Journal of Experimental Biology and Agricultural Sciences, June - 2017; Volume – 5(3)

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

EFFECT OF CUMULATIVE APPLICATION OF NITROGEN AND PGPR ON THE VARIOUS TRAITS OF BARLEY (*Hordeum vulgare* L.) UNDER DROUGHT STRESS CONDITIONS

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Received – October 19, 2016; Revision – December 24, 2016; Accepted – June 17, 2017
Available Online – June 30, 2017

DOI: http://dx.doi.org/10.18006/2017.5(3).302.308

KEYWORDS

Drought stress

Nitrogen, PGPR

Grain yield and biological yield

ABSTRACT

This study was contducted to evaluate the effect of cumulative application of nitrogen and PGPR on various growth attributes of barley under drought stress condition. Study was conducted in factorial split plot design on the basis of randomized complete blocks design with 4 replications in 2011-2012 at the research farm of Islamic Azad University, Shahr-e-Rey Branch, Tehran, Iran. Three irrigation intervals viz 80, 130 and 180 mm and nitrogen levels i.e. 0 (not used), 75 and 150 kg.ha^{-1} was used as main factor while PGPR containing pure strains of *Azotobacter* (strain12), *Pseudomonas* (strainp-169), *Azospirillum* (strain OF) in two levels (not used and used) was usedas a sub factor. Interaction of drought stress with nitrogen application was found significant on grain yield, biological yield and weight of 1000grain (P<0.01). Further maximum grain (4296.17 kgha^{-1}) and biological yield (9292.25kg.ha^{-1}) was reported from the treatment containing 150 kg N ha^{-1} and 80 mm irrigation. In case of dual interaction, drought stress, nitrogen application and PGPR gave maximum grain yield (3061.08kgha^{-1}) while 180 mm irrigation without PGPR treatment has the lowest grain yield (1161.25 kgha^{-1}).

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI]
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1 Introduction

Barley (*Hordeum vulgare* L.) is one of the major cereal in many dry areas of the world and due to its drought resistance, especially in late growing season which is concurrent with drought period, it is widely grown in arid and semi-arid areas (Golzardi et al., 2015). It’s a strategic crop which is important for food and feed security (Bishaw, 2004). Drought stress at different growth stages is the primary restriction in the performance of these products. Further, moisture stress during grain filling and hastens leaf senescence, reduced the grain filling and mean weight of the grains (Austin, 1989). Similarly, Plaut et al. (2004) reported that reduction in the growth of wheat grain is depending on the degree of moisture stress and on the rate of stress development. Svobodová & Míša (2004) reported that spring barley plants exposed to a water deficit at earlier growth stages (from emergence to the beginning of stem elongation) were able to compensate for the stress by the increase in a productive tiller number; while acute drought stressat stem elongation to anthesis stage was much worse and tillers withered away.

Nitrogen is one of the most important element and plays important role in the formation of various protein, enzymes, coenzymes, nucleic acids and cytochrome and also necessary component of chlorophyll (Shafe et al., 2011). According to Cossani et al. (2011) under rainfed conditions, nitrogen deficiency effect the grain yield. Demotes-mainard &Jeuffroy (2004) showed that nitrogen deficiency during wheat growth period resulted in the loss of spike dry weight as well as grain number per spike. In a study Zaongo et al. (1997) studied the effect of different nitrogen rates on the sorghum yield and reported a direct relationship between N and 1000-grain weight. Further, Ryan et al. (2009) reported that higher nitrogen concentration increased the leaf area, tiller formation, LAI and LAD and this augmenting is led to much greater production of dry matter and grain yield. Similarly, Shaban (2013) suggested that for getting highest seed yield addition of nitrogen fertilizer is necessary. Intensive farming practices, that warrant high performance and quality, required extensive use of chemical fertilizers which are costly and also create environmental problems. Recently, there has been a resurgence of interest in eco-friendly, sustainable and organic agricultural practices (Esitken et al., 2005).

Plant growth promoting rhizobacteria (PGPR) are the soil bacteria inhabiting around/on the root surface and facilitate the plant growth (Wu et al., 2005). PGPR can be use as an alternative to mineral fertilizers and help in enhancing soil productivity and plant growth in sustainable agriculture. These PGPR not only increasing nutrient cycling, but also help in suppressing pathogens by producing antibiotics and fungal antagonistic substances. Khalid et al. (2004) reported that bacteria such as *Pseudomonas*, *Azospirillum* and *Azotobacter* have stimulatory effect on the plant growth. The inoculation of PGPR can reduced the famers dependency on the N-fertilizers and also prevent the evacuation of soil organic matter which play an important role in decreasing environmental pollution up to a considerable extent (Kennedy et al., 2004).Yield can be analyzed in terms of different yield components, some of these have higher importance than others, this depending on the timing and intensity of stresses and their temporal development (Aggarwal & Sinha, 1987). This study was formulated to find out the effect of cumulative application of nitrogen fertilization and PGPR on various growths attributes of barley under drought stress conditions.

2 Materials and Methods

Study was conducted in factorial split plot design on the basis of randomized complete blocks design with 4 replications in croping year 2011-2012 at the research station of Islamic Azad University, Shahr-e-Rey Branch, Tehran, Iran. In this experiment, three irrigation intervals viz. 80, 130 and 180 mm evaporation from class A evaporation pan and three nitrogen levels, 0 (not used), 75 and 150 $kg.ha^{-1}$ were used as main factor and plant growth promoting rhizobacteria containing combination of *Azotobacter*(strain 12), *Pseudomonas* (strain p-169), *Azospirillum* (strain OF) at two levels viz 0-PGPR (not used) and PGPR-Inoculation (seed coating) was considered as a sub factor. Cell densities of PGPR per plote were 10^7 CFU/gm. Seeds were coated with gum Arabic as an adhesive and rolled into the suspension of bacteria until uniformly coated. Physicochemical property of pot soil was determine by standard methodology given by various researchers (Table 1). Among this total nitrogen (N) was determined by using Kjeldahl method (Okalebo et al., 1993) while organic carbon (OC), available phosphorus (P), potassium (K), Iron (Fe), Zinc (Zn), Manganese (Mn) and Copper (Cu) was determined by the method given by Sparks et al. (1996).

Adequate plant protection measures and agronomic practices were made during the crop growth. Plot size was maintained 4 × 2 m^2 (row × row distance was 20 cm), among this 50 cm space in either side of each row was left. Data were collected on the maturation of crop (150 days after sowing) and various growth attributes were recorded by standard methodology. Yield was calculated in terms of $kg.ha^{-1}$ while harvesting index was calculated by below method given by Kozak & Mądry (2006).

$$HI = (\text{Economical yield} / \text{Biological yield}) \times 100$$

For measuring the number of grain $spike^{-1}$, 30 spikes $treatment^{-1}$ were randomly selected from each sub plot and it was followed by the counting number of grains $spike^{-1}$ manually, this procedure was repeated for all the collected 30 spikes and average of this were used as number of grain $spike^{-1}$. Number of spike in m^2 was also recorded manually by counting the number of spike in one m^2 in one meter area of the three central rows in each subplot and their mean was then calculated. For calculation of a thousand grain weight after harvesting, 8 samples of 100 seeds in each plots was randomly selected and then the average of a thousand grain weight was estimated by multiplying this average with 10. Statistical analysis was performed using SAS 9.2 (SAS 2009) and EXCEL software and the means were compared using LSD test at 5% probability level.

3 RESULTS AND DISCUSSION

3.1 Effect on the weight of 1000 grain

Analysis of variance with respect to weight of 1000-grain revealed that interaction of drought stress and nitrogen fertilizer, drought stress and PGPR was significant ($P<0.01$); but other interaction were not statistically significant (Table 2). Among various tested treatments, highest weight of 1000-grain was achieved in dual interaction of drought stress and nitrogen fertilizer treatment while the lowest was reported from the untreated control. Treatment containing 80 mm irrigation and 150 kg N ha^{-1} treatment has the higher (42.80 g) weight of 1000 grain while the treatment containing 180 mm irrigation without nitrogen has the lowest weight (26.35 g) of 1000 grain (Table 3). Further in case of treatment containing combined application of drought stress and PGPR, highest weight of 1000 grain weight (37.24 g) was reported from the treatment having 80mm irrigation from evaporation pan and PGPR while treatment containing only 180 irrigation from evaporation pan without PGPR showed lowest weight (27.39 g) of 1000-grain (Table 4). According to Hatfield & Prueger (2004) nitrogen increases the yield of wheat by increasing the number of spikes, grains per spike and weight of 1000-grain. In general, the yield components of wheat are directly affected by nitrogen. Further, Rao et al. (1991) suggested that drought stress at flowering stage can cause reduction in the weight of 1000-grains by reducing the transferring photo assimilates. In addition, these researchers also suggested that weight of 1000 grains also dependent of genetic structure of cultivar also (Rao et al., 1991).

3.2 Effect on Grain Yield

Result of study revealed a significant effect of PGPR and nitrogen fertilizer inoculation on the grain yield. According to the ANOVA, interactive effect of drought stress and nitrogen fertilizer and drought stress and PGPR on the grain yield in barley was significant at 1% probability level (Table 2). Results of study revealed that PGPR can reduce the harmful effects of stress. Nitrogen plays an important role in plant metabolism. It facilitates the vegetative growth of plant by increasing the rate of photosynthesis. Mean comparison of the interactive effects of drought stress and nitrogen fertilizer showed that maximum grain yield belonged to the treatment with consumption of 150 kg N ha^{-1} and irrigation 80 mm irrigation from evaporation pan (4296.17 kg.ha^{-1}) while the lowest grain yield was reported from the treatment containing 180 mm from evaporation pan without N fertilizers (948.85 kg.ha^{-1}) (Table 3). Also in case of dual interaction between drought stress and PGPR, maximum of Grain yield (3061.08 kg.ha^{-1}) was reported from the treatment containing 80 mm irrigation along with PFPR while the treatment containing 180 mm irrigation without PGPR has lowest Grain yield (1161.25 kg.ha^{-1}) (Table 4). These results are in agreement with findings of Zinselmeier et al.(1995), those who reported negative effect of drought stress on grain yield. Results of grain yield and yield components indicated that drought stress during the grain-filling period reduced grain yield by decreasing the number of fertile spikes and grains per plant. González et al. (1999) revealed that drought stress decreased grain yield by decreasing the number of grains per ear and grain weight. Cakmakci et al. (2007) also reported significant effect of PGPR on the plant growth and grain yield in barely plants. Similar types of significant increase in plant growth and final yield on the application of PGPR has been reported by Biswas et al. (2000) and Dobbelaere et al. (2003) in a separate experiments. Kloepper & Beauchamp (1992) stated that plants such as rice, corn and sugar cane inoculated with bacteria had 10 to 30 percent higher yield than the un-inoculated treatments. Similarly, plant treated with PGPR increased dry matter accumulation in plant such as rice (Sudha et al., 1999) and barely (Cakmakci et al., 2007). Pervez et al. (2009) revealed that foliar and soil application of urea significantly increased number of grains spike^{-1}, weight of 1000 grain and grain yield of crop. Mousavi et al. (2011) reported that nitrogen availability at the end of the season in some cases under the nitrogen limitation conditions increases the yield via increasing the grain size. This increase in grain weight is not much able to compensate for the reduction of the number of tillers or the number of grains per spike. Moselhy & Zahran (2002) also reported significant effect of bio and mineral nitrogen fertilization on weight and number of grains spike^{-1} in barely plants.

Table 1. Physico-chemical properties of the study area soil.

depth (cm)	ds.m (EC)	pH	P (ppm)	K (ppm)	N (ppm)	OC (%)	CLAY (%)	SILT (%)	SAND (%)	TEXTURE	Fe (ppm)	Zn (ppm)	Mn (ppm)	Cu (ppm)
0-30	**3.6**	**7.2**	**6.7**	**190**	**1.2**	**1.9**	**21**	**19.5**	**42**	**Sandy-Clay**	**1.5**	**2.4**	**2.5**	**2.5**

Table 2. Analysis of variance for various studied traits of barley

S.O.V.	d.f.	Mean of Squares: 1000 grain weight	Grain yield	Biological yield	Harvest index
Replication	3	13.1038	2685.7029	15606.84	18.38117
Drought (D)	2	266.706**	212814.58**	828467.826**	416.71820**
Nitrogen (N)	2	556.0015**	162835.50**	676399.077**	64.61502*
D × N	4	93.3530**	43429.7073**	161635.25**	63.23841$^{n.s}$
E($_{D×N}$)	24	11.649956	2553.58	14955.153	27.22396
PGPR (P)	1	152.8334$^{n.s}$	12850.28*	39395.309*	116.61190*
D × P	2	20.3718**	514.0232**	2318.902*	12.29553$^{n.s}$
N × P	2	5.23010$^{n.s}$	2964.28$^{n.s}$	14716.417$^{n.s}$	18.47125$^{n.s}$
D × N × P	4	13.4622$^{n.s}$	523.82$^{n.s}$	2818.551$^{n.s}$	13.46320$^{n.s}$
E ($_P$)	27	11.8529	1005.658	5960.384	13.4911
Total	71				
C.V.	-	10.83	15.78	15.57	9.82

n.s. = Non-significant * = Significant at 5% level ** = significant at %1 level

Table 3. Effect of drought stress and nitrogen fertilizers interaction on various studied growth traits

Drought stress	Nitrogen	1000 grain weight (g)	Grain yield (kg.ha^{-1})	Biological yield (kg.ha^{-1})	Harvest index (%)
80 mm	**0 kg.ha^{-1}**	29.88^{e}	1258.51^{e}	3418.86^{ef}	36.95^{cd}
	75 kg.ha^{-1}	35.16^{c}	3281.75^{b}	7654.28^{b}	42.87^{b}
	150 kg.ha^{-1}	42.80^{a}	4296.17^{a}	9292.25^{a}	46.37^{a}
130 mm	**0 kg.ha^{-1}**	27.71^{g}	1045.82^{e}	2936.82^{ef}	3.59^{d}
	75 kg.ha^{-1}	32.55^{d}	2402.77^{d}	6011.06^{d}	39.96^{bc}
	150 kg.ha^{-1}	37.01^{b}	2907.53^{c}	678.01^{c}	42.82^{b}
180 mm	**0 kg.ha^{-1}**	26.35^{h}	948.85^{e}	2754.06^{f}	33.92^{e}
	75 kg.ha^{-1}	28.86^{f}	1143.85^{e}	3377.57^{ef}	34.43^{e}
	150 kg.ha^{-1}	29.28^{ef}	1250.63^{e}	3498.72^{e}	35.80^{d}

Value given in table is mean of four replicates; Values followed by same letter did not differ significantly from LSD test at 5% significance

Table 4. Effect of drought stress and PGPR Interaction on various studied growth traits

Drought stress	PGPR	1000 grain weight (g)	Grain yield (kg.ha^{-1})	Biological yield (kg.ha^{-1})	Harvest index (%)
80 mm	**Not use**	34.65^{b}	2829.87^{b}	6627.76^{b}	41.38^{b}
	used	37.24^{a}	3061.08^{a}	6949.16^{a}	42.75^{a}
130 mm	**Not use**	31.33^{d}	2033.17^{cd}	5137.60^{d}	38.69^{d}
	used	33.52^{c}	2204.25^{c}	5351.66^{c}	40.23^{c}
180 mm	**Not use**	27.39^{f}	1067.64^{e}	3151.24^{f}	33.91^{f}
	used	28.94^{e}	1161.25^{e}	3269.00^{e}	35.52^{e}

Value given in table is mean of four replicates; Values followed by same letter did not differ significantly from LSD test at 5% significance

3.3 Effect on Biological yield (Biomass)

Like weight of one thousand seeds and grain yield, biological yield was also affected by the drought stress and the negative effect of the drought condition can be decline by application of nitrogen fertilizers and PGPR. Analysis of variance suggested that biological yield also significantly influenced by the application of nitrogen fertilizer, PGPR at 1% significance level (Table 2). In dual interaction, combination of drought stress and nitrogen fertilizer have significant effect on plant growth and among various treatments combination, 80 mm irrigation from evaporation pan and use 150 kg.ha^{-1} N treatment has the higher (9292.25kg.ha^{-1}) biological yield while lowest biological yield (2754.06 kg.ha^{-1}) was reported from the individual application of180 mm evaporation pan (Table 3). Similarly, dual interaction of drought stress and PGPR was also proved effective in increasing biological yield (6949.16 kg.ha^{-1}). Here also180 mm irrigation from evaporation pan without PGPR has lowest biological yield (3151.24 kg.ha^{-1}) (Table 4).These results are agreement with the findings of Anbessa &Juskiw (2012), those who has conducted an experiment for finding out the effects of nitrogen on barley cultivars and concluded that biomass related trait was find higher on the application of N fertilizer. Nitrogen availability at the end of the season increases the barley yield by increasing the grain size (Mousavi et al., 2011). Further, Moser et al. (2006), reported negative effect of water stress on the biological yield. Also Stone et al. (2001) suggested that grain yield is strongly related to biological yield especially that accumulated after slicing. Canbolat et al. (2006) reported that inoculation of barley with PGPR strains increased shoot weight up to 40% compared with control.

3.4 Effect on Harvest index

Effect of nitrogen rate and PGPR on harvest index was significant at 5% level; but the effect of irrigation was significant at the 1% level (Table 2). Means comparison for the simple effect of irrigation revealed that the highest and lower Harvest Index percentage (42.07 and 34.72 %) was obtained under 80 mm irrigation and 180 mm irrigation from evaporation pan, respectively (Fig.1). Means comparison indicated that highest harvest index (41.67%) was obtained under application of 150 kg N ha^{-1} while it was reported 35.66% in control (Fig. 2). Inoculation of barley seeds with plant growth-promoting rhizobacteria caused significant differences in the Harvest index of barley. Means comparison of PGPR levels indicated that highest harvest index of barley (39.50%) was obtained under inoculation PGPR than the un-inoculated PGPRcontrol (38%)(Fig. 3). According to Bolaños & Edmeads (1993) value of harvesting index may decrease with decreasing water availability. Water stress reduced the rate of photosynthesis by closing stomata, decreasing leaf area, stomata gravity and chloroplast and protoplast hydration, and protein and chlorophyll synthesis. However, reducing of photosyntate transport accumulates the products in leaves results in diminution in photosynthesis, limiting growth and crop yield (Hornok, 1992). Jayathilake et al. (2006) detected application of *Azospirillum* in combination with vermicompost and chemical fertilizers significantly maximum harvest index (67.3%). Inoculation of seeds with *Azotobacter* and *Azospirillum* in the presence of chemical fertilizers resulted in improving both growth and yield of anise (Gomaa & Abou-Aly, 2001). Lack et al. (2008) also reported that maize HI decreased under drought stress. Perhaps, in addition to decreasing produced dry matter, water deficit disrupts the partitioning of carbohydrates to grains and hence, decreases harvest index.

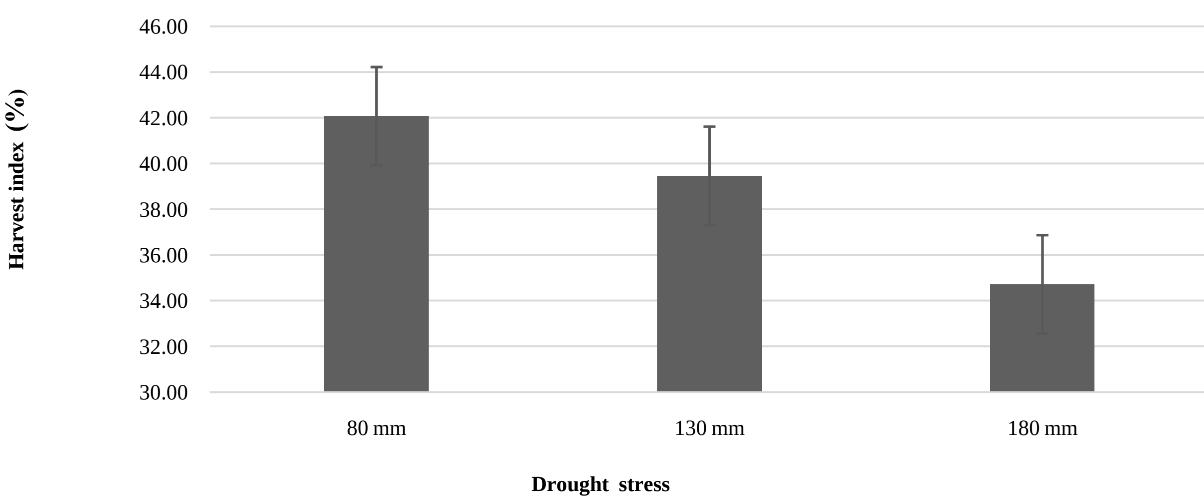

Fig. 1. Effect of drought stress on harvest index of barley

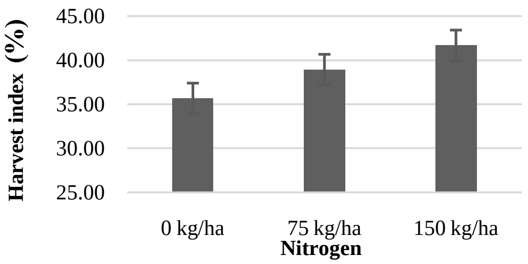

Fig. 2. Effect of nitrogen rate on harvest index of barley

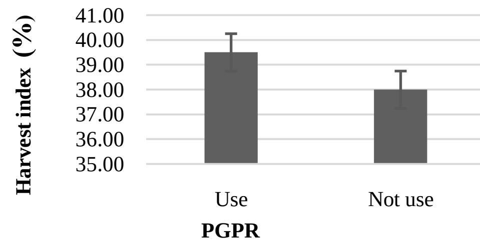

Fig. 3. The effect of PGPR on harvest index of barley

CONCLUSION

Drought stress exertion at seed filling stage decreased yield and yield components of barley. Drought stress decreased amount of photosynthesis transfer to barley grains, there by it decreased yield and yield components. Also the result of study revealed that nitrogen fertilizer had maximum positive impact on yield and yield components of barely grain. In general, it can be conclude that entire growth characteristics were influenced by application of nitrogen fertilizers and PGPR

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