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Effect of Azotobacter and Pseudomonas with Mineral Fertilizer on Yield and Yield Components of Malt Barley (Hordeum vulgare L.)

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Abstract
The effect bio-fertilizer composed of Azotobacter and Pseudomonas spp. was investigated on yield and yield components of malt barley (H. vulgar L). The treatments which were laid at Bekoji, a potential malt barley district in the southeastern highlands of Ethiopia, Azotobacter + Pseudomonas + 46/20.2 N/P kg ha -1, Pseudomonas +46/20.2 N/P kg ha -1, 46/20.2 N/P kg ha -1, Azotobacter +46/20.2 N/P kg ha -1, Azotobacter + Pseudomonas, Azotobacter, Pseudomonas, C- (uninoculated and unfertilized) in randomized complete block design(RCBD) with three replications. Results indicated that application of the bio-fertilizer treatments alone resulted in the minimum performance of different agronomic parameters studied, while inorganic fertilizer application with dual inoculation with Azotobacter + Pseudomonas gives the highest and significant effect on grain yield, harvest index, biological yield, plant height, and thousand seed weight as compared to other treatments. But this trend was not observed in the number of spikes for that case the result was reversed. Hence, the study showed that the use of chemical fertilizer along with dual inoculation (Azotobacter +Pseudomonas) should be considered as a component of inputs especially in inorganic farming systems of malt barley production.
Keywords: Malt barley, Azotobacter, Pseudomonas, Bio-fertilizer.
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1. INTRODUCTION

Malt barley (*Hordeum vulgar L.*) is a major income source to smallholder farmers in the highland areas of Ethiopia, particularly where the agro-ecologies are not more productive than other cereal crops (MoA (Ministry of Agriculture), 2018). However, in Ethiopia, barley productivity (2.66 t/ha) is lower compared to that of other barley-producing countries such as the United Arab Emirates, Belgium, and the Netherlands where mean productivities are 8.0, 7.6, and 7.0 t/ha, respectively) (FAOSTAT, 2018). This is due to the combination of genetic, socioeconomic constraints and inappropriate use of integrated technologies. Besides, malt barley requires optimum environmental factors such as altitude, rainfall, and soil pH which range between 2,300 and 3,000 masl, 500 and 1,000 mm and 5.5 and 6.5, respectively. Prefer soil types well-drained light brown and red soil) due to the quality standards of beer factories (Ferede M. et al., 2020).

Nowadays the practice of boosting yield by inorganic fertilizer is conventional but the impact on long-term soil health and productivity is not promising, so using environmentally friendly soil microbes is gaining momentum. Moreover, intensive cultivation due to population growth has seriously depleted the macro and micronutrients in our soil (Getachew A. and Tilahun A., 2017). A part of rhizospheric bacteria is considered plant growth-promoting bacteria (PGPB) due to their positive effect on plant growth and development. Plant growth-promoting bacteria based on their metabolic activity can be grouped into biofertilizers, phytostimulants, or bio-pesticides. These efficient bacteria due to various direct or indirect effects exerted on plants have a crucial role in agricultural sustainability. Recently were reported diverse genera as PGPB like Acetobacter, Achromobacter, Arthrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Burkholderia, Frankia, Phyllobacterium, Pseudomonas, Serratia, and Rhizobium (Ashok K and Vijay SM, 2019). Bacterial strains have different plant growth-promoting (PGP) activities like multi-stress resistances (temperature, pH, salinity) and other properties such as cellulose, phytin, and lecithin degradation, alkaline phosphatase, and alkaline protease activity, and siderophore production. Plant growth-promoting rhizobia (PGPR) can produce hormones that stimulate plant growth, make nutrients available, fix atmospheric nitrogen, act as bio-control agents, and improve soil structure and Carboxylic acid production, decreasing pH and bound calcium and other nutrient availability (Ashok K and Vijay SM, 2019).

Thus, inoculation of plants with these PGPR is accompanied by a significant increase in productivity that results from two main beneficial mechanisms: stimulation of plant growth and protection of plants against soil-borne diseases (Saida A et al., 2015) and could allow growers to reduce the use of synthetic fertilizers and increase the sustainability of crop production. Strains that possessed traits associated with PGPR have been observed to increase the shoot length, root length, and fresh and dry weight of plants in the growth chamber study. Similarly, under the pot trial, maximum crop traits were observed under a single or different consortium. This confirms that this consortium could provide growers with a sustainable approach to reduce synthetic fertilizer usage in wheat production (AS et al., 2019; Ashok K and Vijay SM, 2019; Satyanarayana T and Johri BN, 2012; Sayyed RZ et al., 2019).

In Ethiopia, only a few studies on tef root-associated microorganisms have been undertaken. Accordingly, the effects of PGPR on growth and yield of tef were evaluated by Delelegn W. and Fassil A (2011). Microbial inoculum of two Bacillus species (Bacillus megatherium and Bacillus mucilaginous) improved the growth of the plant as well as the nutritional assimilation of the plant (Saida A et al., 2015). However, information on little has been known about the use of bio-fertilizers on malt barley in our soils, and hence, the present study was undertaken to generate information on the effects of phosphate solubilizing and nitrogen-fixing microorganisms (Azotobacter sp. and Pseudomonas sp.) on yield and yield components of malt barley.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

The study area, Bekoji, is found in the Arsi zone of Oromia Regional State in the southeastern part of Ethiopia. It is located at an altitude of 2780 m.a.s.l. with a latitude of 7°32'N and a longitude of 39°15'E. The area has a long-term mean annual precipitation of 1020 mm and a mean maximum and minimum temperature of 18.6°C and 7.9°C, respectively. The soil texture of the research site is reddish clay with a pH of 5.4.

2.2. Experimental Design and Treatments,

Field trials were laid out in randomized complete block designs (RCBD) with three replications. The treatments comprised Azotobacter + Pseudomonas + 46/20.2 N/P kg ha -1; Pseudomonas +46/20.2 N/P kg ha -1; 46/20.2 N/P kg ha -1; Azotobacter + 46/20.2 N/P kg ha -1; Azotobacter + Pseudomonas; Azotobacter; Pseudomonas; C- (negative control) which is uninoculated and unfertilized.

2.3. Planting and Agronomic Practices

Field experiments were carried out in the three successive years of growing seasons at Bekoji, in the Arsi zone where barley production has high potential. The experimental plot size was 10.4m2 which is 2.6m x 4m x 13 rows of 20 cm apart. The space between each plot was 50 cm. Di-ammonium phosphate (DAP) containing phosphorus and nitrogen at a ratio of 18N:46P was used as a source of inorganic fertilizer. malt barley variety (cv. Holker) was sown at a seed rate of 150 kg ha-1. To inoculate with biological fertilizers, the seeds were first sterilized with 70% ethanol for two minutes and cleaned five times with distilled water, and were mixed with a sugar solution to serve as a sticker. Then the seeds were inoculated with the powder of Pseudomonas and Azotobacter at a rate of 500 g ha-1. The bacteria were applied with a carrier of fine lignite powder capable of passing through 75-106 µm sieves. The inoculants contain a minimum of 108 (at manufacturing date) up to107 (15 days before the expiry date) of viable cells. The Barley seeds were sown on the experimental plots soon after inoculation with the bio-fertilizers. All agronomic practices were carried out as recommended for the area. At harvest, the data recorded were plant height (cm), number of grain per spike, 1000-grain weight (g), biological yield (kg ha-1), harvest index (grain yield/biological yield × 100), grain yield (kg ha-1). Data were analyzed with Proc GLM procedure, SAS Tukey Honest Significant Difference (HSD) test was used to separate treatment means at a probability level of 0.05 (SAS, 2009).

3. RESULTS AND DISCUSSION

3.1. Number of Spikes

The combined analysis of variance over three years indicated that the number of spikes per m2 was significantly ($p < 0.05$) and positively influenced by inorganic fertilizer application and bio-fertilizer inoculation (Table 3). The highest number of spikes per m2 was obtained from the application of Pseudomonas + 46/20.2 kg NP ha-1. The number of spikes per m2 was increased by integrating bio-fertilizer with chemical fertilizer. Application of Pseudomonas + 46/20.2 kg NP ha-1 increased the number of spikes per m2 by 28.6% and 33.1% compared to the control and Azotobacter alone, respectively. Inoculation of seeds with the bio-fertilizer (Pseudomonas or Azotobacter) treatment along with the application of chemical NP fertilizer increased the number of spikes per m2 by 24.2 % to 28.6% compared to non-inoculated plants (Table 3). These results were consistent with previous findings (Abedi et al., 2010; Kandil et al., 2011; Liu and Shi, 2013).

3.2. Plant Height

Plant height was significantly ($p <0.05$) and positively affected by nitrogen, phosphorus, and bio-fertilizer application as shown in Table 3. The highest plant height (109.2 cm) was obtained from the addition of dual inoculants (Azotobacter + Pseudomonas) with inorganic fertilizer +46/20.2 NP kg ha -1). The next good treatments (Pseudomonas + 46/20.2 NP; NP alone and Azotobacter +NP) were not significantly different from the top performer. The 5th treatment (Azotobacter + pseudomonas) had an intermediate plant height (96.7cm) which is significantly lower than the four top performances. The last three treatments including negative control did not much to plant height increase (Table 3). Previous studies have also reported similar findings (Zorita and Canigia, 2009; Daneshmand et al., 2012; Namvar et al., 2012; Liu and Shi, 2013).

3.3. Grain Yield

Analysis of variance for individual and combined years showed that the mean grain yield of malt barley was significantly ($p < 0.05$) affected by applying bio-fertilizers (Table 2). The highest (3686.0 kg ha-1) and lowest (1924 kg h-1) grain yields were obtained by applying dual bio-fertilizer inoculants along with NP fertilizer, and from the control, respectively. This showed a remarkable 91.6 % of yield increase (Table 2). The inoculants (Azotobacter and Pseudomonas) are effective only when combined with NP fertilizers. Comparing Pseudomonas +NP and Azotobacter +NP treatments, the first is significantly better than the latter in increasing both grain and biomass yields (Table 2). The combination of the two inoculants along with inorganic NP resulted in superior grain and biomass yield increments. Similar results were reported on the effects of N fertilizer (Kizilkaya, 2008; Wortman et al., 2011; Scursoni et al., 2012; Getachew et al., 2014) and bio-fertilizer (Saini et al., 2004; Sary et al., 2009; Namvar et al., 2012) on grain yield of different crops. The increase in grain yield in the inoculated plants could be attributable to the exudation of plant growth regulators (PGRs), such as auxins, gibberellin, and cytokinin by Azotobacter sp., in addition to the increase in nutrient availability (Vessey, 2003). Piccinin et al. (2013) showed that the combined application of chemical NP fertilizers and bio-fertilizer inoculation improved the grain yield of malt barley. Generally, nano bio-fertilizer application increased crop growth and improved yield and yield components in barley (Seyed M. et al., 2013) and extended the growing period in wheat (Mahmoud M. et al., 2014).

3.4. Biomass Yield

Results revealed that treatment effects for either biofertilizers or uninoculated were significant for both grain yield and total biomass yields of malt barley for each year (Table 1). In the combined analysis, application of Azotobacter + Pseudomonas + 46/20.2 N/P kg ha-1 increased total biomass by 35.8 %, compared to the Azotobacter treatments alone, and 50.1% increase to sole Pseudomonas inoculation similarly, Azotobacter + Pseudomonas inoculated plants with NP fertilizer resulted in higher total biomass (9388 kg ha -1) than the control with 5469 kg ha-1.

Table 1. The effect of inorganic and growth-promoting microorganisms on grain yield and biomass in Barley for three years

Treatments	Year 1		Year 2		Year 3	
	GY(kg ha^{-1})	BY(kg ha^{-1})	GY(kg ha^{-1})	BY(kg ha^{-1})	GY(kg ha-1)	BY(kg ha^{-1})
Azotobacter + Pseudomonas +46/20.2 N/P kg ha $^{-1}$	3867.72a	9797.31a	3582.62a	8796.69a	3607.31a	9570.48a
Pseudomonas + 46/20.2 N/P kg ha $^{-1}$	3581.04a	8345.33ab	3166.28b	9644.38a	3195.96b	9191.00ab
46/20.2 N/P kg ha $^{-1}$	3118.23b	7870.69bc	2944.72bc	9326.02a	3249.24ab	9170.33ab
Azotobacter +46/20.2 N/P kg ha $^{-1}$	2928.51b	7512.54bcd	2699.71cd	6530.03b	2957.18bc	7715.50bcd
Azotobacter + Pseudomonas	2401.39c	8746.51ab	2391.69de	6256.95b	2871.41bc	8272.45abc
Azotobacter	2304.82c	6662.64cd	2203.94e	6273.65b	2590.22cd	7202.80cd
Pseudomonas	2125.66cd	6293.78d	2118.07ef	5774.81b	2241.82d	6697.42cd
-C (negative control)	1757.41d	4697.55e	1825.36f	5678.24b	2189.98d	6029.92d
MEAN	2760.60	7490.79	2616.55	7285.10	2862.89	7981.24
CV	8.87	11.84	7.62	17.36	8.02	12.06
LSD	428.69	1553.75	349.06	2215.00	402.30	1686.17

Bio-fertilizer inoculation with NP fertilizer increased straw yield by 60.7 % as compared to the control (Table 2). Several workers have reported similar effects of N fertilizer on total biomass of different crops (Kizilkaya, 2008; Wortman et al., 2011; Scursoni et al., 2012; Getachew et al., 2014) and bio-fertilizer (Saini et al., 2004; Sary et al., 2009; Namvar et al., 2012), Piccinin et al. (2013) also indicated that total biomass of malt barley was improved when malt barley plants were grown with a combination of NP fertilizer and bio-fertilizer inoculation.

Table 2. The effect of inorganic and growth-promoting microorganisms on grain yield and biomass in Barley (Combined over three years)

Treatment	GY(kg ha^{-1})	BY(kg ha^{-1})	HI
Azotobacter + *Pseudomonas* +46/20.2 N/P kg ha^{-1}	3686. 0 ^{a}	9388. 0 ^{a}	40.1 ^{a}
Pseudomonas +46/20.2 N/P kg ha^{-1}	3314. 0 ^{b}	9060. 0 ^{a}	36.9 ^{ab}
46/20.2 N/P kg ha^{-1}	3104.0 ^{bc}	8789. 0 ^{ab}	35.7 ^{ab}
Azotobacter +46/20.2 N/P kg ha^{-1}	2862.0 ^{cd}	7253.0 ^{bc}	40.5 ^{a}
Azotobacter + *Pseudomonas*	2555.0 ^{de}	7759.0 ^{abc}	34.5 ^{ab}
Azotobacter	2366.0 ^{ef}	6910. 0 ^{cd}	33.2 ^{b}
Pseudomonas	2162.0 ^{fg}	6255.0 ^{cd}	35.0 ^{ab}
-C (negative control)	1924.0 ^{g}	5469. 0 ^{d}	35.7 ^{ab}
MEAN	2746.7	7610.4	36.8
CV(%)	8.2	13.6	11.4
LSD	371.4	1700.0	6.9

Means with different letters are significantly different ($p<0.05$); GY=Grain yield, BY =Biomass yield, HI=harvest index.

Table 3. The effect of inorganic and growth-promoting microorganism on yield-related parameters in Barley (Combined over three years)

Treatment	PH(cm)	TSW	NS
1. Azotobacter + Pseudomonas +46/20.2 N/P kg ha^{-1}	109.2 ^{a}	41.9 ^{a}	53.1^{c}
2. Pseudomonas +46/20.2 N/P kg ha^{-1}	108.3 ^{a}	40.4 ^{ab}	71.1^{a}
3. 46/20.2 N/P kg ha^{-1}	107.3 ^{a}	40.1 ^{ab}	57.4 ^{abc}
4. Azotobacter +46/20.2 N/P kg ha^{-1}	105.6 ^{a}	41.6 ^{a}	68.7 ^{ab}
5. Azotobacter + Pseudomonas	96.7 ^{b}	34.1 ^{bc}	65.4 ^{abc}
6. Azotobacter	86.9 ^{c}	36.4 ^{abc}	53.4 ^{c}
7. Pseudomonas	84.0 ^{c}	32.8 ^{c}	63.3 ^{abc}
8. -C (negative control)	80.6 ^{c}	35.2 ^{abc}	55.3 ^{bc}
MEAN	97.3	37.8	60.9
CV(%)	5.4	10.9	14.9
LSD	8.6	6.8	15.2

Means with different letters are significantly different ($p<0.05$); PH=plant height, TSW=thousand seed weight, NS=number of spikes

4. CONCLUSIONS

The results of this study indicated that the combined use of organic and inorganic fertilizers improved yield and yield components of malting barley. The results indicated that the application of Azotobacter and Pseudomonas with chemical fertilizer (nitrogen and phosphorus) has shown a significant influence on yield, and yield components of malt barley. Thus, it seems that inoculating barley with bio-fertilizers (Azotobacter and Pseudomonas) caused the developing root system activity, and increasing plant access ability to nutrients in addition to producing hormones stimulating growth, it also seems that these bio-fertilizers had best and probably synergistic effects on each other which finally led to improving growth traits of barley. So the study results indicated that the application of suitable bio-fertilizers could be efficient in increasing yield, improving growth traits of barley. Thus, we suggested that the combined application of organic and inorganic fertilizers could improve the productivity of malt barley.

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