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Research Paper

Effect of fertility levels and liquid biofertilizers on quality parameters of malt barley (*Hordeum vulgare*)

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ABSTRACT

Barley (*Hordeum vulgare* L.) is crucial for malt production in the beverage industry, driving the need for sustainable practices to improve grain yield and quality. Fertility management and biofertilizers enhance nutrient availability and soil health, but their combined effects on 1malt barley quality require further study. A field experiment was conducted during *rabi* season 2020–21 and 2021–22 with the objective to evaluate the response of malt barley to fertility levels and biofertilizers. The experiment was laid out in randomized block design (Factorial) with 15 treatment combinations comprised of three fertility levels, i.e. application of 70 kg N + 40 kg P_2O_5 + 25 kg K_2O/ha, 60 kg N + 30 kg P_2O_5 + 20 kg K_2O/ha and 50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha with 5 liquid biofertilizers, i.e. control, *Azotobacter*, PSB, KMB and *Azotobacter* + PSB + KMB. The result revealed that malt barley crop fertilized with 70 kg N + 40 kg P_2O_5 + 25 kg K_2O/ha recorded highest protein content (11.6%) and average grain weight (43.8 mg) which was significantly higher over rest of fertility levels. While the highest starch (61.8%) and malt content (82.6%) in grain recorded under application of 50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha. The results showed that seed inoculation with liquid biofertilizers *Azotobacter* + PSB + KMB significantly improved protein content (11.4%) and average grain weight (43.6 mg) of malt barley crop. While the highest starch (62.0%) and malt content (82.7%) in grain recorded under control. The study concludes that higher fertility levels and combined biofertilizer application (*Azotobacter* + PSB + KMB) enhance protein content and grain weight in malt barley, while lower fertility levels favour higher starch and malt content.

Key words: Biofertilizers and fertility levels, Malt barley, Protein, Starch

Barley is generally grown on marginal and sub-marginal land because of its low inputs requirement. In India, barley is mainly grown in the northern plains and concentrated in the states of Utter Pradesh, Haryana and Rajasthan. In India, barley was cultivated on 610.5 thousand ha area with 1,600 thousand t of production at an average productivity of 26 q/ha. In India, Rajasthan is the largest state having more than 52% in production and 46% area followed by Uttar Pradesh. In Rajasthan, barley was cultivated on 312.7 thousand ha area with 1,059.3 thousand t of production at an average productivity of 33.88 q/ha (IIWBR, 2020–21). The use of two-rowed barley for malting and brewing industry has picked up recently with increased consumption of beer and other malt-based products in many countries (Neelam *et al.,* 2018). Presently about 20–25% of total barley production is used in the preparation of malt which is utilized for brewing, distillation, baby foods, confectionaries cocoa- malt drink and medicinal syrups. Adequate mineral fertilization is considered to be one of the most important prerequisite in this respect. Nitrogen supports photosynthesis, phosphorus aids energy transfer and genetic processes, and potassium regulates cellular functions. Biofertilizers enhance soil fertility by fixing nitrogen, solubilizing phosphates and potassium, and promoting nutrient availability (Jat, *et al.*, 2023). Despite the recognized importance of mineral fertilization and biofertilizers in improving crop yield and quality, limited research has focused on their combined effect on the quality parameters of malt barley, particularly in the context of Indian agro-climatic conditions (Terefe *et al.,* 2018). Existing studies have primarily addressed the impact of individual nutrients or biofertilizers but have not adequately explored their integrated use to optimize the

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quality attributes such as protein, starch, and malt content. Furthermore, there is a lack of region-specific recommendations for fertility levels and biofertilizer combinations to enhance the production and quality of malt barley in Rajasthan, where barley cultivation plays a significant role in the agricultural economy.

The combined use of optimized fertility levels and liquid biofertilizers improves the quality of malt barley by enhancing nutrient availability, plant metabolism, and soil health.

Current study was conducted to asess the effect of fertility level and biofertilizer on quality para meters of malt barley and malt yield.

MATERIALS AND METHODS

The field experiment was conducted during *rabi* 2020–21 and 2021–22 at the Instructional Farm, Department of Agronomy, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India. The soil of experimental site was clay loam in texture, slightly alkaline in reaction, low in available nitrogen (278.36 to 279.42 kg/ha), medium in available phosphorus (18.73 to 20.39 kg /ha) and high in available potassium status (328.40 to 332.72 kg/ha). The experiment was laid out in randomized block design (Factorial) with 15 treatment combinations comprised of three fertility levels i.e. application of 70 kg N + 40 kg P_2O_5 + 25 kg K_2O/ha, 60 kg N + 30 kg P_2O_5 + 20 kg K_2O/ha and 50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha with 5 liquid biofertilizers, i.e. control, *Azotobacter*, PSB, KMB and *Azotobacter* + PSB + KMB. The total quantity of phosphorus and potassium with half dose of nitrogen were drilled in furrows before sowing of seed. Remaining half dose of nitrogen was top dressed at the time of first irrigation. The seeds were treated with liquid biofertilizers using 5 ml/kg seed through standard procedure 2–3 hours before sowing as per treatment. The seeds were sown in furrow opened at the depth of about 4–5 cm using seed rate of 100 kg/ha keeping inter row spacing of 20 cm.

Protein content

Grain protein content of malt barley was calculated by multiplying the nitrogen content of grain with a factor 6.25 as proposed by Angelo and Mann (1973). It was expressed in terms of per cent protein content.

Protein content (%) = N content in seed (%) × 6.25

Starch content in grain

Barley flour sample was treated with 80% alcohol to remove sugars and then, the starch was extracted with perchloric acid. In hot acidic medium, starch is hydrolysed to glucose and dehydrated to hydroxymethyl furfural. This compound forms a green colored product with anthrone. In homogenized condition 0.1 to 0.5 g of the sample was taken in hot 80% ethanol to remove sugars, centrifuged and retained the residue. The residue was washed three times with hot 80% ethanol till the washing do not give color with anthrone reagent. The residue was dried well over a water bath. To the residue 5.0 ml of water and 6.5 ml of 52% perchloric acid were added and an extract was made at 0 °C for 20 min. The extraction was repeated using fresh perchloric acid. The pooled supernatants were made up to 100 ml. Pipetted out 0.1 or 0.2 ml of the supernatant and made up the volume to 1.0 ml with water. Prepared the standards by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standards and made up the volume to 1.0 ml in each tube with water. 4.0 ml of anthrone reagent was added to each tube. Heated for 8 min. in a boiling water bath. After cooling, the intensity of green to dark green color was measured at 630 nm to find out the glucose content in the sample using the standard graph. The value was multiplied by a factor 0.9 to calculate the starch content (Sadasivam and Manickam, 1992). Unit expressed in percentage.

Husk content

The husk was removed by treatment with sodium hypochlorite solution. The husk content is calculated from the reduction in weight by sodium hypochlorite solution. An amount of 20 g of malt barley grain was weighed and boiled in 80 ml sodium hypochlorite solution and 20 ml sodium hydroxide solution in a glass beaker of 800 ml. After 30 seconds cold tap water was added to agitate the sample by the action of the water stream. Decanted the water containing the husk remnants and added fresh water. Repeated the rinsing until all the husk remnants were removed. Spread out the grains on filter paper and dried overnight at room temperature. Continued drying for 3 hrs. at 50 °C in a drying oven (Analytica-EBC, 1997). Unit expressed in percentage. The husk content was calculated as per formula given below-

Husk content (%) = Sample weight as % dry-matter – weight of dehusked grains as % dry matter × 100/ Sample weight as % dry matter

Average grain weight

A grain sample was taken from the produce of each of the net plot harvested and average five grains were weighed, calculated for average grain weight (Weston *et al.*, 1993). The unit was expressed in mg.

Malt content

Malting process of barley grains were carried out as per the method suggested by (Singh and Sorsulski, 1985). Properly cleaned, weighted samples of barley grains were steeped in water at 15 °C in an incubator for a period of 60 hours. Water was changed every 8–10 hours and an air rest of 1 hr was given after 24 hours during steeping. Then steeped samples taken in a muslin cloth were placed in a wooden box with wire mesh bottom for germination in an incubator at 15 °C and relative humidity of more than 90 percent. They were allowed to germinate for a period of 5 days. Then the green malt was kilned in an oven at 45 °C for 4 hours. Roots from the kilned malt were removed. Weight of malt and roots was recorded separately to calculate malt yield.

$$\text{Malt content }(\%) = \frac{\text{Malt weight}}{\text{Sample weight}} \times 100$$

RESULTS AND DISCUSSION

Protein content in grain

Fertility levels

Data presented in Table 1 indicates that fertility levels brought about significant variation on protein content in grain during both the years of investigation as well as in pooled analysis. The malt barley crop under the influence of 70 kg N + n 40 kg P_2O_5 + 25 kg K_2O/ha recorded highest protein content in grain over application of 60 kg N + 30 kg P_2O_5 + 20 kg K_2O/ha and 50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha during both years. On pooled basis, compared to application of 60 kg N + 30 kg P_2O_5 + 20 kg K_2O/ha and 50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha, application of 70 kg N + 40 kg P_2O_5 + 25 kg K_2O/ha significantly improved protein content by 7.68 and 14.55%, respectively.

The improvement in protein content with higher fertilizer application, especially nitrogen, can be attributed to increased nitrogen concentration in grain due to nitrogenous compounds. Nitrogen plays a critical role in protein synthesis because it forms more amino acids which directly influencing grain protein content. The results corroborate with the findings of Ghadge and Murumkar (2021) and Kumar *et al*., (2022).

Liquid biofertilizers

Irrespective of the years as well as in pooled analysis, inoculation of malt barley seed with liquid biofertilizers *Azotobacter*, PSB and KMB alone and in combination of *Azotobacter* + PSB + KMB significantly improved protein status of grain over control. On pooled basis, the corresponding increases in protein content of grain was 11.62, 11.42, 11.13 and 16.07 per cent, respectively. Among liquid biofertilizers, co inoculation with liquid biofertilizer *Azotobacter* + PSB + KMB recorded maximum protein content in grain which was significantly higher over inoculation with *Azotobacter*, PSB and KMB alone during both years. On pooled basis, the extent of improvement in grain protein content of grain due to conjoint inoculation of *Azotobacter* + PSB + KMB was by 3.98, 4.17 and 4.46% over single inoculation of *Azotobacter*, PSB and KMB, respectively.

The enhanced protein content from *Azotobacter* + PSB + KMB is likely due to their synergistic effects, including nitrogen fixation, phosphorus solubilization and potassium mobilization. These processes increase nutrient availability, supporting protein synthesis. The results corroborates with the findings of Malik (2018) and Jatinderpal (2021).

Table 1. Effect of fertility levels and biofertilizers on quality parameters of malt barley

Treatment	Protein content in grain (%)			Starch content in grain (%) (%)			Husk content (%)		
	2020–21	2021–22	Pooled	2020–21	2021–22	Pooled	2020–21	2021–22	Pooled
Fertility levels									
50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha	10.07	10.28	10.17	61.60	62.06	61.83	11.50	11.81	11.65
60 kg N + 30 kg P_2O_5 + 20 kg K_2O/ha	10.72	10.92	10.82	60.26	60.72	60.49	11.48	11.76	11.62
70 kg N + 40 kg P_2O_5 + 25 kg K_2O/ha	11.55	11.76	11.65	59.23	59.69	59.46	11.40	11.71	11.55
SEm±	0.10	0.10	0.07	0.33	0.30	0.22	0.06	0.05	0.04
CD (P=0.05)	0.28	0.28	0.19	0.95	0.85	0.63	NS	NS	NS
Liquid biofertilizers									
Control	9.80	9.98	9.89	61.78	62.24	62.01	11.46	11.86	11.66
Azotobacter	10.94	11.14	11.04	60.19	60.65	60.42	11.45	11.72	11.58
PSB	10.92	11.12	11.02	60.40	60.86	60.63	11.44	11.78	11.61
KMB	10.89	11.08	10.99	60.52	60.98	60.75	11.48	11.69	11.59
Azotobacter + PSB + KMB	11.35	11.60	11.48	58.93	59.39	59.16	11.44	11.76	11.60
SEm±	0.13	0.12	0.09	0.43	0.38	0.29	0.07	0.06	0.05
CD (P=0.05)	0.37	0.36	0.25	1.23	1.10	0.81	NS	NS	NS

Starch content in grain

Fertility levels

It is evident from the data (Table 1) that increasing fertility levels had significant influence on starch content in grain during both the years of experimentation as well as on pooled basis. The malt barley crop fertilized with 70 kg N + 40 kg P_2O_5 + 25 kg K_2O/ha recorded least starch content in grain which was significantly lowers it over application of 60 kg N + 30 kg P_2O_5 + 20 kg K_2O/ha and 50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha during both years. On the basis of pooled analysis, application of 70 kg N + 40 kg P_2O_5 + 25 kg K_2O/ha estimated 59.46% starch content in grain which was significantly reduced by 1.70 and 3.83 per cent over application of 60 kg N + 30 kg P_2O_5 + 20 kg K_2O/ha and 50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha, respectively.

The reduction in starch content at higher fertility levels, particularly nitrogen, can be attributed to the inverse relationship between protein and starch synthesis. Grains with higher protein content tend to accumulate less amylose, reducing starch content. These results are consistent with Kumar *et al.,* (2022) who highlighted the negative correlation between protein and starch in malt barley.

Liquid biofertilizers

During both the years as well as in pooled analysis compared to no inoculation, inoculation of malt barley seed with liquid biofertilizers alone and in combination significantly influenced starch content in grain. The mean data indicate that application of liquid biofertilizers *Azotobacter,* PSB, KMB alone and in combination *Azotobacter* + PSB + KMB significantly decreased starch content by 2.56, 2.22, 2.03 and 4.60% over control, respectively. Among liquid biofertilizers, co inoculation with *Azotobacter* + PSB + KMB accumulated least starch content in grain which was significantly inferior over single inoculation with *Azotobacter*, PSB and KMB during both years. On pooled basis, seed inoculation with liquid biofertilizers consisted combination of *Azotobacter* + PSB + KMB recorded 59.16% starch content which was significantly reduced by 2.08, 2.42 and 2.61% over single inoculation of *Azotobacter*, PSB and KMB, respectively.

The decrease in starch content with *Azotobacter* + PSB + KMB is due to the enhanced enzymatic activity and nutrient metabolism promoted by biofertilizers. These results confirm the findings of Malik (2018), Jatinderpal (2021) and Kumar *et al.,* (2022) which emphasize the role of biofertilizers in balancing nutrient uptake.

Husk content

It is explicit from data (Table 1) that application of fertility levels and seed treatment with liquid biofertilizers failed to bring about significant variation on husk content of grain during both the years of study as well as in pooled analysis.

Average grain weight

Fertility levels

Across the years as well as in pooled analysis, fertility levels brought about significant influence on grain weight (Table 2). The malt barley crop fertilized with 70 kg N + 40 kg P_2O_5 + 25 kg K_2O/ha recorded highest average grain weight which showed significant increase over application of 60 kg N + 30 kg P_2O_5 + 20 kg K_2O/ha and 50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha during both years. Thus, when compared to application of 60 kg N + 30 kg P_2O_5 + 20 kg K_2O/ha and 50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha, application of 70 kg N + 40 kg P_2O_5 + 25 kg K_2O/ha significantly improved average grain weight to the extent of 4.95 and 11.22%, respectively.

The increase in grain weight with higher fertility levels can be attributed to improved nutrient availability, leading to enhanced photosynthesis and assimilate partitioning (Kumavat, 2021 and Malik, 2018).

Liquid biofertilizers

Data reflects that inoculation of malt barley seed with liquid biofertilizers alone and in combination significantly influenced average grain weight during both the years of experimentation as well as in pooled analysis. The mean data show that inoculation with *Azotobacter,* PSB, KMB alone and in combination of *Azotobacter* + PSB + KMB significantly enhanced average grain weight to the tune of 5.46, 4.21, 3.83 and 9.30% over control, respectively. Among liquid biofertilizers, conjoint inoculation with *Azotobacter* + PSB + KMB produced grain having highest weight which was significantly higher over single inoculation of *Azotobacter*, PSB and KMB during both years. On the basis of pooled analysis, co inoculation of *Azotobacter* + PSB + KMB significantly increased grain weight to the tune of 3.63, 4.88 and 5.26% over single inoculation of *Azotobacter*, PSB and KMB, respectively.

The synthesis of growth promoting substances produced by these liquid biofertilizers further augmented growth and development which enhance grain filling and ultimately increases average grain weight. Malik (2018), Jatinderpal (2021) and Kumar *et al.,* (2022).

Malt yield

Fertility levels

A reference to data (Table 2) indicates that fertility levels had significant effect on malt yield of crop during both

Table 2. Effect of fertility levels and biofertilizers on quality parameters of malt barley

Treatment	Average grain weight (mg)			Malt yield (%)		
	2020–21	2021–22	Pooled	2020–21	2021–22	Pooled
Fertility levels						
50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha	39.15	39.75	39.45	82.36	82.88	82.62
60 kg N + 30 kg P_2O_5 + 20 kg K_2O/ha	41.51	42.11	41.81	80.54	81.06	80.80
70 kg N + 40 kg P_2O_5 + 25 kg K_2O/h	43.58	44.18	43.88	78.75	79.27	79.01
SEm±	0.41	0.37	0.27	0.49	0.45	0.33
CD (P=0.05)	1.16	1.04	0.77	1.40	1.28	0.94
Liquid biofertilizers						
Control	39.59	40.19	39.89	82.50	83.02	82.76
Azotobacter	41.77	42.37	42.07	80.44	80.96	80.70
PSB	41.27	41.87	41.57	80.56	81.08	80.82
KMB	41.12	41.72	41.42	80.67	81.19	80.93
Azotobacter + PSB + KMB	43.30	43.90	43.60	78.59	79.11	78.85
SEm±	0.52	0.47	0.35	0.63	0.58	0.43
CD (P=0.05)	1.49	1.35	0.99	1.81	1.65	1.21

the years of experimentation as well as in pooled analysis. Among fertility levels, the least malt yield was noticed with the application of 70 kg N + 40 kg P_2O_5 + 25 kg K_2O/ha which recorded significant reduction over application of 60 kg N + 30 kg P_2O_5 + 20 kg K_2O/ha and 50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha during both years. Thus when compared to application of 60 kg N + 30 kg P_2O_5 + 20 kg K_2O/ha and 50 kg N + 25 kg P_2O_5 + 15 kg K_2O/ha, application of 70 kg N + 40 kg P_2O_5 + 25 kg K_2O/ha significantly reduced mean malt yield by 2.20 and 4.37%, respectively.

The lower malt yield at higher fertility levels is linked to excessive protein content, which negatively affects malting quality. The amylose content in grain helps to increase the enzymatic processes during malting and the barley endosperm rich in amylopectin has higher activities of starch hydrolyzing enzymes which ultimately increases the malt production. Both excessive protein and too little protein in the kernels results in reduced malting quality. High protein interferes with enzymatic activity, reducing malt extract, as also reported by Malik (2018), Malik *et al.,* (2019) and Kumavat (2021).

Liquid biofertilizers

A perusal of data shows that crop under the influence of liquid biofertilizers *Azotobacter,* PSB, KMB alone and in combination of *Azotobacter* + PSB + KMB significantly reduced malt yield of crop as compared to control during both the years of study as well as in pooled analysis. The corresponding reduction in malt yield of crop was by 2.48, 2.34, 2.21 and 4.72%, respectively. Among liquid biofertilizers, conjoint inoculation of *Azotobacter* + PSB + KMB produced least malt yield which was significantly reduced over inoculation with *Azotobacter*, PSB and KMB alone during both years. On pooled basis, the significant reduction in malt yield under the influence of seed inoculation with *Azotobacter* + PSB + KMB was by 2.29, 2.43 and 2.57% over single inoculation of *Azotobacter*, PSB and KMB, respectively.

The reduction in malt yield with *Azotobacter* + PSB + KMB may result from its impact on grain composition, particularly the increased protein content, which negatively correlates with malt extract. The results confirms the findings of Malik (2018) and Jatinderpal (2021).

It could be concluded that the application of 70 kg N + 40 kg P_2O_5 + 25 kg K_2O/ha along with inoculation of seed with liquid biofertilizers *Azotobacter* + PSB + KMB represents the most effective practice for improving the quality parameters of the malt barley (*Hordeum vulgare L.*) crop

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