JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2018/22145

Application of Azotobacter and Mycorrihzal Fungus on Yield and Yield Components of Wheat Varieties and Quality of Derived Seeds under Supplementary Irrigation

Abdolghader Enayati[1], Morteza Barmaki[1]*, Raouf Seyed Sharifi[1] and Abdollatif Gholizadeh[2]

[1]Faculty of Agronomy and Plant Breeding, College of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran; m_barmaki@uma.ac.ir, abenayati@gmail.com, raouf_ssharifi@yahoo.com
[2]Department of Plant Production, University of Gonbad Kavous, Gonbad Kavous, Iran; latif_gholizadeh@yahoo.com

Abstract

In order to study effects of Azotobacter and Mycorrhiza fungus on yield and yield components of wheat varieties and quality of derived seeds under supplementary irrigation, an experiment was conducted as a factorial arranged in a RCBD with three replications in Agricultural Research Center of Gorgan. Experimental factors including two irrigated wheat varieties including N-80-20 and Gonbad Cv., six levels of inoculations including control (without biologic fertilizer), application of Mycorrhiza (*Glomus intraradices*), inoculation with *Azotobacter crococum* (powdery), inoculation with commercial liquid Azotobacter and Mycorrhiza, inoculation with *Azotobacter crococum* (powdery) and Mycorrhiza, inoculation with commercial liquid Azotobacter and Mycorrhiza, and three levels of supplementary irrigation (non-irrigated, irrigation at booting and irrigation at full heading phases). Results revealed that inoculation of wheat varieties with a combination of Azotobacter and Mycorrhiza induced seed yield and yield components increment as well as quality improvement of derived seeds. Supplementary irrigation at heading phase had more positive effects on traits of wheat varieties. Considering to the investigated characteristics, N-80-20 had a better response to seed inoculation and supplementary irrigation comparing to Gonbad cultivar. It seems that, application of biological fertilizers and supplementary irrigation is an appropriate and low-cost method for increasing of yield and improvement of seed quality of wheat. In order to increase the seed yield and improvement of seed quality, inoculation of seeds with a joint combination of Azotobacter and Mycorrihza along with supplementary irrigation at heading stage of wheat could be recommended.

Keywords: Biologic Fertilizer, Seed Quality, Terminal Drought Stress, Yield, Yield Component

1. Introduction

Water shortages are becoming prevalent in all plant production systems. More than 35% of the world population uses wheat as a staple food, and arid and semi-arid zones consist of around 60% of land area in the world. In 2016, the cereal production in the world was about 6947 million tons and wheat production was 749 million tons[1]. In Iran, wheat is grown in more than 7 million hectares of agricultural land, with a production value of about 14 million tons[1]. Therefore, considering the growing population of the country and the world, it is important to increase crop production[2].

One of the important factor determining the yield is seed quality because the seed is the main factor in agricultural production[3]. One of the simple and most economical ways to achieve high-quality seeds is adding nutrients to the soil and foliar application[4], as well as supplementary irrigation in wheat fields[5].

*Author for correspondence

Exploring drought resistance physiological mechanisms among different wheat genotypes is the important way for finding new drought resistance gene resources and conventional breeding, and the basis for wheat drought resistance biotechnological breeding and platform[6].

Application of biofertilizers is one of the ways to improve the nutritional status of plants. Biofertilizers also reduce the use of chemical fertilizers. Researchers have reported that the inoculation of Azotobacter and Mycorrhiza increased significantly the spike per square meter compared to without inoculation treatment[7]. According to Zaidi and Khan (2005)[8], the application of *Azotobacter chroococcum, Pseudomonas striata* and *Glomus fasciculatum* caused a positive effect on plant vigor, nutrient uptake, and yield in wheat plants. In the other long-term study, the researchers have reported increase in shoot height, shoot diameter, sugars, proteins, and photosynthetic pigments by application of *Azotobacter chroococcum* and *Arbuscular Mycorrhizal* fungi on *Jatropha curcas*[9].

One of the most important means to achieve the goals of sustainable agriculture is to extent the application of biological fertilizers especially under drought condition. The aim of this study was to investigate the Azotobacter and Mycorrihza fungus effects on yield and yield components of wheat varieties and quality of derived seeds under supplementary irrigation.

2. Materials and Methods

2.1 Experimental Location and Design

The experiment was conducted as a factorial experiment based on randomized complete block design with three replications in the Agricultural Research Center of Golestan (Araghi mahale Research Station) (30 km south east of Caspian Sea), Iran, in 2013. Geographical coordinates of the farm are 36°, 54.00' N, 54°, 25.00' E and altitude of 2m. The 25-year mean temperature, humidity and precipitation recorded were 17.7° C, 70% and 617mm, respectively.

The experiment was carried out as a factorial based on randomized complete block design. First factor was wheat varieties (N-80-20 and Gonbad Cv.), second factor was different kind of biologic fertilizer systems (control) (without biologic fertilizer), application of Mycorrhiza (*Glomus intraradices*), inoculation with *Azotobacter crococum* (powdery), inoculation with commercial liquid Azotobacter, inoculation with *Azotobacter crococum* (powdery) with Mycorrhiza, inoculation with commercial liquid Azotobacter and Mycorrhiza, and third factor was levels of supplementary irrigation, non-irrigated, irrigation at booting and irrigation at full heading phases.

2.2 Crop Management

Before planting, wheat seeds were inoculated according to treaments mentioned with standard methods[10]. The field was as fallow as before year. Field operation was done according to usual of region methods (plow, disc land levelling, and furrow). Fertilizer levels were determined after soil analysis (Table 1). Each plot contained 6 rows with 4-meter length and a distance of 0.2m, with a constant density of 450 plants per square meter. In the stem extension stage of wheat, weeds chemical control were carried out using the Puma super (for the control of grassy weeds) and Granstar herbicide (to fight broadleaf weeds).

In addition, supplementary irrigation was performed in two full booting and full flowering. The final harvest was carried out at the time of drying the stems under the spike and reaching the seeds to hardening and moisture content of about 14%. At the end of the growth period, some properties were measured such as yield, yield components (1000 seed weight, number of spikes per unit area, number of seeds per spike), plant height, harvest index, biological yield. Then the harvested seeds were transferred to the laboratory of seed technology. At the laboratory, some seed traits were determined such as wet gluten, dry gluten, protein, falling number, starch.

2.3 Data Analysis

Data were analyzed using the general linear model (GLM) procedure of the statistical analysis system, SAS

Table 1. Some physicochemical properties of the soil at 0-30cm

Ec (dS/m)	1.7	Mn (ppm)	4
pH	7.6	Fe (ppm)	7.5
OM%	2.4	Clay %	34.86
C	1.65	Silt %	47.23
P (ppm)	13	Sand %	17.9
K (ppm)	370	N (kg/ha)	30.6

9.1. As analysis of variance showed significant treatment effects, Duncan's multiple range tests was applied to compare the means at P ≤ 0.05.

3. Results and Discussion

3.1 Yield and Yield Components

The results of analysis of variance are presented in Table 2. Supplementary irrigation had significant effects on the number of spikes, the number of seeds per spike, 1000-seeds weight, seed yield, biological yield and harvest index (P ≤ 0.01). Wheat cultivars showed a significant difference in terms of plant height, spike length, spike number, number of seeds per spike, 1000-seeds weight, seed yield and biological yield (P ≤ 0.01). Seed inoculation also had a significant difference for all traits listed in Table 2 at the probability level of 1%. The supplementary irrigation with cultivar interaction effects were significant in terms of the number of spikes, the number of seeds per spike, 1000 seed weight, grain yield, biological yield, and harvest index at 1% probability level. The supplementary irrigation with inoculation interaction effects were significant on 1000 seed weight and biological yield at 1% probability level and grain yield at 5% probability level. The cultivar with seed inoculation interaction had significant effects on spike length and grain yield at 5% probability level and 1000 seed weight and biological yield at 1% probability level.

Highest and lowest plant height were obtained by inoculation with commercial liquid Azotobacter and Mycorrhiza (87.71cm) and non-inoculated with Mycorrhiza (80.62cm), respectively. Also, N-80-20 cultivar was 2cm taller than the Gonbad cultivar (Table 3).

Application of biofertilizer led to the increase of spike length in both cultivars. Inoculation of wheat seeds with Mycorrhiza had more positive effects on spike length compare to Azotobacter. But the combination of these two bio-fertilizers showed the longest spike in both wheat cultivars. So the highest spike length (10.03cm) obtained by N-80-20 line and inoculation with commercial liquid azotobacter with Mycorrhiza, also the lowest amount (7.91cm) was observed by Gonbad cultivar and control (Table 4).

In relation to spike number, application of biofertilizer led to the increase of spike number, also supplementary

Table 2. Means squares of treatments effects on yield and yield components

Source of Variation	d.f	Means Square							
		Plant height	Spike length	Number of spike	Seed number per spike	1000-seeds weight	Seed yield	Biological yield	Harvest index
Block	2	230.29**	2.114**	11120**	167.6**	495.25**	2.48**	4460613**	843.5**
Supplementary irrigation	2	0.036	0.033	12433**	150.17**	206.32**	5784958**	3.21**	5.43**
Cultivar	1	126.32**	13.3**	195670**	320.33**	1862**	3913634**	2.93**	0.038
Seed inoculation	5	107.1**	5.134**	14179**	83.79**	73.16**	887577**	3330911**	6.115**
Supplementary irrigation × Cultivar	2	0.203	0.038	4214**	9.75**	9.3**	23336*	1459211**	4.717**
Supplementary irrigation × Seed inoculation	10	0.141	0.013	334.4	2.05**	1.59*	11672*	36112	0.626
Cultivar × Seed inoculation	5	0.034	0.052*	204.1	1.11	2.47**	14353*	191292**	0.697
Supplementary irrigation × Cultivar × Seed inoculation	10	0.165	0.005	408.69	0.56	0.835	5090.03	34842	0.328
Error	70	0.083	0.013	403.05	0.66	0.743	6036.7	45093	0.334
CV%		0.242	1.254	4.546	3.10	1.831	1.456	1.446	1.589

*, **: Significant at 5% and 1% probability levels, respectively.

Table 3. Means comparison between interaction between irrigation and cultivars treatments on yield and yield components

Treatments		Number of spike	Seed number per spike	1000-seeds weight (g)	Seed yield (kg/ha)	Biological yield (kg/ha)	Harvest index (%)
non-irrigated	N-80-20	360.7 e	27.3 b	41.36 f	4735 d	13026 e	36.3 b
	Gonbad	470.7 a	23.0 d	48.50 c	5117 c	14298 c	35.6 c
irrigation at booting	N-80-20	406.7 c	26.6 c	42.26 e	5138 c	14106 d	36.3 b
	Gonbad	481.5 a	22.8 d	51.04 b	5569 b	15384 b	36.1 b
irrigation at full heading phases	N-80-20	373.8 d	29.6 a	45.16 d	5562 b	15254 b	36.3 b
	Gonbad	444.4 b	27.3 b	54.16 a	5892 a	15832 a	37.1 a

Different letters indicate significant difference at P ≤ 0.05, based on Duncan's multiple range test.

Table 4. Means comparison between interaction irrigation and cultivars treatments on yield and yield components

Treatments		Spike length (cm)	1000-seeds weight (g)	Seed yield (kg/ha)	Biological yield (kg/ha)
N-80-20	T_1	8.68 g	48.78 e	5266.89 g	14653.10 e
	T_2	9.38 d	51.77 bc	5549.56 c	15270.00 b
	T_3	8.98 f	50.12 d	5391.33 ef	14951.90 cd
	T_4	9.14 e	50.93 cd	5463.11 de	15164.00 bc
	T_5	9.69 b	52.44 ab	5689.00 b	15416.40 ab
	T_6	10.03 a	53.37 a	5798.56 a	15574.90 a
Gonbad	T_1	7.91 j	39.22 k	4835.78 k	13217.70 i
	T_2	8.66 g	43.76 h	5170.78 h	14299.30 fg
	T_3	8.20 i	41.38 j	4953.44 j	13879.80 h
	T_4	8.40 h	42.69 i	5067.78 i	14165.10 g
	T_5	9.02 f	44.74 g	5331.67 fg	14487.70 ef
	T_6	9.50 c	45.79 f	5514.67 cd	14725.40 de

T1: non-inoculated + mycorrihza as check, T2: application of mycorrihza, T3: seed inoculation with pure race of *Azotobacter crococum* (powdery), T4: inoculation with commercial liquid Azotobacter + mycorrihza, T5: inoculation with pure race of *Azotobacter crococum* + Mycorrihza, T6: inoculation with commercial liquid Azotobacter + Mycorrihza. Different letters indicate significant difference at P ≤ 0.05, based on Duncan's multiple range test.

irrigation had significant effects on spike number and highest means observed by N-80-20 with irrigation at booting. Generally, the number of spike per unit area of N-80-20 lines had more positive responses to supplemental irrigation as compared to Gonbad cultivar (Table 5).

Also, supplementary irrigation at flowering stage of inoculated seeds with Mycorrhiza fungi and Azotobacter bacterium increased the number of seeds per spike than control (non-irrigation supplementation). So, the highest number of seeds per spike (32.33) was observed by liquid Azotobacter with Mycorrhiza, totally liquid Azotobacter had more positive effects on the number of seeds per spike by compared to the pure race of Azotobacter crococum (powdery) (Table 6).

In relation to 1000-seeds weight, it was founded that highest weight (54.16 g) obtained by N-80-20 line under supplementary irrigation at flowering stage, between two studied varieties, N-80-20 had the highest 1000-seeds weight under all irrigation treatment compared to Gonbad variety (Table 5). According to the interaction between irrigation and biofertilizer, the highest 1000-seeds weight was obtained by commercial liquid Azotobacter with Mycorrhiza and irrigation at full heading phases (Table 6).

Table 5. Means comparison between interaction irrigation and cultivars on yield and yield component

Treatments		Number of spike	Seed number per spike	1000-seeds weight	Seed yield (kg/ha)	Biological yield (kg/ha)	Harvest index (%)
non-irrigated	N-80-20	360.7 e	27.3 b	41.36 f	4735 d	13026 e	36.3 b
	Gonbad	470.7 a	23.0 d	48.50 c	5117 c	14298 c	35.6 c
irrigation at booting	N-80-20	406.7 c	26.6 c	42.26 e	5138 c	14106 d	36.3 b
	Gonbad	481.5 a	22.8 d	51.04 b	5569 b	15384 b	36.1 b
irrigation at full heading phases	N-80-20	373.8 d	29.6 a	45.16 d	5562 b	15254 b	36.3 b
	Gonbad	444.4 b	27.3 b	54.16 a	5892 a	15832 a	37.1 a

Different letters indicate significant difference at P ≤ 0.05, based on Duncan's multiple range test.

Table 6. Means comparison between interaction irrigation and inoculations on studied traits

Tretaments		Seed number per spike	1000seed weight (g)	Seed yield (kg/ha)	Harvest index (%)
non-irrigated	T_1	22.83 ij	40.83 m	4684 m	36.38 b-f
	T_2	25.16 fg	46.05 hi	4935 k	35.68 fg
	T_3	23.66 hi	43.38 l	4762 lm	35.19 g
	T_4	25.16 fg	44.7 jk	4832 l	35.17 b-f
	T_5	26.50 de	46.91 gh	5090 j	36.44 a-c
	T_6	27.83 c	47.7 fg	5252 hi	37.09 fg
Irrigation at booting	T_1	22.00 j	43.83 kl	4989 k	35.71 e-g
	T_2	25.33 e-g	47.06 gh	5371 g	35.98 a-f
	T_3	23.16 i	45.41 ij	5178 ij	35.77 a
	T_4	24.5 gh	46.3 hi	5301 gh	35.86 b-f
	T_5	25.83 ef	48.05 e-g	5555 f	36.74 a-d
	T_6	27.5 cd	49.26 cde	5730 cd	37.42 a
Irrigation at full heading phases	T_1	25.16 fg	47.33 f-h	5479 f	36.47 b-f
	T_2	28.33 c	50.16 bc	5773 c	36.85 a-d
	T_3	26.33 ef	48.45 d-f	5576 ef	36.36 c-f
	T_4	27.66 c	49.43 cd	5662 de	36.41 c-f
	T_5	31.00 b	50.81 ab	5885 b	37.15 a-c
	T_6	32.33 a	51.76 a	5987 a	37.29 ab

T1: non-inoculated + mycorrihza as check, T2: application of mycorrihza, T3: seed inoculation with pure race of *Azotobacter crococum* (powdery), T4: inoculation with commercial liquid Azotobacter + mycorrihza, T5: inoculation with pure race of *Azotobacter crococum* + Mycorrihza, T6: inoculation with commercial liquid Azotobacter + Mycorrihza. Different letters indicate significant difference at P ≤ 0.05, based on Duncan's multiple range test.

According to interaction results between irrigation and biofertilizer treatments, the highest seed yield (5987 kg/ha) obtained by commercial liquid Azotobacter with Mycorrhiza and irrigation at full heading phases. Inoculation had the positive effect on the increase of seed yield and N-80-20 showed 10% the increase of seed yield under commercial liquid Azotobacter with Mycorrhiza treatment, irrigation treatments had different effects on genotypes, N-80-20 under irrigation at full heading phases showed 13% increase as compared to control (Table 6).

Biological yield increased by application of supplementary irrigation, between irrigation treatments, irrigation at full heading phases had the highest effect and N-80-20 variety had the highest means (15832 kg/ha) under irrigation at full heading phases (Table 5). Also, it was founded that application of biofertilizer led to the increase of biological yield of studied genotypes and N-80-20 had the highest means with the application of commercial liquid Azotobacter with Mycorrhiza treatment (Table 4).

Further increase in crop yield under water shortage condition is one of the main challenges for farmers. In this study, application of biofertilizer led to the increase of yield and yield components. It seems that inoculation helps the plants to achieve more water under deficit irrigation. The results were in agreement with earlier reports that grain and straw yields improved significantly following inoculation with phosphate solubilizing microorganisms or the vesicular-arbuscular Mycorrhizal fungus especially with the combination of two microorganisms[11]. Khalid *et al.*[12] has reported that growth responses in wheat after the inoculation with biologic fertilizer basically depends on various factors like plant genotype, nature of inoculants, as well as environmental conditions.

Table 7. Means squares of treatments effects on physiological and seed quality of wheat cultivars

Source of Variation	d.f	Means Square				
		Wet gluten	Dry gluten	Protein	Falling number	Starch
Block	2	38.191**	9.69**	3.432**	1696.687**	44.601**
Supplementary irrigation	2	32.69**	8.18**	1.436**	14700.056**	95.011**
Cultivar	1	11.28**	2.55**	13.049**	3886.08**	1092.521**
Seed inoculation	5	11.04**	2.72**	0.525**	2012.483**	125.64**
Supplementary irrigation × Cultivar	2	0.106**	0.058**	0.056**	139.002**	4.508
Supplementary irrigation × Seed inoculation	10	0.037**	0.009**	0.013**	15.992*	2.607
Cultivar × Seed inoculation	5	0.008	0.004	0.016**	7.212	0.47
Supplementary irrigation × Cultivar × Seed inoculation	10	0.028	0.005	0.006	1.293	1.367
Error	70	0.014	0.003	0.005	7.814	2.419
CV%	-	1.4	1.55	1.7	2	3.3

*, **: Significant at 5% and 1% probability levels, respectively.

Many previous studies have also shown plant inoculation with the microorganism from harsh environments resulted in significantly higher survival of drought-stressed plants and in greater photosynthesis and biomass production[13, 14].

Application of biofertilizer led to the increase of biological yield of studied varieties. Increasing of biomass and seedling height of maize with inoculation of *Glomus mosseae* and three bacterial species has been reported by Wu *et al.*[15]. The reason for the increase of biological function is due to the use of biological fertilizers, improving soil quality and increasing the availability of plant root to nutrients as expressed by soil microorganisms[16]. Also, it has been indicated that half the amount of biofertilizer application had similar effects when compared with organic fertilizer or chemical fertilizer treatments. Microbial inoculum not only increased the nutritional assimilation of the plant (total N, P, and K) but also improved soil properties, such as organic matter content and total N in soil. The arbuscular Mycorrhizal fungi had a higher root infection rate in the presence of bacterial inoculation.

According to results, highest grain yield (5987 kg/ha) was obtained by commercial liquid Azotobacter with Mycorrhiza. Mamatha *et al.*[17] mentioned that the crop yield of both mulberry and papaya increased when inoculated with AM fungi alone or together with the bacterium. This may be included in the class of Mycorrhiza helper bacterium. Daei *et al.*[18] concluded that the AM species have the significant effect on root colonization of different wheat cultivars. Biologic fertilizers have great potential in increasing nutrient profiles, plant growth, and productivity, and improving tolerance to environmental stress. Photosynthetic efficiency and the anti-oxidative response of rice plants subjected to drought stress were found to increase after inoculation of Arbuscular Mycorrhiza[19].

3.2 Physiological Traits and Seed Quality of Studied Variety

According to the analysis of variance, supplementary irrigation, cultivar, and seed inoculation had significant effects on stomatal conductivity and chlorophyll ($P \leq 0.01$) (Table 7). N-80-20 line under non-irrigated had the highest value of gluten (31.12 and 10.81% for wet and dry gluten, respectively) and both genotypes showed gluten's reduction by application of irrigation treatments (Table 8).

Irrigation and bio fertilizers interaction treatments showed inoculation with commercial liquid Azotobacter with Mycorrhiza under all irrigation treatments had the highest wet gluten (31.87%) and dry gluten (11.18%), so, this bio fertilizer treatment was the best treatment for the achievement of gluten under all supplementary irrigation (Table 9). These biologic fertilizers are considered as efficient microbial competitors in the root zone, and the net effect of plant-microbe associations on plant growth could be positive, neutral or negative[20]. Increasing nitrogen content in seed treatment with Azotobacter, as well as Mycorrhiza, increased nitrogen content in the plant and consequently increased gluten[21].

The highest protein (12.51%) was achieved by N-80-20 under non-irrigated (Table 8). In all different irrigation treatments, the highest protein percentage produced by N-80-20 line. Variation in protein percentage is the result of differences in genetic constitution.

Between irrigation and inoculations interaction treatments, inoculation with commercial liquid

Table 8. Means comparison between interaction irrigation and cultivars on seed quality of wheat

Treatments		Wet gluten (%)	Dry gluten (%)	Protein (%)	Falling number (s)
non-irrigated	N-80-20	31.12 a	10.81 a	12.51 a	327.00 a
	Gonbad	30.47 b	10.5 b	11.76 d	310.82 b
irrigation at booting	N-80-20	29.94 c	10.23 c	12.25 b	300.10 c
	Gonbad	29.18 d	9.842 d	11.52 e	288.67 d
irrigation at full heading phases	N-80-20	29.19 d	9.831 d	12.04 c	283.04 e
	Gonbad	28.65 e	9.601 e	11.44 f	274.60 f

Different letters indicate significant difference at P ≤ 0.05, based on Duncan's multiple range test.

Table 9. Means comparison between interaction irrigation and inoculation on seed quality of wheat

Treatments		Wet gluten (%)	Dry gluten (%)	Protein (%)	Falling number (s)
Non-irrigated	T_1	29.53 g	10.02 h	11.94 ef	292.7 f
	T_2	30.2 e	10.38 f	12.03 de	285.5 g
	T_3	30.65 d	10.6 d	12.12 c	271.5 i
	T_4	31.03 c	10.74 c	12.18 bc	271.7 i
	T_5	31.48 b	10.99 b	12.23 ab	271.2 i
	T_6	31.87 a	11.18 a	12.31 a	280.4 h
Irrigation at booting	T_1	28.42 j	9.46 j	11.65 jk	311.6 d
	T_2	29.05 h	9.77 j	11.77 hi	302.3 e
	T_3	29.4 g	9.98 h	11.85 gh	285.7 g
	T_4	29.75 f	10.13 g	11.92 fg	286.0 g
	T_5	30.22 e	10.36 f	12.00 ef	285.5 g
	T_6	30.53 d	10.52 e	12.10 cd	295.0 f
Irrigation at full heading phases	T_1	27.78 k	9.14 m	11.40 l	336.8 a
	T_2	28.45 j	9.48 l	11.59 k	328.9 b
	T_3	28.85 i	9.69 k	11.72 ij	309.0 d
	T_4	29.18 h	9.86 i	11.85 gh	309.6 d
	T_5	29.5 g	10.00 h	11.91 fg	308.2 d
	T_6	29.75 f	10.13 g	11.98 ef	321.0 c

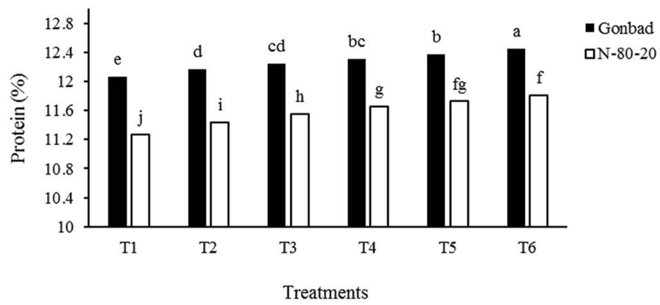
T1: non-inoculated + mycorrihza as check, T2: application of mycorrihza, T3: seed inoculation with pure race of *Azotobacter crococum* (powdery), T4: inoculation with commercial liquid Azotobacter + mycorrihza, T5: inoculation with pure race of *Azotobacter crococum* + Mycorrihza, T6: inoculation with commercial liquid Azotobacter + Mycorrihza. Different letters indicate significant difference at P ≤ 0.05, based on Duncan's multiple range test.

Azotobacter with Mycorrhiza under non-irrigated showed 12.31% as highest protein means. Also, cultivar and seed inoculation interaction treatments showed the highest protein percentage (12.45%) obtained in Gonbad cultivar with the application of inoculation with commercial liquid Azotobacter with Mycorrhiza. In all seed inoculation treatments, Gonbad cultivar produced the highest seed protein percentage (Figure 1).

Kilic and Yagbasanlar[22] has reported increased protein content under drought stress on wheat cultivars. Gooding *et al.*[23] determined the increase in grain protein content in the applied stress before drying, due to the lower effect of stress on nitrogen harvest index compared to dry matter harvest index. Another reason for increase in the percentage of protein in conditions of moisture stress is the accumulation of thermal shock proteins with different molecular weights in growing and reaching seeds[24].

All simple treatments effects showed significant at 1% statistically level on falling number and starch (Table 3). According to the interaction between cultivar and irrigation, line N-80-20 showed 327s as the highest falling number under non-irrigated treatment (Table 8). Also, it was found that application of Mycorrhiza under irrigation at full heading phases had the highest falling number (336.8s) (Table 9).

Application of irrigation at booting and irrigation at full heading phases increased 1 and 3% the starch by compared to the non-irrigated condition. Also, Gonbad cultivar showed 70.77% as the highest starch percentage. Between inoculation treatments, the maximum falling number (336.8s) was achieved by Mycorrhiza application (Table 10).



T1: non-inoculated + mycorrihza as check, T2: application of mycorrihza, T3: seed inoculation with pure race of *Azotobacter crococum* (powdery), T4: inoculation with commercial liquid Azotobacter + mycorrihza, T5: inoculation with pure race of *Azotobacter crococum* + Mycorrihza, T6: inoculation with commercial liquid Azotobacter + Mycorrihza. Different letters indicate significant difference at P ≤ 0.05, based on Duncan's multiple range test.

Figure 1. Effect of different seed inoculation treatments on seed protein of wheat varieties.

Table 10. Means comparison between main effect of factors on Starch

Treatments	Starch (%)
Supplementary Irrigation	
non-irrigated	66.22 c
irrigation at booting	67.16 b
irrigation at full heading phases	69.38 a
Cultivar	
N-80-20	64.61 b
Gonbad	70.77 a
Seed inoculation	
T_1	65.21 d
T_2	71.22 a
T_3	65.32 d
T_4	65.34 d
T_5	68.69 c
T_6	69.74 b

T1: non-inoculated + mycorrihza as check, T2: application of mycorrihza, T3: seed inoculation with pure race of *Azotobacter crococum* (powdery), T4: inoculation with commercial liquid Azotobacter + mycorrihza, T5: inoculation with pure race of *Azotobacter crococum* + Mycorrihza, T6: inoculation with commercial liquid Azotobacter + Mycorrihza. Different letters indicate significant difference at P ≤ 0.05, based on Duncan's multiple range test.

Falling number measures alpha-amylase enzyme activity in bread wheat. In this regard, the researchers have stated that one of the first enzymes for starch synthesis is phosphoryl adenyltransferase, which under deficit irrigation is significantly decreased. Therefore, the phenomena of decreased starch synthesis compared to protein content[25]. Gooding *et al.*[23] reported that the maximum number of falling was observed 15-28 days after pollen stage in wheat cultivars under water deficit.

4. Conclusions

Biofertilizer has been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. Based on the present study, results revealed that inoculation of wheat varieties with the combination of Azotobacter and Mycorrhiza induced seed yield and yield components increment as well as quality improvement of derived seeds. Supplementary irrigation at heading phase had more positive effects on traits of wheat varieties. Considering the investigated characteristics, N-80-20 had a better response to seed inoculation and supplementary irrigation comparing to Gonbad cultivar. It seems that application of biological fertilizers and supplementary irrigation is an appropriate and low-cost method for increasing of yield and improvement of seed quality of wheat. Results suggests that, in order to increase of seed yield and improvement of seed quality, inoculation of seeds with the joint combination of Azotobacter and Mycorrhiza along with supplementary irrigation at heading stage of wheat could be recommended.

5. Acknowledgments

This work was supported by University of Mohaghegh Ardabili, Iran. We are thankful for the financial and laboratory support of this project.

6. References

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