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Evaluation Azospirillum Trends and Urea Fertilizer on Barley Production under Warm and Dry Climate Condition (Ahvaz region, Southwest of Iran)

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ABSTRACT

BACKGROUND: Imbalanced mineral nutrition and scant information about nitrogen (N) in plants may result in reduction in barley morpho-physiological activities. However, farmers use higher or lower fertilizer doses regarding barley growth and yield. Barley is characterized by a rich genetic diversity, making it an important for studies of Azospirillum response with high potential for crop improvement. Moreover, biological fertilizers severely affect barley growth and development, leading to improve yield loss.

OBJECTIVES: Plants of an Azospirillum exposed to 0, 2, and 4 h per 100 kg seed were compared with urea fertilizer levels (75 and 100%) to identify growth pathways to barley's response.

METHODS: Current research was done according split plot experiment based on randomized block design with four replications which urea fertilizer (75% and 100%) was in main plot and Azospirillum trends belonged to sub plots.

RESULT: Regulation of crop growth was severely impacted in leaves, highlighting the complexity of Azospirillum response mechanisms in this tissue. Functional analyses in tissues indicated that response to Azospirillum trends is mainly achieved through sensing and signaling pathways, strong effects to urea amounts and growth, kernel yield and yield components. Azospirillum trends especially lipoferum involved in growth signaling pathways, as well as 100 $kg.ha^{-1}$ urea, were identified. This study provides valuable information on early Azospirillum-responsive in growth indices of barley and identifies several important players in response to chemical fertilizers.

CONCLUSION: Finally based on result of current research apply Azospirillum lipoferum with 100% urea fertilizer led to produce highest seed yield and it can be advised to producers at studied region.

KEYWORDS: *Biofertilizer, Growth indices, Hordeum vulgare L., Nitrogen, Nutrition.*

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1. BACKGROUND

Barley (*Hordeum vulgare* L.) is an important crop grown for different end uses, including food, forage and industrial productions (Acosta-Motos *et al*., 2017). Worldwide, barley is cultivated in 45.53 million hectares with total and average production of 63.44 million tons, respectively (Kwon *et al*., 2019). Chemical fertilizers have several negative impacts on environment and sustainable agriculture. Therefore, bio fertilizers are recommended in these conditions and growth prompting bacteria uses as a replacement of chemical fertilizers (Wu *et al*., 2005). However, its growth and yield is restricted by various biotic and abiotic factors including macronutrients deficiencies especially nitrogen (Li *et al*., 2016). Nitrogen (N) critically plays indispensable role in crop growth, development and yield formation. Adequate supply of N is crucial for maintaining the morpho-physiological and metabolic processes of the crops including nutrients uptake, antioxidant activities, photosynthesis, and respiration (Yousefirad *et al*., 2020). However, it's over supply is a serious problem in intensive agriculture production as it leads to soil acidification, enhancement of reactive N components in the environment as well as modification of soil N structure, with consequent deterioration of the ecosystem (Luo *et al*., 2016) . Therefore, N application in suitable ratio can candidly regulate crop growth and yield, and escape N pollution (Negrao *et al*., 2017). Additionally, the highest effects of N efficiency increased leaf length and width, light interception, and biomass and grains production (Ben Chikha *et al*., 2017). Thus, monitoring of biological fertilizers such as Azospirillum in crops is vital to optimize its accurate rate for recommendation to the growers (Ziemann *et al*, 2020). Substantial consideration has been received recently due to variation in Azospirillum availability caused by human activities (Bahieldin *et al*., 2019). Crop development and grain production vary broadly in response to Azospirillum trends (Hill *et al*., 2019). Growth promoting bacteria induced increasing plant yield as clone in plants root (Gholami *et al*., 2009). Growth prompting bacteria are including Azotobacter, Azospiril-lum and Pseudomonas (Banerjee *et al*., 2006). Tilak (1992) reported positive effects of double-inoculation of Azotobacter and Azospirillum on dry matter of maize and sorghum. To alleviate the problem, integrated plant nutrient management is an option as it utilizes available organic and inorganic nutrients to build ecologically sound and economically viable farming system. Research has suggested that integrated nutrient management strategies involving chemical fertilizers and bio-fertilizers enhance the sustainability of crop production. Integrated plant nutrient management is the combined use of mineral fertilizers with organic resources such as cattle manures, crop residues, urban/rural wastes, composts, green manures and bio-fertilizers (Kemal and Abera, 2015). Recently, significant efforts have been made by researchers to develop Azospirillum trends management tactics for increasing its use efficiency, by taking of accurate source, accurate rate, accurate time, and accurate placement (Ruiz *et al*., 2019). Previous studies have been propounded that barley is pertinent for production on marginal land under suitable trend of Azospirillum (Zhao *et al*., 2020). Application of bio-fertilizers became of great necessity to get a yield with high quality and to avoid the environmental pollution (Shevananda, 2008). Among microorganisms, The Azotobacter has attracted more attention because of their ability to communicate with important crop plants such as wheat, corn, and sorghum (Hegde *et al*., 1999). Azotobacter is a free-living bacteria that stabilizing the molecular nitrogen for stimulating and enhancing plant growth through nitrogen fixation (Pandey *et al*., 1998), increasing the production of hormones (Hegde *et al*, 1999), B vitamins (Rao and Pillai, 1982), the development of the root system and the release of organic acids in the rhizosphere (Gaind and Gaur, 1989). Rai and Caur (1998) studied Azotobacter and Azospirillum and double-inoculation and alone inoculation effects on wheat growth and yield. Double-inoculation of Azotobacter and Azospirillum had positive effects on plant height, spike length, grain yield, biological yield and harvest index in various wheat genotypes. It is proved that hormones such as oxine, giberline and cytokenine are synthesized by many Azotobacter spp (Singh et al., 2004). The most considerable nutrient for enhancing yield and nutritional value of barley is N. Due to wide spread of Azospirillum trends (A.lipoferum, A.irakiens and A.brasilence), modern agriculture is extensively enhance to take the natural environment (Seyfferth *et al*., 2019). Therefore, it is necessary to consume a suitable trend of Azospirillum to achieve higher yield of barley crop. N management at suitable rate is the main significant factor for improving soil fertility and obtaining higher yield (Dodd *et al*., 2017). In spite of the immense importance of N in crop production, impacts of higher levels of N application on morpho-physiological processes of barley growth and development traits are largely unknown. However, much less attention has paid to examining the higher levels of N and Azospirillum trends on barley morpho-physiological activities to achieve higher yield. Deficiency of N in barley crops results in lower biomass production due to reduction in leaf area (Shen *et al*., 2014). Negrao *et al*. (2017) reported that 100 $kg.ha^{-1}$ N applications was optimum to improve barley yield.

2. OBJECTIVES

Based on these premises, a field trial was conducted to optimize a suitable level of N and Azospirillum trends for healthier barley morpho-physiological, and to evaluate appropriate for higher biomass production.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

The unit plot size was 4.0 m × 2.5 m. The spacing between plots and blocks were 0.75 and 1.0 m, respectively. The experiment was conducted in Ahvaz in 2019. Seeds of barley (Sarasari cultivar) were surface-sterilized with 40^{cc} of

Azospirillum trends (A.lipoferum, A.irakiens and A. brasilence) for 1 kg seed solution for 5 min (Ben Chikha, 2017), then thoroughly cultivated in farm in the dark at 25$^{\circ C}$ with split plot based on randomized block design with four replications which urea fertilizer (75% and 100%) was in main plot and azospirillum trends in sub plots.

3.2. Measured Traits

Growth of shoot tissues were measured which CGR (Crop growth rate), LAI (Leaf area index) and NAR (Net Assimilation Rate) at 5 times which a_1, b_1, c_1, a_2, b_2, and c_2 was regression indices. (Soleymani, 2003). Five plants in each time point were harvested, pooled, washed thoroughly and separated for investigating seed yield and 1000 seed weight.

Equ. 1. $LAI=e^{a_1+b_1 t+c_1 t^2}$

Equ. 2. NAR= $(b_2+2c_2t)\, e\, (a_2- a_1) + (b_2+b_1)\, t + (c_2 -c_1)\, t_2$

Equ. 3. CGR=NAR×LAI

3.3. Statistical Analysis

Analysis of variance and mean comparisons were done via Minitab software (Ver. 14.1) and Duncan multiple range test at 5% probability level.

RESULTS AND DISCUSSION

Azospirillum trends and varying levels of N significantly effect on the barley growth parameters including LAI, CGR, NAR, 1000 seed weight and seed yield during growing season (Table 1). Growth analysis is an important characteristic that measures growth status in plants reflecting the ongoing metabolic activities in tissues and that may be used as a reliable indicator of N adjustment. The CGR at 20 to 100 DAA was profoundly affected by N in all Azospirillum trends (Table 2). However, growth indices (CGR, NAR and LAI) were decreased in control, but all recorded varying levels of reduction. As compared to control, the highest seed yield (4745 $kg.ha^{-1}$) due to lipoferum trend was recorded in A. brasilence 4421 $kg.ha^{-1}$ and 3764 $kg.ha^{-1}$, then 1000 seed weight 4745 $kg.ha^{-1}$ and the lowest was in control 29.42 g, and slightly CGR, NAR and LAI higher in A. lipoferun treatments than others.

Table 1. Analysis of variances of mentioned traits

S.O.V	df	Seed yield	1000 seed weight	Crop growth rate	Net assimilation rate	Leaf area index
Replication	3	48.72^{ns}	23.78^{ns}	95.71^{ns}	66.31^{ns}	78.49^{ns}
Nitrogen (A)	1	6445.86**	7336.91**	3022.91**	3447.69**	4118.89**
Error I	3	1572.05	2148.72	924.68	794.55	527.48
Azospirillum (B)	1	3779.55**	5339.61**	2522.69**	2675.24**	3362.74**
A × B	2	2186.42**	3249.55**	1844.65**	1469.72**	1847.91**
Error II	12	948.12	677.63	745.12	589.77	397.43
CV%		6.77	7.54	6.48	6.81	5.86

^{ns} and ** means no significant, significant differences at %1 level.

The results indicated that Azospirillum maintained a greater amount of N in the roots under than the other two trends such as control. A less reduction of growth indices and yield in response to Azospirillum trends has been noted for nitrogen absorption (Zhao *et al*., 2018). The results of our study were in close agreement with the findings obtained with (Roy *et al*., 2020), who reported that barley plants subjected to Azospirillum significantly enhanced the yield due to N uptake among the plants. Increased leaf area index (LAI) through high level of N due to lipoferum trend could be attributed to rolling of leaves, which results in serious to enhance of surface area, and thus might be used as an indicator for determining the growth potential of crop plants (Borghi *et al*., 2019). Azospirillum trends that established high CGR and NAR especially lipoferum had significantly higher potential for preserving N balance in tissues, which reflects their roles on growth (Table 2).

Table 2. Mean comparison effect of treatment on measured traits

Treatment	Seed yield ($kg.ha^{-1}$)	1000 Seed weight (gr)	Crop Growth rate ($gr.m^{-2}.day^{-1}$)	Net assimilation rate ($gr.m^{-2}.day^{-1}$)	Leaf area index
Azospirillum brasilense		g			
75%N	3465^{b}	30.05^{b}	22.84^{b}	7.051^{b}	3.24^{b}
100%N	4421^{a}	33.14^{a}	26.44^{a}	7.388^{a}	3.58^{a}
Azospirillum lipoferum					
75% N	3995^{b}	36.93^{b}	28.35^{b}	8.149^{b}	3.48^{b}
100% N	4745^{a}	40.46^{a}	34.77^{a}	8.894^{a}	3.91^{a}
Azospirillum irakiens					
75% N	3029^{b}	32.83^{b}	24.47^{b}	7.283^{b}	3.36^{b}
100% N	3764^{a}	39.07^{a}	28.98^{a}	7.649^{a}	3.79^{a}
Control					
75% N	2795^{b}	28.74^{b}	14.2^{b}	5.659^{b}	2.51^{b}
100%N	3566^{a}	29.42^{a}	17.48^{a}	5.948^{a}	2.94^{a}

^{a} and ^{b} means significant differences by Duncan test at 5% level, respectively.

As Azospirillum leads to scarcity of nitrogen in the root zone, plants have efficient remobilization. Therefore, N and Azospirillum rolling hold perspectives for utilization in breeding programs aimed at improving the better uptake of elements and genetic potential for higher grain yields (Demidchik, 2018). In addition, Azospirillum showed strong positive with correlations yield, whereas the NAR rate expresses positive correlation with N stress (Table 2) (Gao *et al*., 2018). Growth in leaves is responsive to Azospirillum and correlates with N rate (Wang *et al*., 2019), and is a better indicator of NAR than any indices of plants. Azospirillum trends may have the ability to absorb N and other nutrients from the soil or the ability of root. Plants under N deficiency conditions decreased dry matter production and growth (Farooq *et al*., 2020), showing that CGR and LAI rates were positively correlated (Nazir *et al*., 2021), and high growth (higher leaf area) indicates the role of biological fertilizers. Under N deficit, reduction of traits indicates photosynthesis decline in plant cells, which leads to growth retardation and pheloem efficiency (Yasir *et al*., 2019). In response to Azospirillum, roots generate between the Azospirillum trends may be owing to diverse genetic potential for absorbing of N from the rhizosphere and extending the depth of roots to exploit lower soil horizons for nutrients extraction (Farooq *et al*., 2020). Nouraki *et al*. (2016) reported bacteria have positive role in the production of bio-fertilizers and hormones which play a significant role in regulating plant growth while mixing them with chemical fertilizers as a supplement the level and depth of the roots. This combination also increases the rate of water and nutrient absorbance which raise the rate of growth and photosynthesis. These combination also increase the grain yield, yield components, and biological function, it has been found that bio-fertilizers can be combined with chemical fertilizers in a complementary way to reduce the excessive amount of chemical fertilizers used to grow corn. It was shown that the mixing of biological fertilizers with chemical fertilizers could reduce the needs of chemical fertilizers up to 25% and these results are comparable to the application of 100% chemical fertilizers. Therefore, the best hybrid maze is the single cross 704 that has good yield potential when the chemical fertilizer is used at either 25% or 50% of the current application when mixed with the bio-fertilizer. Azimi *et al*. (2013) found that application of Super nitroplass bio-fertilizer with Phosphate barvar2 treatment has the highest seed yield (7.6 $t.ha^{-1}$) and non-application of bio-fertilizers treatment has the Pishtaz cultivar has the lowest seed yield (6.3 $t.ha^{-1}$). Some researchers also have a significant increase in maize leaf area index of up to 120 kg N ha^{-1} combined with biological fertilizers (Mirshekari *et al*., 2009). Plants strive to alleviate the damaging effects of nutrients stress by altering their metabolism to cope with stress. However, As far as yield and growth indicators is concerned, lipoferum be suggested as N-tolerant, owing to a minimum relative reduction of yield.

CONCLUSION

Finally based on result of current research apply Azospirillum lipoferum with 100% urea fertilizer led to produce highest seed yield and it can be advised to producers at studied region.

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FOOTNOTES

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