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Research article

EFFECT OF SUPERABSORBENT ON PHYSIO-MORPHOLOGICAL TRAITS AND FORAGE YIELD OF MILLET (*PENNISETUM AMERICANUM* L.) UNDER DIFFERENT IRRIGATION TREATMENTS

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ABSTRACT: The present research was carried out to study 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 irrigation levels ($I_1=100\%$, $I_2=80\%$, $I_3=60\%$ and $I_4=40\%$) of field capacity (FC) were allocated to main plots. Three levels of super absorbent polymer [$S_1=$ Control, $S_2=150$ (kg ha⁻¹) and $S_3=300$ (kg ha⁻¹)] were allocated to sub plots. Different characteristics including: plant height, dry forage yield, fresh forage yield, water use efficiency (WUE), Leaf Area Index (LAI), relative water content (RWC), Protein content(%), ash content(%) and chlorophyll index were studied. Analysis of variance showed that there was a significant difference between irrigation and superabsorbent levels for all of the studied traits. The highest value for fresh yield and dry yield of forage (98.48 and 27.75 ton ha⁻¹) obtained in normal irrigation. Superabsorbent application increased forage yield, RWC, WUE and LAI. The highest value for fresh yield and dry yield (93.83 and 23.89 ton ha⁻¹) were obtained in S_3 treatment. This treatment caused the highest value for WUE. Ash and protein content (%) affected by different levels of irrigation and zeolit. The values of RWC and ash (%) had not significant difference between S_2 and S_3 treatments. The obtained result indicated that super absorbent had a remarkable effect on improvement of millet growth, forage yield and its quality under drought stress.

Key words: Irrigation, forage, millet, Quality, Yield, Zeolit.

INTRODUCTION

The objective of well-regulated deficit irrigation is saving water by subjecting crops to periods of water stress with minimal effects on yields. Pearl millet (*Pennisetum americanum* L.) is an important plant that is used as livestock, poultry feed and as raw material in industry [8]. Millet is one of the best plants for green forage production, silage forage and grain [8]. It needs relatively less water than other crops and could grow in hot and dry climates [36]. The usage of millet in animal feeds has made it very important in Iran. Water supply is one of the most important reasons for yield reduction in arid and semi arid areas. Reduction of water potential is one of the major limitations in productivity of natural and agricultural ecosystems [35]. Suitable water management in millet farms could increase forage yield and water use efficiency [36]. The use of superabsorbent polymer is effective on reduction of drought stress effects [7]. Super absorbent polymers could hold 400-1500 (g) of water per each dry gram of hydrogel [15]. Superabsorbent polymers have a great importance due to their role in the increase of water absorption capacity and retention of water content under limiting conditions [26]. Application of polymers to soil could be a perfect strategy [for holding water in arid and semi arid regions] [34]

Super absorbent polymers have been used as water retaining materials in the agricultural and horticultural fields, because when incorporated with soil, they can retain large quantities of water and nutrients [15]. Zeolit, as synthetic and crystalline polymer [17]. It has a high internal surface area [4]. The cation exchange, adsorption, hydration-dehydration, and catalytic properties of natural zeolites have prompted the slow-release of fertilizers and other materials [28]. Natural compound such as zeolite have been reported as ameliorants for coarse soils to modify soil [cation exchange capacity (CEC) to decrease nitrogen leaching and to increase fertilizer recovery] [15]

Zeolite improves soil structure by coupling high CEC with a selective affinity for ammonium and potassium ions [23]. Zeolite, a highly crosslinked polyacrylamide with about 40% of the amide group hydrolysed to carboxylic groups was able to prolong the survival of plants under drought [21]. Nagaz et al. [25] reported a significant increase in water retention capacity with polymers application in soil. Super absorbent polymers have been used as water retaining materials in agricultural fields [7]. Their incorporation with soil, could retain large quantities of water and nutrients [7]. So, these stored water and nutrients are released [slowly as required by the plant to improve growth under limited water supply [17]. In general, hydrophilic polymers applications could enhance plant survival, water use efficiency and dry matter production under drought stress [7]. Karimi and Naderi [17] reported that drought stress decreased leaf dry weight, plant growth rate and leaf area index. Guiwei et al. [13] reported that amendment of soil with super absorbent polymers prolonged the duration of water evaporation. This research was conducted to study the effect of superabsorbent polymer on Physio- Morphological characters of millet under drought stress condition.

MATERIALS AND METHODS

In this study pearl millet hybrid (*P. americanum* var. Nutrifeed) was used as the crop material. This hybrid has very high forage quality for livestock and could be used as hay or silage. A split plot experiment based on a randomized complete block design with three replications was employed. Main plots were included irrigation levels and sub plots were different levels of super absorbent polymer (zeolite). After field preparations, the experimental area was divided into 36 plots of 3 (m) × 2.5 (m) size. The soil of experimental site was sandy-loam. Physical and chemical properties of soil are shown in Table 1. The seeds were sown in 2011. The row spacing between and within rows were 50 (cm) and 10 (cm), respectively. Plants were thinned to acquire the desirable density after emergence. Two harvests were done during the experiment. The first and the second harvests occurred at 70 and 130 days after sowing (DAS), respectively.

Irrigation treatments

Irrigation treatments consisted of irrigation at different levels of field capacity (FC) including: 100 (%), 80 (%), 60 (%) and 40 (%) of FC which were abbreviated to I₁, I₂, I₃ and I₄, respectively. All sides of each plot were closed to control the volume of water in each plot. All of the plots received the same 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 treatments

The plants were grown at three levels of zeolite (0, 150 and 300 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 agronomical traits were studied including plant height, fresh weight and dry weight of forage. Plant height was measured on ten randomly plant in each row. In order to measure dry weight of forage, the fresh samples were placed in oven for 48 (h) at 72° C.

Physiological traits:

Water use efficiency (WUE) can be based on either transpiration or evapotranspiration and grain yield or TDM [27].

In this experiment the WUE was calculated as the total dry matter (TDM) per unit consumed water. So, WUE was calculated according the following formulae:

$$WUE = TDM / ET - 1$$

ET was calculated using by the method of Garrity et al. [11] according 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 surface runoff (mm), D_p is deep percolation (mm) in ΔS is soil water content variation, in crop 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. Since the slope of each plot was near to zero and the amount of irrigation water was only enough to reach to field capacity, so it was also assumed that there was no deep percolation.

Leaf Area Index (LAI) was calculated by the formulae of Gupta and Gupta [12]:

$$LAI = (\text{Surface area of the sample leaves}) / (\text{Ground area occupied by the sampled plants}).$$

RWC was measured on leaf samples. Immediately after cutting the base of lamina, leaves were sealed in plastic bags and quickly transferred to the laboratory. Fresh weights (FW) were determined within 1 h 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 blotting paper to determine turgid weight. Dry weights (DW) were obtained after oven drying for 24 h at 70°C. The RWC was calculated using the following formula [31]:

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

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 was considered as SPAD value.

Nitrogen content was measured by Kjeldahl method. Percentage of protein determined by NIR method AOAC [1]. Ash percentage was measured by the method of Wilson et al. [32].

Statistical analysis

Analysis of variance (ANOVA) was carried out by SAS program [30]. Mean comparison was done by LSD (P<0.05) .test

Table1. Results of Soil analysis for physical and chemical characteristics

Characteristic	Soil depth (cm)	Soil Texture	OC (%)	EC Ds/m ⁻¹	pH	P (ppm)	K (ppm)	N (%)
Value	0-30	Loamy- sand	0.88	1.30	7.6	6.9	240	0.08

Table2. Characteristics of super absorbent polymer (Zeolit)

Color	Humidity (%)	Toxics	Density (g/cm ³)	PH	Water soluble	Dimension (micrometer)
White	3-5	No	1.5	6-7	No	50-150

RESULTS AND DISCUSSION

:Plant height

Analysis of variance showed that various irrigation and super absorbent levels had significant effects on plant height (P<0.01) (Table. 3). The lowest value for plant height belonged to irrigation level of I₄, while the highest was recorded for control treatment (I₁) (Table 4). Application of superabsorbent polymer increased plant height, significantly (Table.5). The highest value for plant height was observed in 300 kg ha⁻¹ of superabsorbent (Table 5). Time of harvest affected plant height, significantly (Table 6). Drought stress affects on physiological and metabolical processes within plants [36, 6]. Plant height acts as a potent indicator for availability of growth resources in its vicinity in plant [6]. The reduction of plant height could be attributed to decline in cell enlargement and increase in leaf senescence under drought stress [28].

Plant height increased under proper utilization of zeolite application, significantly (Table 5). Result obtained in this study, is similar to those reported by Zegada-Lizarazu and Iijima [36]. This trend was similar with the results of Manivannan et al. [22] and in the second harvest, the higher value of plant height could be attributed to positive effect of zeolite.

Fresh Forage Yield:

The yield of fresh forage affected by irrigation levels, super absorbent, irrigation× super-absorbent and harvest× super-absorbent interaction (Table 3). The highest and the least values of fresh forage was observed in I₁ (98.48 Ton ha⁻¹) and I₄ (49.78 ton ha⁻¹) treatments, respectively (Table 4). The highest amount of fresh yield obtained in 300 Kg ha⁻¹ of super absorbent application (Table 5). Fresh forage yield was higher in the second harvest than the first (Table.6). Shortage of water supply will decrease plant turgidity and fresh yield, subsequently [36]. Increase of irrigation intervals, would reduce turgor pressure in plant cells [28]. Subsequently this reduction will reduce weight of cells and fresh weight of forage. Drought stress inhibits the dry matter production through its inhibitory effects on leaf expansion, leaf development and consequently reduction in light interception [10]. Exposure of millet to drought stress in reproductive stage, leads to a substantial reduction in forage yield and its components such as leaf/stem, fresh forage and dry forage [3]. The obtained result indicated that super absorbent had a remarkable effect on millet growth, yield and its quality. The effect of drought stress on yield reduction of millet is reported by previous studies [6], Winkel et al. [33] and Nagaz et al.[25].

The Dry Forage Yield

Dry forage yield significantly affected by irrigation, superabsorbent, harvest, irrigation× harvest and harvest× super absorbent (Table 3). Drought stress reduced the number of leaves per plant, individual leaf size and leaf longevity by decreasing the soil's water potential (Table 4). Leaf area expansion depends on leaf turgor, temperature, and assimilating supply for growth (Table 4). Drought stress caused reduction in leaf area and its expansion through reduction in photosynthesis [10]. Reduction of production in fresh and dry biomass production is a common adverse effect of water stress on crop plants [6]. It was concluded that plant height, stem diameter, leaf area, fresh and dry forage decreased noticeably with increasing water stress [37]. Reduction in dry forage yield was due to reduction in growth and relative water content of leaves under drought stress (Table.4). Application of superabsorbent polymer increased dry forage yield in drought stress in comparison with control treatment (Table 5). The highest (23.89) (tonha⁻¹) and the least (16.38 tonha⁻¹) amount of dry yield was obtained by application of S₃ and S₁ treatments of zeolit, respectively (Table 5). Khadem et al. [18] reported an increase in maize yield by application of super absorbent polymer. Karimi and Naderi [17] declared that using of superabsorbent polymer compensate the negative effects of deficit irrigation in forage corn. Therefore, stored nutrients are released slowly as required by plant to improve growth under limited water supply.

Table 3: Analysis of variance of different studied traits in millet under drought stress and superabsorbent polymer

Source	DF	Plant height	Mean squares							
			Fresh Weight	Dry Weight	WUE	Chlorophyll index	Protein	Ash	RWC	LAI
Replication	2	236.80	44.23	1.13	0.059	47.56	3.05	0.87	9.77	21.25
Irrigation	3	8332.91 ^{***}	2719.89 ^{***}	84.69 ^{***}	3.18 ^{***}	192.10 ^{***}	96.20 ^{***}	16.67 ^{***}	3340.44 ^{***}	33.05 ^{***}
Error (a)	6	341.84	3.20	0.43	0.46	28.75	3.18	0.26	3.85	.59
Superabsorbent	2	3405.93 ^{***}	896.82 ^{***}	70.75 ^{***}	2.07 ^{***}	234.22 ^{***}	94.70 ^{***}	3.50 ^{***}	195.25 ^{***}	22.06 ^{***}
Irrigation× Superabsorbent	6	173.87	136.80 ^{***}	3.75	0.081 ^{***}	7.21	2.41 [*]	1.20 [*]	44.09 ^{***}	2.77 [*]
Error(b)	16	225.87	5.37	0.31	0.12	5.93	1.27	0.86	5.73	0.67
Harvest	1	8352.53 ^{***}	39906.93 ^{***}	1208.78 ^{***}	87.84 ^{***}	186.56 ^{***}	80.57 ^{***}	0.10 [*]	962.36 ^{**}	1.34 [*]
harvest × Irrigation	3	438.14	1101.29	155.26 ^{***}	3.62	17.69	12.83	0.18	204.75	2.47
Error (C ₁)	8	291.63	16.11	0.23	4.81	5.69	1.43	0.20	9.38	0.23
Harvest × superabsorbent	2	311.51	66.87 ^{***}	3.45 ^{***}	0.76 ^{***}	10.96	5.26 ^{***}	0.31	23.06	3.28 ^{***}
Harvest × Irrigation × superabsorbent	6	172.13	93.46	3.47 ^{***}	1.38 ^{***}	5.81	2.54	0.31	34.37	0.28
Error (C ₂)	16	264.13	5.89	0.37	3.15	6.55	1.30	0.29	3.69	0.40
Coeffi variance		6.34	4.81	8.77	8.46	6.69	8.34	4.84	4.4	6.6

*, **: F-test significant at P<0.05 and P<0.01 respectively; ns: not significant

Means followed by the same letter was not significantly different at 0.05 level using LSD test.

Table 4: Mean comparison for different studied traits in millet under different irrigation.

Irrigation treatments	Plant height	Fresh Weight	Dry Weight	WUE (kg DM/m ³)	Chlorophyll index	Protein (%)	Ash (%)	RWC (%)	LAI
Irrigation									
I1: 100% FC	133.63a	94.48a	27.75a	3.07a	34.24c	13.18a	12.56a	90.66a	12.47a
I2: 80% FC	131.23a	96.45ab	24.15b	2.87b	37.11bc	12.62ab	11.15b	79.68b	11.75b
I3: 60% FC	104.09b	69.03c	14.73c	2.72c	39.44ab	9.86c	10.68c	63.59c	10.95c
I4: 40% FC	80.58c	49.78d	12.34d	2.42d	43.03a	8.18d	10.32d	61.32d	9.22d

Means followed by the same letter was not significantly different at 0.05 level using LSD test.. RWC: Relative Water Content, LAI: Leaf Area Index, FC: Field capacity

*, **: F-test significant at P<0.05 and P<0.01 respectively; ns: not significant

Water Use Efficiency (WUE)

Analysis of variance showed that there was a significant difference between harvest time, irrigation, superabsorbent application and irrigation × superabsorbent for water use efficiency (Table 3). The highest value for water use efficiency obtained in control (I₁) (Table 4). Water use efficiency decreased with increasing of drought stress severity (Table 4). I₁ treatment was significantly different from the other treatments of irrigation (Table 5). The highest value for water use efficiency (4.99 DM kgm⁻³) was obtained in the second harvest (Table 6). Application of superabsorbent increased WUE. The highest value for WUE (4.75 DM kgm⁻³) obtained by S₃ treatment (Table 5). In drought-tolerance species, WUE is maintained at an optimum level by reduction in water losses [2]. The high capacity for water retaining by Superabsorbent application, improved the negative effects of deficit irrigation in this experiment. In other hand, superabsorbent application increased the fresh and dry weight of forage in comparison with control (S₁) (Table 5). Karimi and Naderi [17] reported the similar results in corn.

Table 5: Mean comparison for different studied traits in millet under different superabsorbent application

Zeolite	Plant height	Fresh Weight	Dry Weight	WUE (kg DM/m ³)	Chlorophyll index	Protein (%)	Ash (%)	RWC (%)	LAI
S ₃ : 300kg ha ⁻¹	123.78 ^a	93.83 ^a	23.89 ^a	4.75 ^a	36.47 ^b	17.76 ^a	11.25 ^a	79.67 ^{ab}	11.91 ^a
S ₂ : 150kg ha ⁻¹	108.05 ^b	69.78 ^b	17.70 ^b	3.95 ^b	38.28 ^b	11.34 ^b	11.22 ^a	74.72 ^a	11.36 ^b
S ₁ : 0 kg ha ⁻¹	98.56 ^c	61.40 ^c	16.38 ^c	2.62 ^c	42.46 ^a	8.78 ^c	10.76 ^b	70.47 ^b	10.02 ^c

RWC: Relative Water Content, LAI: Leaf Area Index

Means followed by the same letter was not significantly different at 0.05 level using LSD test.

*, **: F-test significant at P<0.05 and P<0.01 respectively; ns: not significant

Table 6: Mean comparison for different studied traits in two different harvest time in millet level

Harvest	Mean squares								
	Plant height	Fresh Weight	Dry Weight	WUE (kg DM/m ³)	Chlorophyll index	Protein (%)	Ash (%)	RWC (%)	LAI
First Harvest	99.51 ^b	42.25 ^b	13.81 ^b	2.73 ^b	39.90 ^a	9.85 ^b	11.22 ^a	70.04 ^b	10.97 ^b
Second Harvest	130.75 ^a	92.42 ^a	24.10 ^a	4.99 ^a	37.02 ^b	12.07 ^a	11.13 ^b	77.53 ^a	12.22 ^a

RWC: Relative Water Content, LAI: Leaf Area Index

Means followed by the same letter was not significantly different at 0.05 level using LSD test.

*, **: F-test significant at P<0.05 and P<0.01 respectively; ns: not significant

Leaf chlorophyll index (SPAD)

Leaf chlorophyll index (SPAD) was significantly influenced by drought stress, superabsorbent polymer and harvest time (Table 3). The highest and the least values of leaf chlorophyll were obtained by severe drought stress and control irrigation treatments respectively (Table 4). Also, the highest and the least chlorophyll index was obtained by application of S₁ and S₃ treatments, respectively (Table 5).

Fist harvest showed a higher value for chlorophyll index than the first (Table 6). Chlorophyll is one of the major components of chloroplast for photosynthesis [9]. Relative chlorophyll content had a positive relationship with photosynthetic rate [9]. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress [29]. It could be a result of pigment photo-oxidation and chlorophyll degradation [29]. Furthermore, water deficit induced reduction in chlorophyll content through the losses of chloroplast membranes, excessive swelling, distortion of the lamellae vesiculation, and the appearance of lipid droplets [14]. Mean comparison showed that leaf chlorophyll index increased by application of superabsorbent polymer, in comparison with control treatment (Table 5). Drought stress decreases photosynthesis rate [16]. Plant growth under drought condition causes a lower stomatal conductance in comparison with normal condition [5]. Consequently, reduction in CO₂ fixation and photosynthetic rate, resulting less assimilate production for growth and yield of plants [2]. Diffusive resistance of stomata to CO₂ absorption, stomatal closure or changes in chlorophyll content could be the main factors that limit photosynthesis rate under drought stress [2]. Zhao et al. [37] reported that leaf chlorophyll content decreases as a result of drought stress. Kulshreshtha et al. [20] 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 [22].

(%) Protein and Ash content

Protein and Ash content (%) had significant differences on irrigation treatments, superabsorbent, drought stress × super absorbent and harvest ($P < 0.01$) (Table 3). Protein (%) was significantly affected by harvests and harvest × superabsorbent (Table 3). The maximum value of protein (%) (13.18) and ash (%) (12.56) was observed in I₁ treatment (Table 4). A significant reduction was observed in protein content with increase of drought tension (Table 4). Decreasing of protein content reported in other forage crops [17]. The similar trend was observed in ash percentage (Table 4.) Khalili Mahalleh et al. [19] reported that with drought stress in forage crops, ash percent decreased significantly. The ash content is a mark of all minerals except I (iodine) and Cl ions, because these elements sublime by burning in electrical furnace. Each deficiency of minerals in food of herbivorous could leads to some disease such as milk fever.

Application of zeolite increased protein and ash content (%) in comparison with control treatment (Table 5). The highest content for protein (17.76) (%) and ash (11.25) (%) were obtained in S₃, while the lowest content of protein (8.78) (%) and ash (10.76) (%) were obtained in S₁ treatment (Table 5). Khadem et al. [18] reported that protein and ash content increased with superabsorbent application. Protein content in second harvest (12.07) was more than the first one (Table 6).

Relative water Content (RWC)

Analysis of variance showed that relative water content (RWC) affected by plant harvests, irrigation, and superabsorbent application (Table 3). Drought stress reduced RWC, significantly. The highest (90.66) and the least values (61.32) for RWC were observed in I₁ and I₄ treatments, respectively (Table 4). Increase in superabsorbent application (from S₁ to S₃) increased RWC, significantly (Table 5). Also, RWC in second harvest (77.53) was higher than the first ones (70.04) (Table 6). In drought stress, reduction of RWC has been reported by Nayar and Gupta [24]. Also, changes in leaf temperature may be an important factor in controlling leaf water status under drought stress [34].

Leaf area index

According to analysis of variance, leaf area index (LAI) was significantly affected ($P < 0.05$) by drought stress, superabsorbent, harvest, superabsorbent × irrigation, superabsorbent × harvest (Table 3). The I₁ treatment had the highest LAI than the others, while in severe drought stress (I₄), LAI was the least (Table 4). Decrease in period of terminal drought stress increased LAI, significantly. Huttermann et al. [15] reported a positive linear relationship between the number of irrigations and LAI value. Super absorbent supply had positive effect on LAI (Table 5). Huttermann et al. [15] reported that application of superabsorbent reduced LAI under adequate irrigation, slightly.

In deficit irrigation, zeolit increased leaf area substantially in levels of S₂ and S₃ rather than control treatment (Table 5). 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 [26]. Zeolite levels significantly influenced LAI, that was similar to the results of Khalili et al. [19]. Zeolite application improved nitrogen uptake, increased nucleic acid, amides and amino acid and hence cell multiplication [26]. The result showed that LAI influenced (P<0.05) by harvest time (Table 6), and the highest value for LAI obtained in second harvest (Table 6). The increase or reduce of LAI has a direct effect on plant growth rate [18]. 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 [15]. Probably, the decrease in leaf area is a response to stress for adapting water deficit conditions and survival through decreasing cell turgor pressure [18].

CONCLUSION

Super absorbent polymer application plays an important role in increasing the 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 soil 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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