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Effect of animal manure and superabsorbent polymer on corn leaf relative water content, cell membrane stability and leaf chlorophyll content under dry condition

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Abstract

The present research was carried out to study effects of animal manure and superabsorbent polymer on leaf relative water content, cell membrane stability and leaf chlorophyll content (SPAD) of corn under drought stress in Fars Agricultural Research Station (Iran). The experimental design was split plot based on randomized complete blocks (RCBD) with three replicates. Water stress was applied by three different irrigation intervals (irrigation after 70, 105 and 140 mm evaporation of basin class A) which were allocated to main plots. A combination of six levels of animal manure and superabsorbent polymer (types: superab A200) allocated to subplots as follows: S1: control, S2: 100% animal manure (40 t/ha), S3: 100% super absorbent polymer (200 kg/ha), S4: 50% animal manure+50% superabsorbent polymer, S5: 35% animal manure+65% superabsorbent polymer, S6: 65% animal manure+35% superabsorbent polymer. Results showed that the highest leaf relative water content (RWC) was obtained with 100% superabsorbent polymer (200 kg/ha), and it reduced by increasing drought stress. Cell membrane stability (CMS) increased with increasing drought stress and decreased by using animal manure and superabsorbent polymer. Leaf chlorophyll index (SPAD values) increased in response to drought stress and by using different combinations of animal manure and superabsorbent polymer. 1000-seed weight and grain yield was decreased by drought stress. Grain yield decreased by yield components reduction, especially 1000-seed weight due to drought stress. 1000-seed weight and grain and biological yield increased by using animal manure and superabsorbent polymer together as maximum yield grain was obtained by using 65% animal manure and 35% superabsorbent polymer.

Keywords: Biological yield; corn (*zea mays)*; drought stress; leaf chlorophyll content; superabsorbent polymer
Abbreviations: RWC - Relative Water Content**;** CMS - Cell Membrane Stability; SPAD - Leaf Chlorophyll Index; EC – Electrical Conductivity

Introduction

Corn (*Zea mays* L.) is an important plant that is used as human food (20-25%), livestock and poultry feed (60-75%) and as raw material in industry (5%) (Emam, 2004). Corn is one of the best plants for producing green forage, silage forages and grain due to its high content of sugar and starch, as well as it yields more than 80 tone forage per hectares (Tajbakhsh and Pour Mirza, 2003), on the other hand, in Iran we have shortage in producing animal feeds, so, corn production is very important in Iran. The dearth of water is one of the important factors for corn production. Plant will experience water stress if transpiration is very high and available water decreases for roots (Ober and Sharp, 2003). The use of organic fertilizers including animal fertilizers and superabsorbent polymer is very effective in reducing drought stress effect and to improve the plants yield and stability in agriculture production. Plant reaction to drought stress is dependent on the intensity of water shortage. Plants have a short term or long term physiological respond to the drought stress. Changes in leaf relative water content and leaf chlorophyll content is a short term reaction to stress and it could be source adapting in drought stress condition (Ahmadi and Ceiocemardeh, 2004). Cell membrane stability is a source indicator of plant resistance to environmental stresses (Saneoka et al., 2004). Puor mousavi et al., (2007) and Costa-Franca et al., (2000) reported that leaf relative content in soybean and bean reduced by drought stress. Saneoka et al., (2004) in a research on *Agrostis palustris* reported that leaf osmotic potential reducing by nitrogen nutrition as a result of leaf water relative content increased. Also potassium nutrition increased leaf water relative under drought stress condition (Azizi and Rashed Mohasel, 1998). Leaf chlorophyll content is an important factor in determination of photosynthesis rates and dry mater production (Ghosh et al., 2004). Chlorophyll A in wheat decreased in heading and 20 days after anthesis and chlorophyll B decreased in heading stage only in drought stress

Table 1. Soil, superabsorbent polymer and animal manure analysis result for physical and chemical characteristics

Soil								
Characteristic	Soil depth (cm)	Soil texture	OC (%)	EC (ds/m^{-1})	PH	P (ppm)	K (ppm)	N (%)
Value	0-30	loamy	1.54	0.68	8.43	6.5	400	0.15
Superabsorbent polymer (types: superab A200)								
Color	Humidity (%)		Toxics	Density (g/cm^3)	PH	Water soluble	Dimension (micrometer)	
White	3-5		No	1.5	6-7	No	50-150	
Animal manure								
Humidity (%)	P (%)	K (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)	
23	0.57	1.43	0.81	12588	119	480	19	

Table 2. Effect of animal manure and superabsorbent polymer in drought stress on RWC in nine stage sampling[1]

S.O.V	df	RAW 1[1]	RAW 2	RAW 3	RAW 4	RAW 5	RAW 6	RAW 7	RAW 8	RAW 9
Replication	2	0.71ns[2]	0.24 ns	4.46 ns	2.95 ns	2.64 ns	33.38 ns	32.42 ns	36.97 ns	0.45 ns
Irrigation (A)	2	107.4*	40.30 ns	97.17*	40.73 ns	43.02 ns	83.91*	96.54*	107.36*	41.17 ns
Error (A)	4	55.16 ns	106.73 ns	48.40 ns	103.99 ns	109.62 ns	46.61 ns	59.74 ns	76.92 ns	107.45 ns
Animal manure and superabsorbent polymer	5	30.01 ns	36.90 ns	36.67 ns	36.50 ns	36.16 ns	34.88 ns	36.25 ns	36.66 ns	35.07 ns
Irrigation × Animal manure and superabsorbent polymer	10	18.72 ns	19.21 ns	19.67 ns	19.57 ns	18.84 ns	19.29 ns	18.60 ns	19.13 ns	18.43 ns
Error (B)	30	26.38 ns	27.49 ns	27.45 ns	27.32 ns	26.96 ns	26.21 ns	26.27 ns	30.63 ns	26.92 ns
CV (%)		5.81	6.26	6.31	6.31	6.18	6.16	6.08	6.52	6.17

1- Sampling after 328, 473, 625, 757, 895, 1028, 1134, 1254, 1371 Growth Degree Day (GDD), respectively

2- Ns= Non significant, and * = $p<0.05$

condition (Ahmadi and Ceiocemardeh, 2004). Ommen, and Donnelly (1999) reported that chlorophyll content in wheat increased in flag leaf by drought stress in anthesis stage in comparison with non stress condition. The result of some researches showed that cell wall was destroyed by increasing fat content in cell wall in drought stress (Macarrone et al., 1995; Hieng et al., 2004). Blum and Ebercon 1981 found that cell wall in wheat has been resistance in drought stress condition. Cell membrane stability increased by drought stress in comparison with nitrogen fertilizer in bent plant; however cell membrane stability decreased by without application of nitrogen fertilizer in drought stress condition (Saneoka et al., 2004). Ouattar et al., (1997) reported yield reduction in corn by drought stress due to decrease grain weight by lessen during grain filling. Tollenaar and Lee (2002) believed that the most drought stress effect on during grain filling was on grain weight that grain weight decreased by drought stress. Plants light absorption decreased by leaf area reduction due to drought stress (Ghosh et al., 2004). This research carried out to study the effect of animal manure and superabsorbent polymer on leaf relative water content, cell membrane stability and leaf chlorophyll content of corn under drought stress condition.

Materials and methods

Description of the Project Site

The experiment was conducted in 2004 at Zarghan Agricultural and Natural Resources Research Station in Fars province (in Iran). Experimental design was a split plot arrangement based on randomized complete blocks with three replicates. Physical and chemical properties of soil in experimental field were presented in Table 1. Main plots were included drought levels as irrigation after 70, 105 and 140 mm evaporation from basin class A, and a combination of six levels of animal manure and superabsorbent polymer at S1: control (without application of animal manure and superabsorbent polymer), S2: 100% animal manure (40 t/ha), S3: 100% superabsorbent polymer (200 kg/ha), S4: 50% animal manure + 50% superabsorbent polymer, S5: 35% animal manure + 65% superabsorbent polymer, S6: 65% animal manure + 35% superabsorbent polymer were assigned to subplots of 10 × 3m (physical and chemical characteristic of superabsorbent polymer (types: superab A200) and animal manure were presented in Table 1). Each plot was consisted of four rows and to prevent water leakage, there was 2m distance between main plots and 1m between subplots. Animal manure and superabsorbent polymer were added in plots before sowing handy. Method of irrigation was siphons; for all treatments irrigation was the same until growing third leaves and irrigation treatments carried out after this stage.

Crop Sampling and Calculation

Leaf relative water content was obtained by following formula (Dopte and Manuel 2002):

$$RWC\ \% = \frac{FW - DW}{TW - DW} \times 100$$

FW = fresh weight
DW = dry weight
TW = total weight
Sampling of youth and developed leaves was conducted nine times after water stress in six leaves and early of physiological maturity. Poly ethylene glycol solution (PEG) was used for

Table 3. Effects of animal manure and superabsorbent polymer in drought stress on leaf RWC during nine stages of sampling. Comparison of means was done using Duncan Multiple Range Test

Irrigation	animal manure and superabsorbent polymer	RWC 1	RWC 2	RWC 3	RWC 4	RWC 5	RWC 6	RWC 7	RWC 8	RWC 9
70		89.9a[1]	85.3	84.7a	83.9	84.0	85.0a	86.0a	87.1a	87.9
105		88.5a	83.9	83.0a	82.2	81.3	82.2b	82.9b	83.5b	84.8
140		85.7b	82.4	80.5b	80.2	80.9	81.6b	82.5b	83.0b	83.7
LSD		3.14	2.88	2.93	3.20	3.02	3.34	3.09	3.12	3.53

Row means followed by the same letter are not significantly different at 0.05 probability level

Table 4. ANOVA of the effect of animal manure and superabsorbent polymer in drought stress on 1000-seed weight, grain and biological yield and cell membrane stability, leaf chlorophyll (SPAD) in flowering and ripening stage

SOV	df	Cell membrane stability (%)		Leaf chlorophyll index		yield		
		flowering	ripening	flowering	ripening	1000-seed weight	Grain yield	Biological yield
Replication	2	51.24ns	85.37ns	11.48ns	11.55ns	8.00 ns	0.34 ns	0.14ns
Irrigation (A)	2	94.56**	212.67**	164.53**	173.46**	4681.1**	8.82**	13.16**
Error (A)	4	8.24	11.18	11.06	12.39	11.7	1.20	0.69
Animal manure and superabsorbent polymer (B)	5	17.44**	21.52**	22.15**	11.64**	1207.2**	3.92**	9.46**
Irrigation × animal manure and superabsorbent polymer (A×B)	10	6.46*	7.24*	3.62**	4.02**	304.3**	1.11ns	3.15**
Error (B)	30	1.49	1.72	1.11	0.54	22.1	0.56	0.95
CV (%)		1.85	2.08	2.50	1.87	1.37	6.38	3.38

ns= Non significant, ** = p < 0.01 and * = p < 0.05

measuring cell membrane stability with following formulas (Saneoka et al., 2004).

$$\%\ Injury = \{1 - [\frac{1 - \frac{T_1}{T_2}}{1 - \frac{C_1}{C_2}}]\} \times 100$$

%Cell membrane stability (CMS) = 1- % Injury
T1 = sample EC before autoclaving
T2 = sample EC after autoclaving
C1 = control samples EC before autoclaving
C2 = control sample EC after autoclaving
Leaf chlorophyll at two stages (1- end of flowering stage and 2- eight days after first measuring) was measured by "SPAD 502" chlorophyll-meter system (Minolta Company). Measurement was carried out at grain formation stage, on three up leaves. The sensor was placed between leaves third terminal and within two centimeters of leaf margin. Grain and biological yield was measured on 4 square meters after physiological maturity stage. Data analysis was done by using SAS software. The ANOVA test was used to determine significant ($p \leq 0.05$) treatment effect and Duncan Multiple Range Test to determine significant difference between individual means.

Results and discussion

Leaf relative water content (RWC)

Result showed that leaf relative water content decreased by increasing drought intensity (Table 5). Reduction in leaf relative water content had a positive correlation with soil relative water content (Nautiyal et al., 2002). RWC decreased by increasing evapotranspiration in plant society and reducing root growth and activity (Tarumingkeng and Coto, 2003). Saneoka et al., (2004) reported that RWC in lenti decreased by drought stress in compare with non stress conditions.

Cell membrane stability

The percentage of cell membrane stability affected by drought stress in flowering and ripening stages, as far as it increased by increasing drought stress intensity (Table 4). Pour mousavi et al., (2007) and Saneoka et al., (2004) in separate studies obtained same result, however Covarrubias et al., (1995) and Hieng et al., (2004) in their studies reported different results. Maybe intensity of drought stress had a different effect on plant response. Cell membrane stability decreased by severe drought stress in short term, in other words in this condition destruction of cell wall increased, while plant has enough time to respond to stress in gradual drought stress in long term (Pour mousavi et al., 2007). In this research with regard to irrigation intervals it seems that plant could adjust with drought stress. The percentage of cell membrane stability decreased significantly by animal manure and superabsorbent polymer. Cell membrane stability percentage was reduced by superabsorbent polymer more than animal manure. In other words drought stress intensity decreased by animal manure and superabsorbent (Table 4 and 5). On the other hand cell membrane stability decreased by increasing leaf relative water content. The pressure inserter from inside the cell for cell growth provide by increasing relative water content, and ultimately cell membrane stability decreased by cell wall stretch. Soil physical condition and preserving water capacity in soil was improved by superabsorbent polymer and it cause that plant has been a less would to invest to increase the cell membrane stability. Also with the favorable conditions for the growth and development such as enough water and nutrients, plant has a less would for

Table 5. Mean values of animal manure and superabsorbent polymer in drought stress on 1000-seed weight, grain and biological yield and cell membrane stability, leaf chlorophyll (SPAD) in flowering and ripening stage

irrigation	Animal manure and superabsorbent polymer	Cell membrane stability (%)		Leaf chlorophyll index		yield		
		flowering	ripening	flowering	ripening	1000-seed weight (g)	Grain yield (ton/ha^{-1})	Biological yield (ton/ha^{-1})
70		80.63c[1]	76.23c	38.81c	35.96c	353.77a	12.43a	29.40a
04		81.93b	77.14b	43.24b	40.61b	339.23b	11.74b	29.14a
140		80.09a	82.59a	44.58a	41.84a	324.21c	11.03c	27.80b
LSD		0.48	0.58	0.72	0.50	3.20	0.51	0.66
	0:0	83.26a	79.42a	40.28c	38.04e	327.07c	10.77c	27.50d
	0:100	82.94a	79.29a	44.17a	41.18a	351.73ab	11.15bc	27.73cd
	100:0	80.95c	77.42c	40.51c	38.61de	355.11a	11.72ab	28.57bc
	50:50	82.46ab	78.65ab	42.43b	39.39c	343.23b	12.20a	29.73a
	65:35	82.01b	78.06bc	42.32b	39.28cd	348.82b	12.08a	29.21ab
	35:65	82.76a	79.08a	43.54a	40.32b	340.92bc	12.49a	29.96a
LSD		0.68	0.82	1.02	0.71	4.52	0.72	0.94

Row means followed by the same letter are not significantly different at 0.01 probability level

cell wall strength, and provided condition for cell wall growth (Saneoka et al., 2004). Cell membrane stability measuring in ripening stage had similar results (Table 5). Cell membrane stability significantly affected by interaction of irrigation, animal manure and superabsorbent polymer (Table 4). Result showed that cell membrane stability in flowering and ripening stages increased by drought stress (Table 6). Minimum cell membrane stability was obtained by applying complete superabsorbent polymer (79.49% and 75.16% in flowering and ripening stages respectively), and maximum cell membrane stability was obtained in treatment without applying animal manure and superabsorbent polymer (81.27% and 77.32% in flowering and ripening stages, respectively). Probably this phenomenon was due to high ability of superabsorbent polymer to absorb water and conserve it in the soil.

Leaf chlorophyll index (SPAD)

Leaf chlorophyll index (SPAD) was significantly influenced by drought stress, animal manure and superabsorbent polymer (Table4 and 5). In flowering and ripening stage maximum and minimum leaf chlorophylls were obtained by severe drought stress and irrigation treatments respectively (Table 5). Leaf area reduced by drought stress in this condition Plants for using of maximum sunlight increased the leaf chlorophyll; therefore plants could use of maximum sunlight with minimum leaf area (Heydari, 2007). Means comparison showed that leaf chlorophyll index increased by applying animal manure and superabsorbent polymer, and animal manure had high effect on leaf chlorophyll index compared to superabsorbent polymer. Maximum and minimum leaf chlorophyll index was obtained by applying complete animal manure and control (without applying animal manure and superabsorbent polymer) treatments respectively (Table 5). Leaf chlorophyll increased by applying animal manure (Table 5). Essential elements for plant and microorganisms in soil provided by application of animal manure could be the possible reasons, as soils pH was reduced by microorganism activity. In these soils solvability of microelements is high, and thereupon chlorophyll synthesis increases by increasing uptake of some elements such as Fe, Mn, Zn, and Mg (Fallah et al., 2007). Table 6 showed that leaf chlorophyll index (SPAD) increased by drought stress. Maximum leaf chlorophyll index was obtained by applying complete animal manure and minimum index was obtained at no animal manure and superabsorbent polymer treatment (Table 6). Movahedi dehnavi et al., (2004) also reported similar results.

1000-seed weight

1000-Seed weight significantly affected irrigation, animal manure and superabsorbent polymer treatments and interaction (Table 5). Comparison of means showed that 1000-seed weight decreased by drought stress (Table 6). Grain weight reduction can be because of reduced growth due to leaves relative water content reduction under drought stress. Grain weight reduction by drought stresses in Ouattar et al., (1997) research confirmation these findings. Use of animal manure and superabsorbent polymer increased grain weight in compare with control treatment, so that the highest grain weight (363.1 g) was obtained by complete superabsorbent polymer consumption with irrigation and the lowest (301.7 g) was obtained by naught use of animal manure and superabsorbent polymer treatment in severe drought stress (Table 6). Silva et al., (2006) reported increasing in maize 1000-seed weight by animal manure.

Grain yield

Grain yield significantly affected by irrigation and use of animal manure and superabsorbent polymer (Table 4) and grain yield decreased significantly by increasing drought stress intensity (Table 5). But the interaction between irrigation and animal manure and superabsorbent polymer was not significant on grain yield (Table 4). Yield reduction by drought stress due to reduced number of grain per ear and grain weight in response to decreased leaf relative water content and increased cell membrane stability reported by Majidian and ghadiri (2002). Application of animal manure and superabsorbent polymer together increased grain yield in compare with control significantly (Table 4), so that the highest grain yield was obtained by using animal manure and superabsorbent polymer 65% and 35% respectively. Animal manure increased grain yield by provide nutrients and increase the available moisture in the soil (Brar et al., 2001). Significant increasing in corn yield

Table 6. Effect of irrigation, animal manure and superabsorbent polymer interaction on 1000-seed weight, biological yield and cell membrane stability and leaf chlorophyll content (SPAD) in flowering and ripening stages

treatment	Cell membrane stability (%)		Leaf chlorophyll index		yield	
	flowering	ripening	flowering	ripening	1000-seed weight (g)	Biological yield (ton/ha-1)
I1S1	81.27e	77.32bcd	37.10g	35.81hi	341.0ef	28.27cdef
I1S2	80.96ef	76.01de	41.50cde	41.29bcd	354.0bc	28.47cdef
I1S3	79.49fg	75.16e	38.60f	37.55g	363.1a	28.82cdef
I1S4	80.56ef	75.54e	40.30e	39.00f	341.4ef	30.00bc
I1S5	80.48ef	75.35e	39.70e	37.65g	361.8b	29.62bcd
I1S6	80.96ef	75.97de	41.05e	40.12bcde	352.2cde	31.20a
I2S1	83.05c	78.67b	39.10f	38.00g	333.8fgh	27.40efg
I2S2	83.10c	78.03b	45.10ab	41.99bcd	343.1de	27.99efg
I2S3	81.17e	76.02de	40.30e	39.30ef	353.5bcd	28.56cdef
I2S4	82.53cd	77.55bc	43.00bcd	39.86cde	333.0ghi	29.56bcd
I2S5	81.75de	77.06bcd	41.67bcd	39.21f	354.0bc	29.26cde
I2S6	82.68cd	77.86b	44.43bc	41.12bcd	335.0fgh	30.81ab
I3S1	85.89a	80.70a	39.70f	39.33de	301.7k	26.38g
I3S2	85.54ab	80.40a	46.67a	43.78a	311.0j	26.74fg
I3S3	84.00b	79.14ab	41.37de	40.40bcde	338.6fg	28.03defg
I3S4	85.20ab	79.67ab	45.09ab	42.14bc	323.0i	29.20cde
I3S5	84.31b	79.36ab	43.86bc	41.27bcd	330.0hi	28.26cde
I3S6	85.24ab	80.27a	45.15ab	42.29b	317.1j	29.82bcd

Row means followed by the same letter are not significantly different at 0.01 or 0.05 probability level

I1 = Complete irrigation	S1 = nought applying animal manure and superabsorbent polymer	S4 = 50% animal manure + 50% superabsorbent polymer
I2 = medium drought stress	S2 = complete animal manure	S5 = 35% animal manure + 65% superabsorbent polymer
I3 = severe drought stress	S3 = complete superabsorbent polymer	S6 = 65% animal manure + 35% superabsorbent polymer

by consumption of chemical and animal fertilizers has been reported (Brar et al., 2001; Fallah et al., 2007).

Biological yield

Biological yield significantly affected by irrigation, animal manure and superabsorbent polymer and their interaction (Table 4). Biological yield decreased by increasing drought stress intensity. Use of animal manure and superabsorbent polymer together increased biological yield (Table 5). Biological yield was 18.27 percent higher in animal manure 65% and superabsorbent polymer 35% treatment in full irrigation in compare with naught use of animal manure and superabsorbent polymer in the drought stress treatment (Table 6). Biological yield reduction by drought stress (Majidian and ghadiri 2002; Osborne et al., 2002) and biological yield increasing by consumption of animal and chemical fertilizers (Brar et al., 2001) have been reported.

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