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Research Article

EFFECT OF BIOINOCULANTS ON WHEAT (*Triticum aestivum* L.) AT DIFFERENT GROWTH STAGES UNDER VARYING SOIL MOISTURE REGIMES

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Abstract: Wheat is cool season crop, widely cultivated under varied agro-climatic condition. To overcome the problem of drought, biofertilizers have been used to improve water uptake efficiency and plant nutrients in sustainable agriculture. Some microorganisms like *Arbuscular mycorrhizae* (AM) have positive effects on plant growth by improving physiological and biochemical activities. An experiment was conducted on wheat to find suitable growth responses in genotype WH 1142 under restricted irrigation. The experiment was designed as split plot consisting of three irrigation schedules *viz*., one irrigation at crown root initiation (CRI), two irrigations at CRI and heading stage and three irrigations at CRI, tillering, heading stage in main plot and five bio-inoculants treatments *viz*., recommended dose of fertilizers (RDF), *Arbuscular mycorrhizae*(AM) with *Azotobacter* and PSB (75% of RDF), AM fungi with RDF, *Azotobacter* with PSB and RDF, AM fungi with 75% of RDF in the sub-plots with three replication. Arbuscular mycorrhizal (AM) fungi inoculation significantly enhanced water relating parameters by increasing the severity of drought from three irrigations to single irrigation.

Keywords: Biofertilizers, Drought, Fungi inoculation

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Introduction

Improvement Wheat (*Triticum aestivum* L.) is the second-most produced crop on earth, lagging behind only corn and India is second largest consumer of wheat after China [1]. As a major cereal crop, wheat accounts for about 30% of the world cereal area to provide food for 36% of the global population. India contributing 30.37% million ha with 96.64 % million tons yield and productivity is 29.88 q/ha, while Haryana contributes 25.40 lakh ha with 10.74 million tons and productivity is 42.28 q/ha. Drought stress not only affects plant growth and development but ultimately productivity in almost all the cereals, thus it is one of the most serious threats to world agriculture [2]. Crops demonstrate various morphological, physiological, biochemical, and molecular responses to tackle drought stress resulting growth inhibition, stomatal closure with consecutive reduction of transpiration, decrease in osmotic potential and inhibition of various plant water relating parameters [3]. In plants, metabolism of reactive oxygen species (ROS), such as superoxide radicals (O_2.-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) is kept in dynamic balance [4]. Under water stress conditions, this balance is disturbed and antioxidant systems are needed to decrease the damage to tissues. To achieve the targeted production of 321 million tonnes of food grain by 2020, the requirement of nutrient will be 28.8 million tonnes, while their availability will be only 21.6 million tonnes being a deficit of about 7.2 million tons. The increasing yield potential has indisputable importance in solving world hunger issue. To overcome this problem, indiscriminate use of synthetic fertilizers has led to the pollution and contamination of the soil, water basins, destroyed micro-organisms and friendly insects, making the crop more prone to diseases and reduced soil fertility. Bio-fertilizers contains microorganism which promote the adequate supply of nutrients to the host plants and ensure their proper development of growth and regulation in their physiology [5]. *Arbuscular mycorrhizae* (AM) fungi are obligate symbionts that colonize the roots of terrestrial plants and from the arbuscular that is a specialized hyphae structure that develops inside cortex cells, and represents the main site of nutrients exchange between partners to alleviate the problem of drought stress, there are many strategies, of which arbuscular mycorrhizal fungi is an efficient and new way to enable plants to grow well under drought-prone environments [6]. The AM fungi enhance growth and reproductive success when associated with AM fungi. Moreover, AM fungi ameliorate soil quality and improve the ability of host plants to withstand water stress and disease thus increasing plant performances [7]. However, the application of AM fungi inoculation to mitigate the adverse effect of water stress on growth, physiology and yield of wheat plants is still unexplored.

Material and Methods

Wheat variety WH 1142 was selected to study the response of biofertilizers under varying level of soil moisture. The seeds were obtained from Wheat Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. The AM fungi obtained from Mycorrhizal Section, The Energy and Resources Institute (TERI), New Delhi, while the *Azotobacter* biofertilizers (azoteeka) and PSB biofertilizers (phosphoteeka) were obtained from Department of Microbiology, CCS Haryana Agricultural University, Hisar. Before sowing the seeds were inoculated with AM fungi, *Azotobacter*and PSB(Phosphate Solubilizing Bacteria).Strains used for AM Fungi and *Azotobacter*were Glomus mosseaeand *Azotobacter*croococum, respectively. Recommended dose of fertilizers and crop protection measures was adopted as per packages and practices. The experiment was conducted in field at Crop Physiology Research Area, Department of Agronomy, CCS Haryana Agricultural University, Hisar. The plot size for each treatment was 2.7 x 5 m with 12 rows of 5 m length at 22.5 cm spacing. Research area is situated in semi-arid and sub-tropical region at 29º-10º N latitude and 75º-46º E longitudes with an altitude of 215.2 m above mean sea level. In the experiment replicates are used. Samples from each plot were collected for various physiological parameters *i.e.,* plant water relation were recorded at two stages *i.e*., anthesis and 20 days after anthesis (in flag leaf only). Water potential (Ψw) of flag leaf was measured with the help of Pressure Chamber (Model 3005, Soil Moisture Equipment Corporation, Santa Barbara, CA, USA), between 10 to 11 AM.

Table-1 Effect of bio-inoculants and soil moisture on leaf water potential and leaf osmotic potential at different growth stages of wheat

Moisture Level	Bioinoclulants	Leaf water potential (-MPa)				Leaf osmotic potential (-MPa)			
		2015-16		2016-17		2015-16		2016-17	
		Anthesis	20DAA	Anthesis	20DAA	Anthesis	20DAA	Anthesis	20DAA
One irrigation	RDF	0.85	1.26	0.77	1.19	1.30	1.65	1.22	1.47
	RDF+Azoto +PSB	0.82	1.24	0.75	1.18	1.28	1.59	1.25	1.27
	RDF+AM fungi	0.74	1.16	0.62	0.93	1.13	1.26	1.10	1.31
	75 RDF+AM fungi	0.91	1.28	0.86	1.21	1.38	1.76	1.27	1.22
	75RDF+AMfungi+Azoto +PSB	0.86	1.26	0.83	1.19	1.33	1.72	1.16	1.34
	Mean	0.84	1.24	0.77	1.13	1.28	1.60	1.20	1.32
Two irrigations	RDF	0.70	1.02	0.67	1.00	1.10	1.83	1.28	1.55
	RDF+Azoto +PSB	0.65	0.97	0.62	0.97	1.03	1.74	1.33	1.46
	RDF+AM fungi	0.58	0.89	0.56	0.83	0.86	1.78	1.29	1.50
	75RDF+AM fungi	0.72	1.14	0.71	1.09	1.17	1.69	1.28	1.41
	75RDF+AMfungi +Azoto +PSB	0.71	1.08	0.69	1.05	1.12	1.72	1.34	1.44
	Mean	0.67	1.02	0.65	0.97	1.06	1.75	1.31	1.47
Three irrigations	RDF	0.63	0.95	0.57	0.68	0.86	1.60	1.33	1.32
	RDF+Azoto +PSB	0.62	0.92	0.54	0.67	0.84	1.58	1.34	1.30
	RDF+AM fungi	0.56	0.81	0.47	0.61	0.68	1.45	1.39	1.17
	75 RDF+AM fungi	0.68	1.07	0.64	0.77	0.97	1.61	1.30	1.33
	75RDF+AMfungi +Azoto +PSB	0.64	1.04	0.61	0.72	0.91	1.48	1.33	1.20
	Mean	0.63	0.96	0.57	0.66	0.85	1.54	1.34	1.26
CD at 5 % level	Moisture levels (I)	0.12	0.10	0.13	0.15	0.20	0.10	0.08	0.12
	Bio-inoculates (B)	0.13	0.11	0.15	0.17	0.26	0.12	NS	0.12
	I x B	0.18	0.12	0.17	0.20	0.31	0.18	NS	0.16

*RDF- Recommended dose of fertilizer; 75 RDF - 75 % of Recommended dose of fertilizer; AM fungi- Arbuscular mycorrhizae fungi; Azoto- Azotobacter; PSB- Phosphate solubilizing bacteria; DAA- days after anthesis

Table-2 Effect of bio-inoculants and soil moisture on relative membrane injury and canopy temperature depression at different growth stages of wheat

Moisture Level	Bioinoclulants	Relative membrane Injury (%)				CTD(^{0}C)			
		2015-16		2016-17		2015-16		2016-17	
		Anthesis	20DAA	Anthesis	20DAA	Anthesis	20DAA	Anthesis	20DAA
One Irrigation	RDF	26.3	38.1	22.1	30.8	1.33	3.33	1.23	2.83
	RDF+Azoto +PSB	24.4	36.6	20.7	28.3	1.02	3.07	0.95	2.56
	RDF+AM fungi	21.7	31.3	16.8	20.7	0.58	2.26	0.82	2.14
	75 RDF+AM fungi	29.8	41.8	26.6	35.1	1.91	3.91	1.80	3.53
	75RDF+AM+Azoto +PSB	27.4	40.2	24.5	33.7	1.69	3.74	1.61	3.19
	Mean	25.9	37.6	22.2	29.7	1.31	3.26	1.28	2.85
Two Irrigations	RDF	22.5	27.4	17.5	23.6	0.88	1.88	0.77	1.53
	RDF+Azoto +PSB	20.1	25.4	16.0	21.4	0.68	1.68	0.69	1.27
	RDF+AM fungi	17.7	20.4	14.9	17.7	-0.47	0.47	-0.67	0.83
	75RDF+AM fungi	26.4	32.0	19.9	30.3	1.39	2.39	1.32	2.16
	75RDF+AM fungi+Azoto +PSB	25.0	29.0	19.1	28.4	1.21	2.10	1.01	2.03
	Mean	22.3	26.8	17.5	24.3	0.74	1.70	0.62	1.57
Three Irrigations	RDF	20.7	22.8	14.7	20.6	0.09	1.03	-0.94	1.06
	RDF+Azoto +PSB	18.8	20.1	14.0	19.8	-0.08	0.81	-1.14	0.95
	RDF+AM fungi	15.6	18.6	13.1	17.0	-1.09	0.43	-1.49	0.42
	75 RDF+AM fungi	22.1	25.8	17.3	25.7	1.07	1.90	0.90	1.76
	75 RDF+AM fungi +Azoto +PSB	20.7	23.3	16.8	23.5	0.99	1.60	0.70	1.53
	Mean	19.6	22.1	15.2	21.3	0.20	1.15	-0.39	1.15
CD at 5 % Level	Moisture levels (I)	1.6	1.8	1.5	1.6	0.41	0.36	0.26	0.32
	Bio-inoculates (B)	2.4	2.7	2.1	2.4	0.53	0.43	0.35	0.43
	I x B	2.8	3.2	2.7	3.1	0.82	0.65	0.47	0.52

*RDF- Recommended dose of fertilizer; 75 RDF - 75 % of Recommended dose of fertilizer; AM fungi- Arbuscular mycorrhizae fungi; AzotoAzotobacter; PSB- Phosphate solubilizing bacteria;DAA- days after anthesis

The osmotic potential of flag leaf was estimated with psychrometric technique using Vapour Pressure Osmometer (Wescor INC., Lorganan, Utah, USA). Relative water content (RWC) of flag leaf was measured and Canopy temperature (CT) measurements were made using hand held Infrared Thermometer (IRT), Model AG-42, Tele temp Crop, Fullerton. Five measurements were taken per plot at approximately 0.5 m from the edge of the plot and approximately 1.0 m above the canopy with an approximately 30-60° from the horizontal. The canopy temperature (CT) and ambient temperature (AT) were measured between 13:00 to 14:00 hours on cloudless, bright and no windy day. Analysis of variance of phenotypic traits was done by using OPSTAT software available on CCS HAU, Hisar, website hau.ernet.in [8].

Results and Discussion

Water relation was measured in terms of leaf water potential, leaf osmotic potential and relative water content leaf water potential decreased significantly under restricted irrigation as compared to normal irrigated condition [Table-1]. The decreased was more in one irrigation as compare to two and three irrigations during both the years and times of observation. The decrease in leaf water potential was more pronounced at 20 DAA than at anthesis stage in all the irrigation environments. Among the bio-inoculants, AM fungi with recommended dose of fertilizers showed highest leaf water potential in all irrigation environments. Reduction in fertilizer dose to 75 % of recommended dose significantly reduced the leaf water potential. Application of AM fungi, *Azotobacter* and PSB compensated the leaf water potential to some extent the fertilizer reduction influence. The interaction among irrigation levels and bioinoculants were significant at both stages and year of observations. The similar response of bio-inoculants under drought [9]. Osmotic potential of leaf decreased under moisture stress condition was also accompanied by change in relative water content and

Table-3 Effect of moisture levels and bio-inoculants on grain yield, biological yield and harvest index of wheat

Moisture Level	Bioinoclulants Treatment	Grain yield (kg/ha)		Biological yield (kg/ha)		Harvest index (%)	
		2015-16	2016-17	2015-16	2016-17	2015-16	2016-17
One irrigation	RDF	3897	3944	11268	11620	34.6	34.0
	RDF+Azoto +PSB	3979	4073	11185	11737	35.6	34.8
	RDF+AM fungi	4143	4214	11549	12089	35.9	34.9
	75 RDF+AM fungi	3345	3439	10751	11033	31.1	31.2
	75RDF+AM fungi+Azoto +PSB	3415	3509	10927	11268	31.3	31.2
	Mean	3756	3836	11136	11549	33.7	33.2
Two irrigations	RDF	4026	4190	11608	11971	34.7	35.0
	RDF+Azoto +PSB	4167	4249	11925	12207	34.9	34.9
	RDF+AM fungi	4319	4484	12453	12617	34.7	35.5
	75RDF+AM fungi	3592	3662	11138	11268	32.3	32.5
	75RDF+AM fungi+Azoto +PSB	3638	3744	11221	11549	32.4	32.4
	Mean	3948	4066	11669	11923	33.8	34.1
Three irrigations	RDF	4906	5000	12582	12676	39.0	39.4
	RDF+Azoto +PSB	5000	5106	12793	12887	39.1	39.6
	RDF+AM fungi	5235	5364	13087	13333	40.0	40.3
	75 RDF+AM fungi	4390	4542	11925	11737	36.8	38.7
	75 RDF+AM fungi+Azoto +PSB	4448	4636	12031	11972	37.0	38.7
	Mean	4796	4930	12484	12521	38.4	39.3
CD at 5 % Level	Moisture levels (I)	184	98	184	201	2.4	3.1
	Bio-inoculates (B)	112	124	232	286	3.2	3.6
	I x B	136	185	306	374	4.3	4.8

*RDF- Recommended dose of fertilizer; 75 RDF - 75 % of Recommended dose of fertilizer; AM fungi- Arbuscular mycorrhizae fungi; AzotoAzotobacter; PSB- Phosphate solubilizing bacteria; DAA- days after anthesis

thus pointing to a higher or lower osmoregulation. The value of osmotic potential decreased from anthesis to 20 days after anthesis mentioned in [Table-2]. The highest value of osmotic potential was recorded under irrigated control than restricted irrigation treatment, which may be due to ample water supply to the tissue resulting from increased water absorption from the soil [10]. The more negative value of osmotic potential was contributed to maintain the turgor and it could be one of the reasons for performing various physiological functions even at low water potential. Similar, decrease in osmotic potential under water stress [11,12]. The inoculation with AM fungi proved to the best performer in terms of maintaining higher leaf osmotic potential among the other bio-inoculants used in the study. The increased plant water status by use of AM fungi [13]. Membrane stability was estimated on percent leakage of electrolytes *i.e.*, relative stress injury. The results revealed that membrane stability significantly decrease and relative stress injury increased under water stress. Effect of water was more pronounced at 20 days after anthesis stage as compare to anthesis stage [Table-3]. The AM fungi inoculation maintained the maximum membrane stability followed by *Azotobacter* with PSB. The lowest membrane stability was recorded in one irrigation with 75 % RDF + AM fungi inoculation. Results obtained in this investigation are in accordance with the findings of other researchers where they reported that the electrolyte leakage activity increased under drought stress and bio-inoculation activities seem to be co-ordinately functioning in the plant as an adaptive response to modulated water stress tolerance and minimizing the stress damage [14]. The crop canopy was warmer in one irrigation treatment as compared to two and three irrigation at anthesis and 20 days after anthesis [Table-2]. Canopy temperature depression decreased significantly from anthesis to 20 days after anthesis stage of observation (0.20 to 1.15) during 2015-16 and (-0.39 to 1.15) during 2016-17 in normal irrigated condition. Crop subjected to one irrigation environment showed 84.7 % and 64.8 % lower CTD at anthesis and 20 days after anthesis stages as compare to irrigated control condition during 2015-16. The bioinoculants RDF +AM fungi showed 56.3 % and 32.1 % higher in CTD, whereas RDF+*Azotobacter*+PSB increased the canopy temperature depression by 23.3 % and 7.8 % over RDF at both stages during 2015-16 season. The interaction of irrigation environment and bio-inoculants treatment was found significant at both stages of observation and years of studies. Drought stress is the most wide spread environmental stress, that affect growth and productivity resulting reduction in grain yield. The drought conditions could decline its yield between 23 to 27% in world's leading wheat belts by 2050 [15]. Other researchers also expressed that the average of some morpho-physiological traits was decreased under drought stress results in reduction of yield and related components of wheat include number of tillers per plant, number of spikelet's per spike, number of grains per spike, grains weight per spike, test weight [16,17]. The inoculation with AM fungi provided an important enhancement to yield in wheat cultivars and an increase in straw and grains yield, and test weight of wheat plant [18]. The reduction in grain yield was maximum in one irrigation environment (28.5 %) and two irrigation (6 %) over normal irrigation level irrespective of years. Crop subjected to normal irrigation condition showed higher grain yield (4796 and 4930 kg/ha) as compared to the plants grown in restricted irrigated environment during both years [Table-3]. The addition of bioinoculants increased the grain yield. Maximum response was observed in AM fungi followed by *Azotobacter* and PSB. Among the bioinoculants, maximum increase was observed with RDF with AM fungi (5235 and 5364 kg/ha) followed by RDF+ *Azotobacter* +PSB (5000 and 5106 kg/ha) and lowest was found by 75 % RDF with AM fungi (4390 and 4542 kg/ha) in the normal irrigation condition. The interaction of bio-inoculants and irrigation environment was found significant during both years. Therefore, the present studies on symbiotic relationship between arbuscular mycorrhizal fungi (AM fungi) and the roots of higher plants have demonstrated that AM fungi symbiosis is a key component in helping plants to cope with water stress and in increasing drought resistance by enhancing the plant growth by maintaining water relations parameter.

Conclusion

The research was aimed to study and analyze differential behaviour of bio-inoculants under varying soil moisture regimes in relation to plant water related parameters. The bio-inoculants along with recommended dose of fertilizer (RDF) were used include AM fungi, *Azotobacter* and Phosphate Solubilizing Bacteria (PSB) and with reduced dose of fertilizers to 75% RDF. The physiological parameters related to plant water relations were observed in flag leaves at anthesis and 20 days after anthesis while yield characteristics were recorded at harvest. Results showed that various physiological and yield characteristics were adversely affected under drought stress (one irrigation environment), while treatments of bio-inoculants with arbuscular mycorrhizal fungi could alleviate the effect of drought stress and thus played a protective role under stress conditions. The plant water relation parameters such as water potential, relative water content of leaves and canopy temperature depression decreased significantly under drought stress. Application of bio-inoculants improved relative water content, water potential and canopy temperature depression under drought stress. Absolute value of water potential and osmotic potential were comparatively high (less negative) with AM fungi + *Azotobacter* with PSB under all the irrigation environments. The percent reduction in leaf water potential and leaf osmotic potential was more in one irrigation than three irrigations environment at anthesis stage as well as at 20 days after anthesis stage. The effect of soil moisture stress was compensated by use of AM fungi along with *Azotobacter* and PSB treatment to some extent. Significant shift in plant water status under drought stress occurs in one irrigation environment, involved a decline in relative membrane injury and canopy temperature depression. A significant increase was observed in all the above parameters with bio-inoculants treatment. Response of AM fungi with *Azotobacter* and PSB was relatively more than other bio-inoculants treatments under two and three irrigation environments.

Application of research: This research is a part of bioorganic farming.
Research Category: Wheat, bioinoculants

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