of two grain sorghum (Sorghum bicolor (L.) Moench) cultivars. The layout of the experiment was a split-split-plot, where the whole plot portion was a randomized complete block design with three replicates. Main plots were the irrigation regime (irrigation after 50 (control), 100, 150, and 200 mm evaporation from evaporation pan), subplots were foliar application of chlormequat chloride (0 and 1370 mg l<sup>-1</sup>), and sub-sub plots were two sorghum cultivars ('Sistan' landrace and 'Payam' cultivar). The results showed that delay in irrigation led to a significant reduction in grain yield. The highest grain yield (3393.3 kg ha<sup>-1</sup>) was observed in plots irrigated after 50 mm evaporation from evaporation pan, while the lowest one (2841.5 kg ha<sup>-1</sup>) was recorded in plots irrigated after 200 mm evaporation from evaporation pan. For both cultivars, foliar application of CCC significantly increased grain yield, however, the increases in grain yield for 'Payam' cultivar was much greater than 'Sistan' landrace. Grain number per panicle was significantly higher at IR50 and IR100 than at IR150 and IR200 irrigation regimes. The results also showed that delay in irrigation led to a significant reduction in the thousand grain weight. The highest and the lowest 1000-grain weights were recorded for plots irrigated after 50 and 200 mm evaporation from evaporation pan, respectively. Thousand grain weight was significantly increased after foliar application of CCC. Moreover, 'Payam' cultivar had significantly greater 1000-grain weight than 'Sistan' landrace.

Key words: drought stress, grain yield, plant growth retardant, sorghum cultivars

# INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop grown primarily in arid and semi-arid regions in the world because of its drought tolerance (Rosenow *et al.* 1983) and its performance in marginal lands with low fertility. World wide, sorghum is produced on 42.1 million ha, with an estimated total production of 61.3 million tons in 2013 (FAO 2013). Sorghum grain has a high nutritive value, with 70-80% carbohydrate, 11-13% protein (free of gluten), 2-5% fat, and 1-2% ash. In southern Iran, sorghum is usually cultivated with limited irrigation. Sorghum has C4 type of photosynthetic pathway which allows it to be more efficient to water, radiation, and nutrient use, thus allowing it to survive in harsh climatic conditions. Drought tolerance of sorghum plants may be attributed to well developed and finely branched root system, limited transpiration because of small leaf area per plant, lower transpiration ratio compared to many cereals, corky and waxy epidermis which protects the plant form desiccation (Robinson *et al.* 1977). Moreover, sorghum has the ability to remain in a virtually dormant stage during drought stress and resume growth as soon as conditions become favorable. Under limited irrigation, achieving greater yield is one of the most important goals in arid and semi-arid regions.

Chlormequat chloride (CCC; 2-chloroethyl-trimethyl-ammonium chloride) was first described by Tolbert in 1960 as a plant growth regulator on wheat (Tolbert 1960). It also used to reduce the risk of lodging, and to increase yields of cereals (wheat, rye, oats, and barley), and to alleviate the adverse effects of drought stress in plants. It inhibits the cyclization of trans-geranylgeranyl-pyrophosphate, which leads to a low concentration of gibberellic acid inside the plant (Jung 1984), and thereby plant height is reduced by slowing both cell division and elongation. It has been reported that exogenous application of CCC resulted in increased grain yield in barley (Ma and Smith, 1991), and rice (Akinrinde 2006). Rademacher and Brahm (2012) stated that foliar application of CCC reduced the leaf area, while increased the chlorophyll content, leaves thickness and root growth. The beneficial effects of CCC is attributed to improving stomatal regulation, maintaining leaf chlorophyll content, increasing water use efficiency, increasing the production of the various antioxidant enzymes, and stimulating root growth.

This experiment was conducted to evaluate the response of two grain sorghum cultivars, 'Sistan' landreac and 'Payam', to different irrigation regimes and foliar application of chlormequat chloride.

# MATERIALS AND METHODS

A field experiment was conducted at Agricultural Research and Natural Resources Station, Chabahar, Iran, in 2013. Soil properties of the experimental field were 13% clay, 69% silt, 18% sand, 0.29% total nitrogen content, 7.4 mg kg<sup>-1</sup> phosphorus, 45 mg kg<sup>-1</sup> exchangeable potassium and 7.1 pH. The layout of the experiment was a split-split-plot, where the whole plot portion was a randomized complete block design with three replicates. Main plots were the irrigation regimes [irrigation after 50 (control), 100, 150, and 200 mm evaporation from

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evaporation pan], subplots were foliar application of chlormequat chloride (0 and 1370 mg l<sup>-1</sup>), and sub-sub plots were two sorghum cultivars ('Sistan' landrace and 'Payam' cultivar).

For both cultivars, seeds were sown on 5 September in five 3-m rows spaced 60 cm apart at a density of 16.6 seeds  $m^{-2}$  (30 seeds in each row). Fertilizer was applied as per recommended doses i.e. 90 kg N ha<sup>-1</sup> (as urea), 25 kg P ha<sup>-1</sup> (as triple superphosphate), and 70 kg K ha<sup>-1</sup> (as potassium sulphate). 1/2 of N and total P and K were applied as basal, just before final land preparation. The rest amount of N was top dressed 25 days after planting. Weeds were controlled manually during the experiment. 'Sistan' landrace and 'Payam' cultivar were harvested at 74 and 82 days after sowing, respectively.

Plant height was measured from the soil surface to the tip of the panicle at harvest stage. Grain yield was determined by hand-harvesting the sorghum plants from 2.5 m<sup>2</sup> per plot at the hard dough stage and was adjusted to 120 g kg<sup>-1</sup> grain moisture content. Ten randomly selected plants were harvested from each plot at ground level for measuring number of grains per panicle, and 1000-grain weight. For measuring biological yield, shoot biomass from each plot was dried at  $70^{\circ}$ C for 96 h, and weighted.

Leaf chlorophyll index was measured using Minolta's hand-held SPAD-502 meter (Minolta Corp., Ramsey, NJ). Twenty plants were chosen at random in each plot, and at each plant three readings were taken from the uppermost fully expanded leaf.

Analyses of variance were conducted using SAS procedures (SAS Institute 2004) and means were compared using Fisher's protected LSD test at 5% probability level.

## **RESULTS AND DISCUSSION**

## Plant height

Plant height was significantly influenced by irrigation regime (IR), foliar application of chlormequat chloride (CCC), and cultivar (V) (Table 1). Plant height was significantly higher at IR50 and IR100 compared to IR150 and IR200 when averaged across chlormequat chloride treatments and sorghum cultivars (Table 2). Moreover, IR  $\times$  CCC and IR  $\times$  V interactions were significant, while V  $\times$  CCC and IR  $\times$  CCC  $\times$  V interactions were not significant (Table 1). This indicates that 'Sistan' landrace and 'Payam' had different response to irrigation regime in terms of plant height. At IR50 and IR100, 'Sistan' landrace was taller than 'Payam', while there were no significant differences in plant height between 'Sistan' landrace and 'Payam' cultivar at IR150 and IR200 irrigation regimes (Fig. 1). Regardless of sorghum cultivar, foliar application of CCC reduced significantly plant height at IR50 and IR100, while no significant differences were observed for plant height between CCC-treated and -untreated plants at IR50 and IR100 (Fig. 2). The reduction in cell division and elongation due to CCC, a plant growth retardant, foliar application reduces plant height. This result agrees with those obtained by Ma and Smith (1991) in barley, and rice (Akinrinde 2006).

Table 1. Mean squares of ANOVA for plant height (H), seed yield (Y), grain number per panicle (GN), 1000grain weight (ThGW), biological yield (BY), SPAD readings as affected by irrigation regime (IR), foliar application of chlormequat chloride (CCC), and cultivar (V)

S.O.V	df	Н	Y	GN	ThGW	BY	SPAD readings
R	2	25 <sup>ns</sup>	78365 <sup>ns</sup>	4094**	0.1 <sup>ns</sup>	39134586 <sup>ns</sup>	43.3 <sup>ns</sup>
Irrigation Regime (IR)	3	339**	$661790^{**}$	$1441^{**}$	13.6**	965279 <sup>ns</sup>	95.5 <sup>*</sup>
Error (a)	6	36	152406	193	0.9	16598783	17.8
Chlormequat chloride (CCC)	1	$79^*$	213520 <sup>ns</sup>	188 <sup>ns</sup>	24.3 **	$1688625^{**}$	$26.0^{**}$
$IR \times CCC$	3	$68^{*}$	35244 <sup>ns</sup>	69 <sup>ns</sup>	0.24 <sup>ns</sup>	13444 <sup>ns</sup>	6.3 <sup>ns</sup>
Error (b)	8	14	72954	499	0.68	154915	2.0
Cultivar (V)	1	$109^{**}$	1222 <sup>ns</sup>	3500 <sup>ns</sup>	$17.5^{**}$	3312226**	0.9 <sup>ns</sup>
$IR \times V$	3	$41^{*}$	26406 <sup>ns</sup>	$36^{ns}$	$0.4^{ns}$	158782 <sup>ns</sup>	$1.2^{ns}$
$CCC \times V$	1	$4^{ns}$	$813437^{*}$	356 <sup>ns</sup>	1.9 *	284438 <sup>ns</sup>	$0.2^{ns}$
$IR \times CCC \times V$	3	$1^{ns}$	55893 <sup>ns</sup>	97	0.3 <sup>ns</sup>	87567 <sup>ns</sup>	0.9 <sup>ns</sup>
Error	16	10	90759	1339	0.3	140139	1.1
CV (%)	-	5	10	5	3	9	3

\*, \*\* represent significance at 0.05 and 0.01 probability level, respectively.

ns represents no significant difference

### Grain yield

Analysis of variance showed that only the main effect of irrigation regime (IR) was significant for sorghum grain yield (Table 1). Seed yield was significantly greater for IR50 (3393 kg ha<sup>-1</sup>) and IR100 (3239 kg ha<sup>-1</sup>) than for IR50 (2791 kg ha<sup>-1</sup>) and IR100 (2741 kg ha<sup>-1</sup>) as averaged across CCC applications and sorghum cultivars (Table 2). Photosynthesizing of higher plants are seriously subjected to oxygen toxicity under drought stress, resulting in a drastic decline in the levels of  $CO_2/NADP$  and increased transfer of electrons to oxygen, leading to

the formation of the superoxide radical. The superoxide radical and hydrogen peroxide can directly attack membrane lipids and inactivate SH-containing enzymes (Navari-Izzo et al. 1994). In the presence of trace amounts of iron salts, the combination of superoxide radical and hydrogen peroxide lead rapidly to the formation of the hydroxyl radical (Halliwell 1987). The hydroxyl radical damages DNA, proteins, lipids, chlorophyll and almost every other organic constituent of the living cell (Becana et al. 1998). It has been reported that stomatal conductance of  $C_4$  plants decreases with declining leaf water status, which in turn reduces photosynthetic rates (Ghannoum et al. 2003; Carmo-Silva et al. 2008). Also, Chlorophyll level is an important factor in photosynthesis capacity of plants (Jiang and Huang, 2001). It appears that the loss of chlorophyll under drought stress is brought about by the increase in production of oxygen radicals which causes peroxidation (De La Luz, 2004) and decomposition of these pigments (Vorasoot et al. 2001). Ranibarfordoei et al. (2002) stated that drought stress also reduces plant photosynthesis by degrading chlorophyll pigment. At the same time, for  $C_4$ plants, nitrate assimilation and nitrate uptake are considerably reduced under drought stress (Foyer et al. 1998). This may explain the decreases in chlorophyll and protein content in a number of  $C_4$  species subjected to water stress (Fover et al. 1998; Carmo-Silva et al. 2008). The decrease in chlorophyll and protein contents under water stress may also be due to generalized protein degradation as a result of induced senescence as suggested by increased contents of amino acids (Becker and Fock, 1986). Analysis of variance (Table 1) also showed that the interaction between foliar application of chlormequat chloride (CCC) and cultivar (V) was significant, indicating that sorghum cultivars had different response to foliar application of CCC in terms of grain production. Other 2- and 3-way interactions were not significant for sorghum grain yield (Table 1). For both cultivars, grain yield was significantly increased after CCC foliar application when averaged across irrigation regimes. However, the increases in grain yield for 'Payam' cultivar (14%) was greater than that for 'Sistan' landrace (9%) after CCC foliar application (Fig. 3). These results may be attributed to the promoting effect of CCC on numerous physiological processes, leading to improvement of all yield components. CCC has also been associated with increased photosynthesis in study by Nepomuceno et al. (1997), through increased total chlorophyll concentration of plant leaves. De et al. (1982) reported that application of CCC on wheat plants promote root growth under arid conditions, resulting in more efficient water absorption from the deeper layers of soil, which in turn increased grain yield (Emam and Moaied, 2000). At the same time, yield enhancement for wheat cultivars after CCC foliar application was attributed to the increase in leaf area (Miranzadeh et al. 2011), photosynthesis rate, and photo-assimilate partitioning to the grain (Ma and Smith, 1991; Emam and Karimi, 1996) of the CCC-treated plants.

## Grain number per panicle

The main effect of irrigation regime (IR) was significant for grain number per panicle, while foliar application of chlormequat chloride (CCC), and cultivar (V) main effects were not significant (Table 1). Moreover, all 2and 3-way interactions were not significant. Grain number per panicle was significantly higher at IR50 and IR100 than at IR150 and IR200 (Table 2). Water deficits during anthesis can cause floret abortion in the lower branches of the panicle, thus reduces grain number per panicle. Moreover, the reduction in grain number per panicle under drought stress may be due to pollen sterility, which in turn reduces grain number per panicle. This finding is similar to the results of Manjarrez-sandoval *et al.* (1989), who reported that number of grains per mature panicle was reduced by 25–55% under drought stress.

Table 2.	Plant	height (	(H), s	eed yiel	d (Y), gr	ain nur	nber per pa	anic	ele (GN), 10	000-grai	n weig	ht (Th	GW), biolog	ical
	yield	(BY),	and	SPAD	reading	(SR),	response	to	irrigation	regime	(IR),	foliar	application	of
	chlori	mequat	chlor	ide (CC	C), and c	ultivar	(V)							

Traits Factors	H (cm)	Y (kg ha <sup>-1</sup> )	GN panicle <sup>-1</sup>	ThGW (g)	BY (kg ha <sup>-1</sup> )	SR
Irrigation regime						
IR 50	96	3393	781	21.38	17688	46.7
IR 100	91	3239	772	20.08	17556	44.0
IR 150	84	2791	721	19.66	17483	43.0
IR 200	84	2741	715	18.82	17034	39.8
LSD (0.05)	6	389	43	0.99	4609	4.2
Chlormequat chloride						
Application	88	3207	770	20.7	17252	44.1
No application	91	3074	766	19.2	17627	42.6
LSD (0.05)	2	179	14	0.5	262	0.9
Cultivar						
'Sistan' landrace	91	3146	777	20.5	17703	43.2
'Payam'	88	3136	760	19.3	17177	43.5
LSD (0.05)	2	184	22	0.3	229	0.6

IR 50, IR 100, IR 150, and IR 200 indicate irrigation after 50, 100, 150, and 200 mm evaporation from evaporation pan



Fig. 1. Irrigation regime  $\times$  cultivar interaction effect on plant height as average across foliar application of chlormequat chloride. Vertical bars represent  $\pm 1$  SE of means



Fig. 2. Irrigation regime  $\times$  chlormequat chloride (CCC) interaction effect on plant height as average across sorghum cultivars. Vertical bars represent  $\pm 1$  SE of means

# Thousand grain weight

Irrigation regime (IR), foliar application of chlormequat chloride (CCC), and cultivar (V) had significant effect on 1000-grain weight (Table 1). Regardless of CCC and sorghum cultivar, the highest (21.38 g) and the lowest (18.82 g) 1000-grain weights were recorded for plots irrigated after 50 and 200 mm evaporation from evaporation pan, respectively (Table 2). The reduction in photosynthesis during grain filling period due to water deficit reduces grain size and weight. Moreover, drought stress accelerates panicle development and, therefore, shortens grain filling period. A shorter grain filling period is associated with smaller grains. Manjarrez-sandoval *et al.* (1989) reported that late drought stress reduced individual grain weight in sorghum by as much 50%. A significant CCC × V interaction ( $P \le 0.05$ ) was found for thousand grain weight, while other 2- and 3-way interaction were not significant (Table 1). This indicates that sorghum cultivars had different response to foliar application of CCC in terms of 1000-grain weight. For 'Sistan' landrace, 1000-grain weight increased form 20.0 g to 21.3 g (6.5%) due to foliar application of CCC as averaged across irrigation regimes, while 1000-grain weight for 'Payam' cultivar increased from 18.8 to 19.6 (4%) due to foliar application of CCC as averaged across irrigation regimes (Fig. 4).

# **Biological yield**

There were significant effects of foliar application of chlormequat chloride (CCC), and cultivar (V) on biological yield. At the same time, all 2- and 3-way interactions were not significant (Table 1). Regardless of irrigation regime and cultivar, untreated plants produced greater biomass compared to CCC-treated plants (Table 2). CCC is a plant growth retardant which reduces cell devising and elongation. Therefore, control (untreated) plants produced greater biomass compared to CCC treatments and irrigation regimes, 'Sistan' landrace produced grater biomass compared to 'Payam' cultivar (Table 2).







## Sorghum cultivar

Fig. 4. Cultivar  $\times$  chlormequat chloride (CCC) interaction effect on 1000-grain weight as average across sorghum irrigation regimes. Vertical bars represent  $\pm 1$  SE of means

## SPAD reading

SPAD reading significantly ( $P \le 0.05$ ) affected by irrigation regime (IR), and foliar application of chlormequat chloride (CCC). All 2- and 3-way interactions were not significant (Table 1). Averaged across foliar application of chlormequat chloride treatments and sorghum cultivars, the highest (46.6) and the lowest (39.8) SPAD readings were recorded for plots irrigated after 50 and 200 mm evaporation from evaporation pan, respectively (Table 2). A positive correlation between SPAD readings and leaf chlorophyll concentration was repeatedly reported by researchers for many crops (Monje and Bugbee, 1992; Samdur *et al.* 2000; Akkasaeng *et al.* 2003; Uddling *et al.* 2007; Ling *et al.* 2011). So this result can be attributed to chlorophyll degradation under water deficit. Contrary to this result, Barraclough and Kyte (2001) reported that critical SPAD values for wheat were 53 and 44 at low and high water supply, respectively. Regardless of irrigation regime and cultivar, CCC-treated plants had greater SPAD reading compared to untreated plants (Table 2). This result can be attributed to increased chlorophyll content and leaves thickness in CCC treated plants. Similar result was reported by (Rademacher and Brahm, 2012).

# CONCLUSION

The experiment illustrated that grain yield reduced by 16% when plots irrigated after 200 mm evaporation from evaporation pan compared to when plots irrigated after 50 mm evaporation from evaporation pan. Regardless of irrigation regime, grain yield of both cultivars was significantly increased with CCC foliar application, however the increases in grain yield for 'Payam' cultivar was much greater than 'Sistan' landrace. Moreover, there was no significant difference in grain yield between 'Payam' cultivar and 'Sistan' landrace as averaged across irrigation regimes and CCC foliar application.

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