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Effects of Nitroxin Fertilizer on Physiological Characters Forage Millet under Irrigation Cessation

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KEYWORDS

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ABSTRACT: An experiment in the split plot factorial design using the randomized complete block design was conducted in Damghan, Semnan Province, Iran in the cropping year 2012-2013 to study the effects of irrigation cessation (based on the phenological stages of the plants) on physiological characteristics of forage millet cultivars. The treatments included three irrigation levels (the control with full irrigation, irrigation cessation when flowering started, and irrigation cessation when flowering ended) in the main plots, and applying nitroxin biofertilizer (+) and not applying nitroxin biofertilizer (control) and forage millet cultivars (Bastan, Pishahang, and Isfahan) in the subplots. The maximum water-soluble carbohydrates contents were observed in the cultivar Bastan (8.91%, respectively), the highest contents of fiber and water (74.17 and 48.83%, respectively) in the treatment of irrigation cessation when flowering started, and the largest proline concentration (1.90µmol/$g^{-1}$$ww^{-1}$) in the treatment of irrigation cessation when flowering started. Millet tolerated high levels of drought under conditions of irrigation cessation and Nitroxin, as a biological fertilizer, was useful in producing a good quality crop. The very rapid growth of millet, its short growing season, drought tolerance, unique feature with regard to harvest time, and its response to nitroxin biofertilizer can help to expand its cultivation in arid and semi-arid regions of Iran.

INTRODUCTION

Millet and sugar beet are the most important forage crops in arid and semi-arid regions and have relatively high tolerance to unfavorable climatic conditions. Their adaptation to drought and their high water use efficiency enable them to have high yields under these environmental conditions [1]. Drought is one of the most important limiting factors for plant growth and for crop plan production in most agricultural lands throughout the world [2]. Plant response to drought stress differs at different levels from cells to whole plants, and depends on drought severity and duration, on the species, and even on the various genotypes in a given species [3]. Nitroxin biofertilizer contains nitrogen-fixing complexes of bacteria belonging to the

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Azotobacter and *Azospirillum* genera that cause the growth and development of roots and aerial parts of plants [4]. One of the most important changes resulting from drought stress is the reduced relative water content in leaves, and this index can exhibit the capability of plants in tolerating drought [5]. Leaf area and plant's relative water content decreased with increasing drought stress [6].

Proline accumulation is another response observed and reported in most crop plants faced with drought, referred to as an index of drought tolerance [7]. Undoubtedly, proline accumulation plays a role in increasing a plant's survival capability under stressful environmental conditions [8]. Application of mycorrhiza (which is a biological fertilizer) had significant effects on ash content in barley [9]. Ash and percentage in forage millet did not change with increasing drought stress [10]. In studies conducted in Australia on drought stress in three forage grains, in treatments under stress, the qualitative characteristics of the forage crops declined in addition to the reduction in their yields [11].

In Damghan region, Iran [10], the percentage of fiber (and that of soluble sugars) increased under severe drought stress. Considering rainfall reduction all over the world, the decline in the water levels in wells, and the drop in groundwater levels (especially in arid and semi-arid regions like Damghan in Iran), this research was conducted with the following purposes: (1) To test millet cultivars under such climatic conditions, and (2), given the uncontrolled use of chemical fertilizers, to apply the biological fertilizer Nitroxin for improving the qualitative characteristics of millet (so that it can be introduced for cultivation in such regions, especially in Iran).

MATERIALS AND METHODS

This research was carried out in the form of an experiment in split plot factorial design using the complete randomized block design with three replications in a field six kilometers south of Damghan in Semnan Province, Iran in the cropping year 2012-2013. The average long-term rainfall in the region is 286 mm and the annual absolute maximum and minimum temperatures is 42 and – 10 °C respectively.

The treatments included three irrigation levels (the control with full irrigation, irrigation cessation when flowering started, and irrigation cessation when flowering ended) in the main plots, and three millet cultivars (Bastan, Pishahang, and Isfahan) and two nitroxin levels, application(+) and non-application (-), in the subplots. The subplots were

2×2 meters and each cultivar was planted in 4 rows 40 centimeters part, Primary tillage operations were performed in winter and secondary tillage operations (which included two disking operations in perpendicular directions) in mid-May 2013. The quantity of seeds planted was determined based on the density of 200 plants/m^2. Seeds were treated with the nitroxin biofertilizer and planted before sunrise to prevent their exposure to sunlight.

Based on results in Table 1 (analysis of soil samples taken at depths of 30 and 60 cm), ammonium phosphate at 200 kg/ha and half of the nitrogen fertilizer (75 kg of urea) were applied together before planting, and the rest of the nitrogen fertilizer was used at tillering as a topdressing. Plant density was adjusted at the 4-leaf stage, and hand weeding was carried out twice to control weeds.

Table 1. Specifications physical and Chmical of soil

Soil No	PH	EC (Dsm^{-1})	P(ppm)	K(ppm)	N%	O.M%	T.N.V%	Sand%	Silt%	Clay%	Texture
0-30 cm	7.5	4.4	2.7	128	0.088	0.94	42.5	58	23	19	Sandy Loam
0-60 cm	7.7	7.2	2.5	130	0.088	1.1	40.8	60	21	19	Sandy Loam

Four samples were taken from each plot and from each of the three replications, the samples were completely dried and ground, and an NIR machine (Near Infrared Reflectance Spectroscopy, model DA 7200, made in Sweden) was employed to measure water-soluble carbohydrates (WSC), and fiber content (CF) [12]. Previous methods [13, 14] were used to measure the proline content and the relative water content percentage, respectively.

SAS 9.1 was used to perform the calculations, and Duncan's test was employed for comparison of the means of the data at 1 and 5 percent probability levels.

RESULTS AND DISCUSSION

Water-soluble carbohydrates (WSC)

The maximum water-soluble carbohydrates content (8.91%) was observed in the Bastan cultivar and the minimum (7.49%) in the Isfahan cultivar (Table 2). Water-soluble carbohydrates are required for the continuation of plant growth and development. Therefore, determination of the suitable time for forage consumption is very important [15]. Drought stress increases the percentage of water soluble- carbohydrates [10]. Drought increased the content of soluble carbohydrates in sesame leaves [16]. Reduced duration of seed filling stage (because it coincided with the warm and dry conditions at the end of the growing season) caused a limited increase in seed carbohydrate content, increased protein percentage and reduced seed weight [17].

Table 2. Comparison of the means related to the individual effects on physiological characteristics of millet cultivars under irrigation cessation and nitroxin application

Individual effects of irrigation, cultivar, and fertilizer		Water Soluble carbohydrates (%)	Fiber (%)	Proline ($\mu mol/g^{-1}ww^{-1}$)	Relative water content (%)
Irrigation cessation	Control	8.05667	68.4006^{b}	0.69567^{c}	35.5556^{c}
	Start of flowering	7.72056	74.1744^{a}	1.90633^{a}	48.8333^{a}
	End of flowering	8.22833	70.525^{b}	1.10356^{b}	42.8333^{b}
Cultivar	Bastan	8.91167^{a}	70.1289	1.13722^{b}	42^{b}
	Pishahang	7.59444^{b}	71.3767	1.34728^{a}	40.6111^{b}
	Isfahan	7.49944^{b}	71.5944	1.22106^{ab}	44.6111^{a}
Nitroxin	Applied (+)	8.10963	70.8352	1.34478^{a}	43.2222^{a}
	Not applied (-)	7.89407	71.2315	1.12559^{b}	41.5926^{b}

Treatments with common letters are not significantly different based on Duncan's multiple range tests at the 1% and 5% probability level

Fiber Content (FC)

Comparison of the means related to the individual effects (Table 2) suggests that the highest fiber content (74.17%) was obtained in the treatment of irrigation cessation when flowering started, and that severe stress increased fiber content percentage [10]. Fiber content percentage in pearl forage millet and sorghum produced under drought stress decreased compared to the fiber content of these crops when they were grown under irrigation [4]. With increasing plant age, their digestibility does not change, while the chemical structure of cell walls changes and plant total fiber content increases [18]. The effects of nitrogen on silage corn and reported that increasing the nitrogen application rate reduced fiber content.

Table 3. ANOVA (mean squares) concerning the effects of irrigation cessation, fertilizer, and cultivar on agronomic and physiological characteristics of forage millet cultivars

Sources of change	DF	Water soluble carbohydrates	Fiber	Proline	Relative water content
Block	2	5.873	57.323	10	36.685
Irrigation cessation (B)	2	1.201	153.509*	6.830**	795.796**
Main error	4	1.686	13.109	0.020	8.269
Cultivar (A)	2	11.215**	11.257	0.201**	74.241**
Fertilizer (C)	1	0.627	2.120	0.649**	35.852*
A*B	4	1.339	8.972	0.104*	7.491
B*C	2	0.315	4.995	0.210**	6.685
A*C	2	0.168	1.297	0.642**	88.353**
B*C*A	4	0.883	16.391	0.801**	21.602*
Minor error	30	0.542	10.098	0.027	6.807

The symbols ns, *, and ** represent not significant, significant at 5%, and significant at 1% probability levels, respectively

Relative water content (RWC)

Result of ANOVA in Table 3 show that the effects of drought stress, cultivar, and the mutual effects of cultivar and fertilizer were significant at 1% probability level. Irrigation cessation had its maximum effect (48.83%) at the start of flowering; and, the cultivar Isfahan had the highest relative water content (44.61%) among the cultivars (Table 2). Compared to the other treatments, Nitroxin biofertilizer application (with 43.22%) had the greatest effect on RWC (Table 2). Cultivars sensitive to drought mainly used the mechanism of drought evasion by closing their stomata and through preserving their water (thus having greater RWC), while cultivars semi-resistant and resistant to drought mainly depended on the drought tolerance mechanism [19]. Drought stress caused greater decrease in the relative water content (RWC) of the susceptible cultivar [20]. Water loss severely reduced the relative water content of the plants [21]. Khazaei and Borzooei [19] carried out research on wheat and observed that drought stress significantly reduced leaf chlorophyll and relative water contents.

Proline

The highest proline content (1.90 $\mu mol/g^{-1}ww^{-1}$) was observed in the treatment in which irrigation cessation happened at the start of flowering (Table 3), but Nitroxin biofertilizer application had a greater effect on proline concentration and raised it to 1.34 μmol/g-1ww-1 (Table 2). In this research (and in other studies too) increased proline concentration in plant organs became evident when water shortage worsened, and in most studies this rise in proline concentration was attributed to osmosis regulation by plants. Undoubtedly, proline accumulation under stressful environmental conditions plays a part in enhancing the survival capability of plants [8]. Drought stress increased leaf proline content in wheat [22]. Irrigation cessation and the resultant drought stress increased proline content in the aerial organs of sesame [23].

CONCLUSIONS

In general, the cultivar Bastan performed better than the other two cultivars. The biofertilizer nitroxin also had meaningful effects on the studied characteristics. Considering that millet is a forgotten crop in the region, and that farmers prefer to plant crops such as corn and alfalfa instead of millet and there is also this very important point that an autumn crop can be planted after millet is harvested, which is an added reason why millet should be planted. The climatic conditions in this region (the high temperature) and millet's short growing season improve its growth. Necessary recommendations can be made for farmers to plant fast growing millet cultivars (and to apply the Biofertilizer Nitroxin) instead of the crops that are commonly planted in the region.

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The authors declare that there is no conflict of interests.

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