Influence of Azotobacter chroococcum, Azospirillum brasilense, Trichoderma harzianum and Tri-calcium phosphate on hydroponically-grown barley grains

Abbas Fadhil Ali1, Faris M. Suhail1, Hussein Ali salim2, Asmaa Hussein Abed1

1College of Agriculture, University of Diyala, Iraq.
2Directorate of Diyala Agriculture, Ministry of Agriculture, Iraq.

Email: h_salim11111@yahoo.com

Abstract

This study investigated to evaluate the effect of Azotobacter chroococcum, Azospirillum brasilense, Trichoderma harzianum and Tri-calcium phosphate on sprouted barley grains under hydroponic culture conditions. The results showed that increase of a total weight of sprouted barley grains in treatments of A. chroococcum 257.3 g, Tri-calcium phosphate (TCP) 255.6 g and A. brasilense 254.3 g, whereas T. harzianum + TCP, A. brasilense + TCP and TCP recorded highest height of sprouted barley 25.8, 25.7 and 25.6 cm respectively, the protein ratio in the roots of sprouted barley was increased in A. chroococcum 19.8% followed by T. harzianum 18.4%, while the protein ratio in the vegetative portion was increased in T. harzianum + TCP, A. brasilense + TCP, A. chroococcum and A. chroococcum + TCP which reached 36.4, 32.7, 32.5 and 31.0 % respectively.

Keywords: barley grains, Azotobacter chroococcum, Azospirillum brasilense, Trichoderma harzianum.

1. Introduction

Barley (Hordeum vulgare L.) belongs to Poaceae family and it is a very important crop after wheat, rice and maize in the world [1]. There is a known truth, don't complete the animals nutrition in their diet unless there is green fodder [2]. Green fodder can be defined as produced sprouts through hydroponic (soil-less) with heights 15 to 20 cm by germinating of edible grains for livestock [3]. The green fodders are germinated grains by hydroponic culture in appropriate circumstances inside specific chambers for growing [4]. The forage grains production by hydroponic gives a height germination percentage in sprouted barley for a short time [5]. Cereal sprouts contribute a large percentage in increment of livestock birth rates and improve their health [6]. For centuries, sprouting grains have been consumed by humans in Asian countries to increase their food value [7]. [8] reported that 1 kg of barley grains produces 7 to 10 kg of green fodder by hydroponic. There are many changes occurring in seeds result of sprouting activities by an increase in enzyme levels, such as convert of fats to essential fatty acids, seed protein to essential amino acids and carbohydrates to sugars [9, 10]. Sprouted barley by hydroponic is distinguished through increasing of vitamin contents, ash, minerals and crude fiber then crude protein [11, 12]. Tri-calcium phosphate (TCP) Ca₃(PO₄)₂ is a natural fertilizer and not costly and it can be alternative of synthetic fertilizer [13], thus Tri-calcium phosphate is a rare and non-renewable resource for all phosphorus fertilizers, where many researchers reported that the reserve rock phosphate may be consumed for 50 years [14]. Trichoderma has potential factors in plant development such as solubilization of phosphate and insoluble minor nutrients in soil [15,16]. Azotobacter and Azospirillum possess some of mechanisms that use to fix atmospheric nitrogen and secretion of auxins, amino acids and vitamins [17-19].

In order to increase the nutritional value of sprouting barley fodder, the current study aimed to evaluate the effect of Azotobacter chroococcum, Azospirillum brasilense, Trichoderma harzianum and Tri-calcium phosphate on sprouted barley grains.

2. Materials and methods

This study was carried out at Soil microbiology Laboratory, Agriculture college, Diyala University under room temperature (20-25 °C) with normal and artificial illumination by using soil-less hydroponic culture, 24 plastic trays at size of 10×15 cm were equipped for barley grains growing. The grains were cleaned and sterilized with sodium hypochlorite by adding 10 mm to 10 liters of water, then the grains soaked for 5 minutes in this solution and washed with tap water for several times in order
to remove the effects of sterilized substance then the grains soaked for 12 hours in water before distributed on the trays. The experiment included 8 treatments such as T1 = Control by adding only water, T2 = A. chroococcum, T3 = A. brasilense, T4 = T. harzianum, T5 = TCP, T6 = A. chroococcum + TCP , T7 = A. brasilense + TCP, T8 = T. harzianum + TCP, each treatment repeated three times. The data of the experiment was analysis in a randomized complete design according to the analysis of variance at P≤0.05 [20]. Each of A. chroococcum, A. brasilense and T. harzianum mixed at rate 100 ml / 200 g of barley grains in addition Arabic gum at a rate 1:10 (gum : water) as the adhesive material then left for one hour before planting, Tri-calcium phosphate (TCP) was added at a rate 2 g /35 g of barley grains, thereafter barley grains were added at a rate of 35 g per replication, then added the water as needed. After 14 days of planting, the following traits were calculated, total weight of sprouted barley grains, total height of sprouted barley grains and estimation of protein ratio for root and vegetative portion, where 0.2 g of the plant sample was dried and ground, then 10 ml of a mixture of perchloric acid (HClO4) and sulfuric acid (H2SO4) was added to the sample and heated on a hot plate to complete the digestion process until the color of the solution becomes clear (colorless) as a sign of complete digestion, then the samples were added in a bottles 50 ml and complete the volume to the mark by adding distilled water, nitrogen was estimated by using a Micro Kjeldahl apparatus [21], the protein content was estimated based on dry weight, as in the following equation:

\[
\% \text{ Protein} = \text{nitrogen percentage} \times 6.25 \quad [22].
\]

3. Results

The total weight of sprouted barley grains was significantly increased in A. chroococcum followed by TCP and A. brasilense where was 257.3, 255.6 and 254.3 g respectively (figure 1).

![Figure 1. Effect of A. chroococcum, A. brasilense, T. harzianum and tri-calcium phosphate on total weight of sprouted barley grains](image_url)

The highest height of sprouted barley was obtained in T. harzianum + TCP, A. brasilense + TCP and TCP where was 25.8, 25.7 and 25.6 cm respectively, followed by A. chroococcum + TCP 24.8 cm, T. harzianum 24.7 cm, A. chroococcum 24.7 cm and A. brasilense 24.1 cm compared to control 22.6 cm (figure 2).
Figure 2. Effect of *A. chroococcum*, *A. brasilense*, *T. harzianum* and tri-calcium phosphate on height of sprouted barley.

The protein ratio in roots was also significantly increased in *A. chroococcum* 19.8% followed by *T. harzianum* 18.4% compared to other treatments (figure 3).

Figure 3. Effect of *A. chroococcum*, *A. brasilense*, *T. harzianum* and tri-calcium phosphate on protein ratio in roots of sprouted barley grains.

The highest of protein ratio in the vegetative portion of sprouted barley was obtained in *T. harzianum* + TCP, *A. brasilense* + TCP, *A. chroococcum* and *A. chroococcum* + TCP which reached 36.4, 32.7, 32.5 and 31.0 % respectively (figure 4).
Figure 4. Effect of *A. chroococcum*, *A. brasilense*, *T. harzianum* and tri-calcium phosphate on protein ratio in vegetative portion of sprouted barley grains

4. Discussion

The results indicated that application of *A. chroococcum*, *A. brasilense*, *T. harzianum* and Tri-calcium phosphate led to a significant increase in the studied traits on barley grains. This finding is accordance with the results of [23] found that some of microorganisms possess technique which act P availability through solubilizing of phosphor in soils that contain rock phosphates. Addition of rock phosphate with three microorganisms led to increase in P content and enhance of wheat plant growth [24]. There are some of direct mechanisms that contribute in development of root and plant growth by the bacteria Azotobacter and Azospirillum such as fixing of nitrogen and secretion auxins, amino acids and some vitamins [17,18,19]. Seeds inoculation with Azotobacter, Azespirillum and Pseudomonas led to increasing of N content in soil and enhancing of plant growth [25]. *A. chroococcum* and *A. barasilense* may applied as a bio-fertilizer for barley cultivation [26]. Applying of Azotobacter and Pseudomonas on the growing seed led to increase a height of the corn plant [27]. The inoculation of Azospirillum with synthetic fertilizers resulted to increase the height of roots and shoots in maize and wheat plants [28]. Inoculation of *Trichoderma* sp result in enhancing of the microbial community and increases nutrient content in the soil [29,30]. Application of *A. chroococcum* and *T. harzianum* led to a significant increase in germination percentage of cucumber seeds reached 80% [31].

Conclusion

It can be concluded that addition of *A. chroococcum*, *A. brasilense*, *T. harzianum* and Tri-calcium phosphate to barley grains under hydroponic system resulted in better improvements in traits of sprouted barley grains which lead to increase in fodder production.

References

[1] Kumar P, Singh S, Shukla R D and Singh V 2017 Effect of NPK and biofertilizer on growth and yield of barley (Hordium vulgare L.) In western uttar Pradesh, Progressive Research – An International Journal, Volume 12 (Special-IV) : 2434-2437.
[2] Shah V D, Makwana M, and Sharma S 2011 Economics of production, processing and marketing of fodder crops in Gujarat, India Research Study No.144.
[3] FAO 2001 Organización de las Naciones Unidas para la Agricultura yla Alimentación. Manual técnico forraje verde hidropónico. Santiago de Chile, Chile. Feed Sci. Technol. 13, 191–202.
[4] Sneath R and McIntosh F 2003 Review of Hydroponic Fodder Production for Beef Cattle 84. Department of Primary Industries, Queensland, Australia, pp. 54.
[5] AL-Saadi M. J, Al-Zubiadi I A H 2016 Effects of Substitution Barley By 10%, 30% of Sprouted Barley on Rumen Characters, Digestibility and Feed Efficiency in Diet of Awassi Male Lambs, International Journal of Science and Research, Volume 5 Issue 4, April, 2228-2233.
[6] Mohsen M K., Abdel-Raouf E.M. Gaafar H.M.A and Yousif A.M. 2015 Nutritional evaluation of sprouted barley grains on agricultural by-products on performance of growing New Zealand white rabbits. World Rural Obs. 7, 96–107.
[7] Resh H M. 2001 Hydroponic Food Production. 6th ed., 567 pp., Woodbridge Press, Santa Barbara, CA.
[8] Gebremedhin W K 2015 Nutritional benefit and economic value of feeding hydroponically grown maize and barley fodder for Konkan Kanyal goats. J. Agric. Vet. Sci. 8, 24–30.
[9] Alsultani, M., Abed, H., Ghazi, R., & Mohammed, M.A. (2020). Electrical Characterization of Thin Films (TiO2: ZnO)x-y (GO)x / FTO Heterojunction Prepared by Spray Pyrolysis Technique. Journal Of Physics: Conference Series, 1591, 012002. doi: 10.1088/1755-1315/1591/1/012002
[10] Prakash D S 2017 Effect of Replacement of Concentrate Mixture by Maize Hydroponic Fodder on Performance of Goat. Master thesis. Maharashtra Animal and Fishery Sciences University, Nagpur.
[11] Peer D and Leeson S 1985 Nutrient content of hydroponically sprouted barley. Animal Feed Science and Technology, 13: 191-202.
[12] Morgan J, Hunter R R and O’Hare R 1992 Limiting factors in hydroponic barley grass production. In the proceeding of the 8th International congress on soil less culture. pp. 241–261.
[13] Medina A and Azcon R 1981 Effectiveness of the application of arbuscular mycorrhiza fungi and organic amendments to improve soil quality and plant performance under stress conditions, J Soil Sci Plant Nutr, Vol. 10, pp. 354-372.
[14] Krey T, Vassilev N, Baum C and Eichler-Löbermann B 2013 “Effects of long-term phosphorus application and plant-growth promoting rhizobacteria on maize phosphorus nutrition under field conditions., Eur J Soil Biol Vol. 55, pp. 124-130.
[15] Altomare C, Norvell W A and Bjorkma T 1999 Solubilization of phosphate and micronutrients by the plant-growth promoting and biocontrol fungus Trichoderma harzianum Rifai 1295–22. Applied and Environmental Microbiology, 65, 2926–2933.
[16] Rawat L, Singh Y and Shukla N 2011 Alleviation of the adverse effects of salinity stress in wheat (Triticum aestivum L.) by seed biopriming with salinity tolerant isolates of Trichoderma harzianum. Plant and Soil, 347, 387–400.
[17] Radwan F I 2002 Response of some maize cultivars to VA mycorrhizal inoculation, bio-fertilization and soil nitrogen application. Alexandria Journal of Agricultural Research 43, 43-56.
[18] Mohammed, M.A., Abdulridha, W.M., Abd, A.N. (2018). Thickness effect on some physical properties of the Ag thin films prepared by thermal evaporation technique. Journal of Global Pharma Technology, 10(3), 613-619.
[19] Akbari G A, Arab S M, Alihkhani H A, Allahdadi I and Arzanesh M H 2007 Isolation and selection of indigenous Azospirillum spp. and the IAA of superior strains effects on wheat roots. World Journal of Agricultural Sciences 3, 523-529.
[20] Fisher R A and Yates 1968 Statistical method for research workers. Oliver and Boyd Ltd. Edinburgh and London, 10.
[21] Haynes R J 1980 A Comparison of two modified kjeldhal digestion techniques for Multi-element plant analysis with conventional wet and dry ashing methods. Comm Soi. Sci. Plant Analysis., 11(5): 459-467.
[22] A.O.A.C. 1970 Official Methods of Analysis II the d . Washington . D.C. Association of official Analytical chemists. P. 1015.
[23] Reyes I, Bernard L and Antoun H 2002 Rock phosphate solubilization and colonization of maize rhizosphere by wild and genetically modified strains of Penicillium rugulosum. Microbial Ecology, v.44, p.39-48.
[24] Omer S A 1998 The role of rock phosphate solubilizing fungi and vesicular-arbuscular mycorrhiza in growth of wheat plants fertilized with rock phosphate. World J. Microbiol. Biotechnol. 14, 211-218.
[25] Foudou Houssein Kamel, Chuman Hameed Saeed, Ashti M. Amin and Saleem Saeed Qader. (2018). Development of in-vitro Sensitivity Testing for Pathogenic Bacteria. Al-Qadisiyah Journal For Agriculture Sciences, 8(2) 1-5.
[26] -Otkay S M 2009 Impact of Bio-fertilizers Application on Improving Barley (Hordeum vulgare) and the Presence of Dehydrin Genes, Middle Eastern and Russian Journal of Plant Science and Biotechnology 58-61.
[27] Zahir A Z, Arshad M and Khalid A 1998 Improving maize yield by inoculation with plant growth promoting rhizobacteria. Pakistan Journal of Soil Science, 15, 7-11.
[28] Intethar Abbas Al-Zuobidy and Majeed K. Abbas Al-Hamzawi. (2015). The Effect of Foliar Application of Seaweed Extract and NPK Fertilizer on some Growth Characteristics and Yield of Cucumber (Cucumis sativus L.). Al-Qadisiyah Journal For Agriculture Sciences, 5(1), 14-23.
[29] Yadav R L, Shukla S K, Suman A and Singh P N. 2009 Trichoderma inoculation and trash management effects on soil microbial biomass, soil respiration, nutrient uptake and yield of ratoon sugarcane under subtropical conditions. Biol. Fertil. Soils, 45, 461-468.
[30] Wagner K, Apostolakis A, Daliakopoulos I and Tsanis I 2016 Can Tomato inoculation with Trichoderma compensate yield and soil health deficiency due to soil salinity, in proceedings of the Egu General Assembly Conference Abstracts, Vienna, Austria, 17–22.
[31] Salmi H A. Sahil F M , Ibrahim M K , Isaac H S, Hussein H H, and Fahmy A H 2012 Integrated control of Rhizoctonia sp causing cucumber damping off disease. Journal of Al-Mustansiriya Sciences Volume 23, No. 1.