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Effects of Different Irrigation and Superabsorbent Levels on Physio-Morphological Traits and Forage Yield of Millet (*Pennisetum americanum* L.)

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Abstract: This study was carried out to investigate the effects of superabsorbent polymer and irrigation treatments on different morph-physiological traits of pearl millet. The experiment was conducted as a split plot based on a Randomized Complete Block Design (RCBD) with three replications. Four different levels of irrigation (I_1= 100%, I_2= 80%, I_3= 60% and I_4=40% of field capacity (FC) and three levels of super absorbent polymer [S_1= Control, S_2= 150 (kgha^{-1}) and S_3= 300 (kgha^{-1}) of zeolite] were allocated to main plots and sub plots, respectively. The studied traits were as follow: Plant Height (PH), Forage Dry Yield (FDY), Forage Fresh Yield (FFY), Water Use Efficiency (WUE), Leaf Area Index (LAI), Relative Water Content (RWC), Protein Content (%), Ash content (%) and Chlorophyll index (Chl). Analysis of variance showed that there were significant differences between irrigation and superabsorbent levels for all of the studied traits. The highest value for FFY and FDY (78433.20 and 26744.4 kgha^{-1},respectively) obtained in I_1. Superabsorbent application increased forage yield, RWC, WUE and LAI. The results indicated that superabsorbent had a remarkable effect on enhancement of millet growth, forage yield and its quality under drought stress.

Key words: Forage · Irrigation · Millet · Yield · Zeolite

INTRODUCTION

Agronomic applications could be a good strategy for crop survival in water-limited environments. Pearl millet (*Pennisetum americanum* L.) is a forage crop that is used as livestock, poultry feed and raw material in industry [1]. Millet is one of the best crops for production of green and silage forage besides its grain [1]. It needs relatively less water than other crops and could grow in hot and arid climates [2]. Drought stress is an important environmental tension in productivity of agricultural ecosystems [3]. Water management in millet farms could increase forage yield and WUE [3]. Superabsorbent polymer (SAP) application is effective to reduce hazardous effects of drought stress [4]. Super absorbent polymers could hold 400-1500 (g) of water per one gram of dry hydrogel [5]. Superabsorbent polymers could increase water absorption and its retention capacity under water-limited environments [4, 6]. Stored water and nutrients are released slowly as required by plant to improve its growth under limited water supply [7]. Polymers application could be a perfect strategy for water and nutrients holding in arid and semi arid climates [8].

Superabsorbant have been used as water container in agricultural and horticultural fields [5]. Zeolit is known as a synthetic and crystalline polymer [7]. Modification of cation exchange capacity (CEC), adsorption, hydration-dehydration and catalytic properties of natural zeolites have prompted slow-release of fertilizers and other materials [5]. Natural compound such as zeolite could decrease nitrogen leaching and increase fertilizer recovery [5]. Nagaz *et al.* [9] reported a significant increase in capacity of water retention with polymers application in soil. In general, hydrophilic polymers

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application could enhance plant survival, water use efficiency and dry matter production under drought stress [4]. Karimi and Naderi [7] reported that drought stress decreased leaf dry weight, plant growth rate and leaf area index in corn. Guiwei *et al.* [10] reported that application of SAP inhibits from water deficiencies for plant.

This study was conducted to study the effect of superabsorbent polymer on Physio-Morphological traits of millet under drought stress condition.

MATERIALS AND METHODS

In this study pearl millet hybrid (*Pennisetum americanum* L. var. Nutrifeed) was used as the crop material. This hybrid has high forage quality for livestock. This experiment was conducted as a split plot experiment based on a randomized complete block design (RCBD) with three replications. This study was conducted on experimental field of Shahid Bahonar University of Kerman (56°58′ longitude and 30°15′, 2044 asl) in 2011. Four different treatments of irrigation and three levels of superabsorbent polymer (Zeolite) were considered as main plots and subplots, respectively. Soil texture of experimental site was sandy-loam. Physical and chemical properties of soil are shown in Table 1. The row spacing was 50 (cm) and 10 (cm) between and within rows, respectively. Two harvests were done during this experiment. The first and the second harvests occurred at 70 and 130 days after sowing (DAS), respectively.

Irrigation Treatments: Irrigation treatments consisted of four different levels that including: 100 (%), 80 (%), 60 (%) and 40 (%) of field capacity (FC) which were abbreviated to I_1, I_2, I_3 and I_4, respectively. All sides of each plot were closed to control water content in each plot. All plots received equal content of water for germination. The irrigation treatments started 12 days after sowing, when the plants completely were established with 4 leaves on their main stem. Irrigation was conducted by polyethylene tubes.

Super Absorbent Polymer (SAP) Treatments: The plants were grown at three levels of zeolit ($0_{(S1)}$, $150_{(S2)}$ and $300_{(S3)}$ kg ha^{-1}). Super absorbent was applied at depth of 15 (cm) in soil before planting. Physical and chemical characteristic of super absorbent polymer is presented in Table 2.

Data Collection

Agronomic Traits: Different physio-morphological traits were studied that includes: Plant height(PH), Forage Fresh Yield (FFY), Forage Dry weight (FDY), Water Use Efficiency (WUE), Chlorophyll index (Chl), Protein content (%), Ash content (%), Relative Water Content (RWC) and Leaf Area Index (LAI).

Different traits were measured on ten randomly plant in each plot. To measure forage dry yield, fresh samples were placed in oven for 48 (h) at 72°C.

Physiological Traits: Water Use Efficiency (WUE) was calculated as the total dry matter (TDM) per unit of water consumed using the following formulae: WUE= TDM / ET-1

ET was calculated by the method of Garrity *et al.* [11] using the following formulae:

$ET = P + I - R - D_P \pm \Delta S$, Where, ET is crop water consumption (mm), P is rainfall (mm), I is irrigation water (mm), S is runoff (mm), D_P is deep percolation (mm) and ΔS is soil water content variation in root depth (mm). Therefore, total ET were calculated by summation of all ET during growing season. In this study, D_P and R were assumed to be negligible. It was also assumed that there was no deep percolation.

Leaf Area Index (LAI) was calculated by the formulae of Guerfel *et al.* [12]:

LAI = (Surface area of the sample leaves) / (Ground area occupied by the sampled plants).

Relative Water Content (RWC) was measured on ten random leaf samples of each plot. Immediately after cutting the base of lamina, leaves were sealed in plastic bags and quickly transferred to the laboratory.

Table 1: Results of Soil analysis for physical and chemical characteristics.

Characteristic	Soil depth(cm)	Soil texture	OC(%)	EC(dSm^{-1})	pH	P (ppm)	K(ppm)	N(%)
Value	0-30	Loamy-sand	0.88	1.30	7.6	6.9	240	0.08

OC: Organic Content

Table 2: Characteristics of super absorbent polymer (Zeolit)

Color	Humidity (%)	Toxics	Density (gcm^{-3})	pH	Water soluble	Dimension (micrometer)
White	3-5	No	1.5	6-7	No	50-150

Fresh Forage Yield (FFY) was determined within one hour after excision. Turgid weights (TW) were obtained after soaking leaves with distilled water in test tubes for 4 to 6 (h) at room temperature (20°C) under low light condition. After soaking, leaves were carefully blotted dry with bloating paper to determine turgid weight. Forage Dry Yield (FDY) was obtained after oven drying for 24 h at 70°C. The RWC was calculated using the following formula [13]:

$$RWC (\%) = [(FW - DW) / (TW - DW)] \times 100,$$

where: FW= Fresh weight, DW= Dry weight, TW= Total dry weight.

Chlorophyll content was assessed using a chlorophyll meter (SPAD-502, Minolta) and measurements were done at three points of each leaf (upper, middle and lower part). Average of these three readings for ten randomly plant was considered as SPAD value in each plot. Protein content (%) determined by NIR method AOAC, (1990). Ash content (%)was measured by the method of Wilson (1983).

Statistical Analysis: Analysis of variance (ANOVA) was carried out by SAS Ver.9. Mean comparison was done by Least Significant Difference (LSD) (P<0.05) test.

RESULTS

Analysis of variance showed that various irrigation and superabsorbent levels had significant effects on plant height (P<0.01) (Table 3). The lowest value for PH belonged to irrigation level of I_4, while the highest was recorded for control treatment (I_1) (Table 4). Application of super absorbent polymer increased PH significantly (Table 4). The highest value for plant height was observed with 300 kg ha^{-1} of super absorbent (Table 4). Harvest time affected significantly on plant height (Table 4).

The forage fresh yield (FFY) was affected by irrigation levels, super absorbent, irrigation× super-absorbent, harvest and harvest× superabsorbent interaction (Table 3). The highest and the lowest values of FFY was observed in I_1 (78433.20 kg ha^{-1}) and I_4 (4977.20 kg ha^{-1}) treatments, respectively (Table 4). The highest amount of FFY obtained in S_3 (Table 4). FFY was higher in the second harvest than the first (Table 4).

Forage Dry Yield (FDY) significantly affected by irrigation, superabsorbent polymer, harvest and interactions of superabsorbent polymer× irrigation, harvest× irrigation, harvest× super absorbent and superabsorbent × irrigation× harvest (Table 3).

Table 3: Analysis of variance of for studied traits under different irrigation levels and superabsorbent application in millet.

		Mean squares								
Source of variation	df	PH	FFY	FDY	WUE	Chl	Protein(%)	Ash (%)	RWC	LAI
Replication	2	236.80	62577053	2972612	0.059	47.56	3.05	0.87	9.77	21.25
Irrigation (I)	3	8332.91**	2495378**	421163277**	3.18**	192.10**	96.20**	16.67**	3340.44**	33.05**
Error (a)	6	63001	4993912	63001	0.46	28.75	3.18	0.26	3.85	0.59
Superabsorbent(S)	2	84338963**	7261798**	84338963**	2.07**	234.22**	94.70**	3.50**	195.25*	22.06*
I× S	6	4198187	14484864**	4198187	0.081**	7.21	2.41*	1.20*	44.09**	2.77**
Error (b)	16	166216	53087	166216	0.12	5.93	1.27	0.86	5.73	0.67
Harvest (H)	1	824767747**	42739235179**	824767747**	87.84**	186.56**	80.57**	0.10	962.36*	1.34*
I× H	3	108508767	763079550	108508767**	3.62	17.69	12.83	0.18	204.75**	2.47
Error (C_1)	8	354767	53613722	354767	4.81	5.69	1.43	0.20	9.38	0.23
H×S	2	6722523	61559902**	6722523**	0.76**	10.96	5.26**	0.31	23.06**	3.28**
I×S×H	6	580254	78114336	580254**	1.38**	5.81	2.54	0.31	34.37**	0.28
Error (C_2)	16	469633	18623163	469633	3.15	6.55	1. 30	0.29	3.69	0.40
C.V(%)		6.34	4.81	8.77	8.46	6.69	8.34	4.84	4.4	6.6

Different studied traits are abbreviated as: The mean of Plant height(PH), Forage Fresh Yield (FFY), Forage Dry weight (FDY), water use efficiency (WUE), Chlorophyll (Chl) Protein percentage, Ash percentage, Relative Water Content(RWC) and Leaf Area Index (LAI)

*, **: Significant at 5% and 1% probability levels, respectively, and n.s.: non-significant

Table 4: The means of studied traits under different irrigation levels and superabsorbent application in millet

	PH(cm)	FFY(kga^{-1})	FDY(kga^{-1})	WUE(kgDMm^{-3})	Chl	Protein(%)	Ash(%)	RWC	LAI
Irrigation levels									
I_1	133.63^{a}	78433.20^{a}	26744.4^{a}	3.07^{a}	34.24^{c}	24.3^{a}	12.56^{a}	90.66a	12.47^{a}
I_2	131.23^{a}	76033.20^{ab}	23250^{b}	2.87^{b}	37.11b^{c}	23.3^{ab}	11.15^{b}	79.68 ^{ab}	11.75 ^{ab}
I_3	104.09^{b}	69661.10^{c}	14697^{c}	2.72^{c}	39.44^{ab}	20. 07^{b}	10.68^{c}	63.59 ^{b}	10.95 ^{b}
I_4	80.58^{c}	4977.20^{d}	12231.1^{d}	2.42^{d}	43.03^{a}	15^{c}	10.32^{c}	61.32^{c}	9.22 ^{c}
Super absorbent levels(kg ha-1)									
S1	98.56^{c}	60904^{c}	15048.30c	2.62^{c}	42.46^{a}	19.6^{c}	10.76^{b}	70.47^{b}	10.02^{b}
S_2	118.05 ^{ab}	687771^{b}	16991.7b	3.95^{b}	38.28^{b}	23.1 ^{a}	11.22^{a}	74.72^{ab}	11.36 ^{ab}
S_3	123.78^{a}	73830^{a}	22902.5a	4.75^{a}	36.47^{b}	20.9 ^{b}	11.55^{a}	79.67^{a}	11.91^{a}
Harvest									
1	99.51 ^{b}	40556^{b}	13513.78b	2.73^{b}	39.90^{a}	18. 5^{b}	11.22^{a}	70.04^{b}	10.97^{b}
2	130.75 ^{a}	79894^{a}	23447.78a	4.99^{a}	37.02^{b}	23.8^{a}	11.13^{b}	77.53^{a}	12.22^{a}

Different studied traits are abbreviated as : Plant height(PH), Forage Fresh Yield (FFY), Forage Dry weight (FDY), water use efficiency (WUE), Chlorophyll (Chl) Protein percentage, Ash percentage, Relative Water Content(RWC) and Leaf Area Index (LAI).

Values within the column followed by the different letters are significantly different according to the LSD test at 0.05

Application of superabsorbent polymer increased FDY under drought stress conditions in comparison with control treatment (Table 4). The highest (22902.5 kg ha^{-1}) and the lowest (15048.30 kg ha^{-1}) value for FDY were obtained by application of S_3 and S_1 treatments of zeolit, respectively (Table 4). Analysis of variance showed that there was a significant difference between irrigation, harvest, superabsorbent and interactions of irrigation × superabsorbent, harvest× superabsorbent and harvest × superabsorbent× irrigation for water use efficiency (WUE) (Table 3). The highest value for water use efficiency obtained in control (I_1) (Table 4). WEU decreased with increasing of drought stress severity (Table 4). I_1 treatment was significantly different from others (Table 4). Application of super absorbent increased WUE, significantly (Table 4). The highest value for WUE (4.75 DM kgm^{-3}) obtained in S_3. The highest value for water use efficiency (4.99 DM kgm^{-3}) was obtained in the second harvest (Table 4).

Leaf chlorophyll index (SPAD) was significantly influenced by irrigation, superabsorbent, harvest, harvest× irrigation (Table 3). The highest and the lowest values of leaf chlorophyll were obtained by I_4 and I_1 treatments, respectively (Table 4). Also, the highest and the lowest chlorophyll index was obtained by application of S_1 and S_3 treatments, respectively (Table 4). Frist harvest showed a higher value for chlorophyll index than the first (Table 4). Protein and ash content (%) showed significant differences with irrigation, superabsorbent and interaction of irrigation × super absorbent (P<0.01) (Table 3). Protein (%) was significantly affected by harvests and harvest × superabsorbent interaction (Table 3). The maximum value for protein (%) (24.3) and ash (%) (12.56) was observed in I_1 treatment (Table 4). Increase of drought tension, reduced protein content, significantly (Table4). The similar trend was observed in ash percentage (Table 4). Application of zeolite increased protein and ash content (%) in comparison with control treatment (S_1) (Table 4). The highest content for protein (23.1) (%) and ash (11.55) (%) were obtained in S_2 and S_3 respectively, while the lowest content of protein (19.6) (%) and ash (10.76) (%) were obtained in S_1 treatment (Table 4). Protein content in second harvest (23.8) (%) was more that the first one (Table 4).

Analysis of variance showed that relative water content (RWC) was affected by irrigation, superabsorbent, harvest and interactions of irrigation × superabsorbent, harvest× irrigation, harvest× superabsorbent and irrigation× superabsorbent ×harvest (Table 3). Drought stress reduced RWC, significantly (Table 4). The highest (90.66) and the lowest values (61.32) for RWC were observed in I_1 and I_4 treatments, respectively (Table 4). Increase in superabsorbent application increased RWC, significantly (Table 4). Also, RWC in second harvest (77.53) was higher than the first ones (70.04) (Table 4).

Analysis of variance showed that there were significant effects between irrigation, harvest and interactions of irrigation × superabsorbent, superabsorbent×harvest and irrigation× harvest for leaf area index (LAI) (Table 3). Leaf area expansion depends on leaf turgor, temperature, and assimilating supply for growth. The I_1 treatment had the highest LAI in comparison with others (Table 4). Under deficit irrigation, zeolit increased leaf area substantially in levels of S_3 and S_2 rather than control S_1 (Table 4). The result showed that LAI influenced ($P<0.05$) by harvest time (Table 3) and the highest value for LAI obtained in second harvest (Table 4).

DISCUSSION

Drought stress affects physiological and metabolic processes of plants [2, 16]. Plant height acts as a potent indicator for availability of growth resources in its vicinity in plant [16]. The reduction of plant height could be attributed to decline in cell enlargement and increase in leaf senescence under drought stress [16]. In this study, plant height increased significantly under proper utilization of zeolite (Table 4). The obtained result is similar to those reported by Zegada-Lizarazu and Iijima [2] and Manivannan *et al.* [17]. Enhancement of plant height in second harvest could be attributed to positive effect of zeolit. Drought stress caused reduction in leaf area and its expansion through reduction in photosynthesis [18]. Similar with our findings, reduction in fresh and dry biomass production and leaf area is a common adverse effect of water stress on plants [16, 19]. The reduction in forage dry yield was due to reduction in growth and relative water content of leaves under drought stress (Table 4). Khadem *et al.* [20] reported an increase in maize yield by application of super absorbent polymer. Karimi and Naderi [7] declared that using of superabsorbent polymer compensate the negative effects of deficit irrigation in forage corn. In drought-tolerance species, WUE is maintained at an optimum level by reduction of water evapo-transpiration [21]. According to our results, Karimi and Naderi [7] reported that high capacity of water retention via superabsorbent application, improved the negative effects of deficit irrigation in this experiment. On the other hand, superabsorbent application increased fresh and dry weight of forage in comparison with normal treatment. Chlorophyll is one of the major components of chloroplast for photosynthesis [22]. Relative chlorophyll content had a positive relationship with photosynthetic rate [22]. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress [23, 24]. It could be a result of pigment photo-oxidation and chlorophyll degradation [23]. Mean comparison showed that leaf chlorophyll index increased by application of superabsorbent polymer, in comparison with control treatment (Table 4). Plant growth under drought condition causes a lower stomatal conductance in comparison with normal condition [25]. Consequently, reduction in CO_2 fixation and photosynthetic rate, resulting less assimilate production for growth and yield of plants [21]. Diffusive resistance of stomata to CO_2 absorption, stomatal closure or changes in chlorophyll content could be the main factors that limit photosynthesis rate under drought stress [21]. Zhao *et al.* [19] reported that leaf chlorophyll content was decreased under drought stress conditions. Kulshreshtha *et al.* [26] reported that drought stress caused a significant decline in total chlorophyll and its components (*a* and *b)* and total chlorophyll content in sunflower. The decrease in chlorophyll content in drought stress could be mainly the result of damage to chloroplasts damages that is caused by reactive oxygen species [17]. Decreasing of protein content reported in other forage crops [7]. Khalili Mahalleh *et al.* [27] reported that with drought stress in forage crops, ash percent decreased significantly. The ash content is a mark of all minerals except I^{-1} and Cl^{-1}, because these elements sublimate by burning in electrical furnace. Each deficiency of minerals in food of herbivorous could leads to some disease such as milk fever. Khadem *et al.* [20] reported that protein and ash content increased with superabsorbent application. In drought stress, reduction of RWC has been reported by Nayyar and Gupta [28]. Also, changes in leaf temperature may be an important factor in controlling leaf water status under drought stress [8]. Huttermann *et al.* [5] reported a positive linear relationship between the number of irrigations and LAI value. Super absorbent supply had positive effect on LAI (Table 4). Huttermann *et al.* [5] reported that application of superabsorbent reduced LAI under adequate irrigation, slightly. The reduction of leaf area at terminal growth stages could be due to senescence of older leaves that associated with remobilization of the stored metabolites from the leaf [6]. Zeolite levels significantly influenced LAI, that was similar to the results of Khalili *et al.* [27]. Zeolite application improved nitrogen uptake, increased nucleic acid, amides and amino acid and hence cell multiplication [6]. The increase or reduce of LAI has a direct effect on plant growth rate [20]. This index is the main tool for enhancing photosynthesis power and assimilates production. LAI reduction under water deficit condition is a main reason for forage yield reduction [5, 29] Probably, the decrease in leaf area is a response to stress for adapting water deficit conditions and survival through decreasing cell turgor pressure [20].

CONCLUSION

Super absorbent polymer plays an important role in enhancement of absorption capacity and retention of water in soil, fighting against water shortage and decreasing harmful effects of drought stress. Super absorbent polymers may have great potential in restoration and reclamation of s oil and storing water available for plant growth and production. Super absorbent polymer works by absorbing and storing water and nutrients in a gel form, hydrating and dehydrating as the demand for moisture fluctuates.

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