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Assessing the role of fertility levels and biofertilizers on growing degree days, NDVI and chlorophyll in malt barley (*Hordeum vulgare*)

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ABSTRACT

The experiment was conducted during 2020–2022 at Maharana Pratap University of Agriculture and Technology, Udaipur, Rajasthan to assess the impact of different fertility levels and biofertilizer treatments on malt barley (*Hordeum vulgare* L.). The experiment followed a factorial randomized block design (Factorial-RBD) and included 15 treatment combinations comprised of three fertility levels, viz. application of 70 kg N+40 kg P_2O_5 +25 kg K_2O/ha ($N_{70}P_{40}K_{25}$), 60 kg N+30 kg P_2O_5 +20 kg K_2O/ha ($N_{60}P_{30}K_{20}$) and 50 kg N+25 kg P_2O_5 +15 kg K_2O/ha ($N_{50}P_{25}K_{15}$) with five liquid biofertilizers i.e. control, *Azotobacter*, PSB, KMB and *Azotobacter* + PSB + KMB. The malt barley cultivar 'DWRB-137' served as the experimental crop. The findings indicated that the malt barley, when supplemented with ($N_{70}P_{40}K_{25}$) kg/ha recorded the highest growing degree at heading stage, 50% anthesis, 7, 14, 21 days after 50% anthesis and physiological maturity (1601.57°C day), normalized difference vegetation index at 50% anthesis, 7, 14 and 21 days after 50% anthesis (0.734) and chlorophyll content in leaves represented significant increase as compared to other fertility levels. Furthermore seed inoculated with *Azotobacter* + PSB + KMB significantly increased growing degree at heading stage, 50% anthesis, 7, 14 and 21 days after 50% anthesis (1264.49°C day) and chlorophyll content in leaves.

Keywords: Fertility levels, Growing degree day, Malt barley, NDVI

Malt barley (*Hordeum vulgare* L.) is an important cereal crop, widely cultivated for its use in the brewing industry as well as for animal feed and human consumption. Among the various factors influencing the growth of barley, fertility management and biofertilizer application play a pivotal role in determining crop performance (Neelam *et al.* 2018). Malt barley is predominantly grown in Rajasthan, and is primarily used in the brewing industry, as well as for food and fodder. Barley suits the state's dry, harsh areas due to its drought tolerance and resilience to extreme conditions (Singh *et al.* 2012). Malt barley is extensively used in brewing, food, and feed industries. Its successful cultivation and high-quality yield are determined by various agronomic practices and environmental conditions. Among these, fertility management and the use of biofertilizers make a substantial impact in influencing the physiological and biochemical processes of the plant (Gupta *et al.* 2010).

The shift to sustainable agriculture has increased the use of liquid biofertilizers, which improve nutrient delivery by boosting soil microbial activity and uptake. When integrated with appropriate fertility levels, biofertilizers can positively influence crop growth and productivity. Comprehending the relationship between fertility levels and liquid biofertilizers is critical for optimizing growth parameters such as Growing Degree Days (GDD), Normalized Difference Vegetation Index (NDVI), and chlorophyll content, which are key indicators of crop health and productivity.

GDD serve as an essential tool in predicting the growth stages and maturation of crops, providing valuable insights into temperature accumulation over time (Chhabra *et al.* 2018). The NDVI is a widely used remote sensing metric that helps assess the vegetative vigour and health of crops, reflecting their overall productivity potential. Chlorophyll content, a direct measure of photosynthetic efficiency, is another critical factor influencing crop growth and yield quality (Brown and Garcia 2021).

This research will provide insights into the interactions between traditional fertilizers and biofertilizers, guiding balanced nutrient use to enhance barley productivity. Ultimately, this study aims to contribute to the broader goals of sustainable agriculture by identifying practices that not only increase yield but also reduce environmental impact,

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promote soil health, and maintain long-term agricultural productivity. The findings will help farmers and agronomists optimize barley cultivation for ecological sustainability and rising food production demands. Under these circumstances, the present research aims to evaluate the influence of different fertility levels and liquid biofertilizers on GDD, NDVI, and chlorophyll content in malt barley. This study seeks to provide insights into optimizing nutrient management for improving barley growth and maximizing yield, contributing to the broader goals of sustainable agriculture.

MATERIALS AND METHODS

The experiment was conducted during 2020–2022 at Maharana Pratap University of Agriculture and Technology, Udaipur (24°35’ N latitude and 73°42’ E longitude, and height of 581.35 m amsl), Rajasthan. The experimental soil was clay loam in texture, with a composition of 37.34% sand, 26.65% silt, and 34.64% clay. The soil's *p*H was measured at 7.9, indicating a slightly alkaline condition, while the Electrical Conductivity (EC) was recorded at 0.52 ds/m at 25°C in the furrow soil layer, reflecting the soil's salinity level. The study consisted 15 treatment comibinations having three fertility levels [70 kg N+40 kg P_2O_5 +25 kg K_2O/h ($N_{70}P_{40}K_{25}$); 60 kg N+30 kg P_2O_5 +20 kg K_2O/ha ($N_{60}P_{30}K_{20}$) and 50 kg N+25 kg P_2O_5 +15 kg K_2O/ha ($N_{50}P_{25}K_{15}$)] and five liquid biofertilizers (Control, *Azotobacter*, PSB, KMB and *Azotobacter* + PSB + KMB). The experiment was laid out in a factorial randomized block design (F-RBD), each repeated four times.

Urea, di-ammonium phosphate (DAP), and muriate of potash (MOP) served as the nitrogen, phosphorus, and potassium sources, respectively. The entire amount of phosphorus and potassium, along with half of the nitrogen dose, was placed into furrows prior to seed sowing. The remaining portion of the nitrogen application was made as a top-dressing during the initial irrigation. The malt barley cultivar 'DWRB-137' served as the experimental crop. The seeds were sown at a depth of approximately 4–5 cm utilizing a seeding rate of 100 kg/ha, with a row spacing of 20 cm. Seeds were covered with liquid biofertilizers *Azotobacter*, PSB, KMB, and a mixture of *Azotobacter* + PSB + KMB @5 ml/kg of seed, following standard procedures 2–3 h prior to sowing. The seeds were thoroughly mixed with the biofertilizers to ensure uniform coating and were then allowed to dry in the shade before planting.

The duration required to achieve various growth stages was measured by randomly picking five plants from each plot, noting the duration from planting to the attainment of each phenological phase until full maturity. The highest and lowest temperature data used in the research were sourced from the agro-meteorological station situated close to the investigation area. The growing degree days or accumulated heat units (°C day) were worked out by the following formulae which was described by Cao and Moss (1989):

$$\text{GDD or accumulated heat units (°C day)} = \sum_{i=1} \{(T_{max} + T_{min})/2 - T_b\}$$

Where T_{max}, Day's maximum temperature; $T_{min,}$ Minimum temperature, and $T_{b,}$ Base temperature, which is the lowest point at which growth ceases, it is taken as 4°C for malt barley. The NDVI reading was taken at 50% anthesis, 7, 14 and 21 days after 50% anthesis with the help of green seeker. The sensor was placed approximately 0.7–0.9 m above the plant canopy, while moving steadily throughout all trial plots. The path of the sensor was oriented parallel to the rows of seeds, with its light beam directed perpendicular to them. The built-in software of the Green Seeker immediately calculates the NDVI values by measuring the difference between infrared and visible light. The sensor uses sunlight as a source of passive light. The sensor releases short pulses of red and infrared light, subsequently measuring the quantity of each type of light that is reflected back from the plant. The NDVI is associated with vegetation since healthy green plants absorb a majority of the red light while reflecting a significant amount of infrared light.

The chlorophyll levels in leaves at 50 and 75 days after sowing (DAS) were assessed by gathering fresh leaf samples from every plot. These samples were quickly brought to the laboratory, rinsed with distilled water, and dried using absorbent paper. After removing mid ribs, the leaves were chopped into pieces to make homogenous sample before processing for chlorophyll estimation. One gram of the prepared sample was thoroughly crushed with 80% acetone and poured into a 25 ml volumetric flask. The volume was adjusted to the calibration mark, and absorbance was measured at specific wavelengths of 645–663 nm using a spectrophotometer for chlorophyll 'a' and 'b,' respectively, and presented as mg/g of fresh leaf weight (Arnon 1949). The total chlorophyll content at different stages was computed by following formula:

$$\text{Total chlorophyll (mg/g)} = \frac{20.2(\text{A652}) + 8.02(\text{A663}) \times \text{Total volume (ml)}}{\text{a} \times 1000 \times \text{weight of sample (g)}}$$

Where α, Path length = 1 cm

RESULT AND DISCUSSION

Growing degree day (°C day): A reference to data (Table 1) indicated that application of fertility levels failed to bring about significant variation on GDD values at boot leaf stage of malt barley crop during first year of investigation and in combined analysis. Application of $N_{70}P_{40}K_{25}$ kg/ha recorded highest GDD value that was notably greater over $N_{50}P_{25}K_{15}$ kg/ha but at par with $N_{60}P_{30}K_{20}$ kg/ha at heading, 50% anthesis, 7,14 and 21 days after 50% anthesis.

The mean of two years showed that use of $N_{70}P_{40}K_{25}$ kg/ha and $N_{60}P_{30}K_{20}$ kg/ha notably improved GDD value at heading stage by 6.07 and 3.45%, at 50% anthesis by 5.81 and 3.42%, at 7 days after 50% anthesis by 6.01 and 3.33%, at 14 days after 50% anthesis by 6.13 and 3.51% and at 21 days after 50% anthesis by 6.23 and 3.55% over application of $N_{50}P_{25}K_{15}$ kg/ha, separately.

At physiological maturity, application of $N_{70}P_{40}K_{25}$ kg/ha

Table 1 Effect of fertility levels and biofertilizers on growing degree day of malt barley

Treatment	GDD (°C day)						
	Pooled						
	At boot leaf stage	At heading stage	At 50 % anthesis	7 days after 50% anthesis	14 days after 50% anthesis	21 days after 50% anthesis	At physiological maturity
Fertility levels							
$N_{50}P_{25}K_{15}$	668	820	895	975	1065	1166	1502
$N_{60}P_{30}K_{20}$	683	848	926	1007	1102	1207	1533
$N_{70}P_{40}K_{25}$	697	870	947	1033	1130	1239	1602
SEM±	9.6	9.5	10.4	11.5	12.9	14.6	17.0
CD (*P*=0.05)	NS	26.7	29.3	32.4	36.4	40.9	47.8
Liquid biofertilizers							
Control	656	804	878	954	1044	1142	1490
Azotobacter	695	858	936	1020	1116	1223	1564
PSB	682	844	919	1002	1095	1199	1550
KMB	677	838	914	995	1087	1190	1544
Azotobacter + PSB + KMB	704	886	965	1054	1153	1265	1578
SEM±	12.4	12.3	13.5	14.9	16.7	18.8	21.9
CD (*P*=0.05)	NS	34.5	37.8	41.9	47.0	52.9	NS

GDD, Growing degree day. Treatment details are given under Materials and Methods.

recorded highest GDD values that were notably greater than the other fertility levels across both years except during 2021–22, application of $N_{60}P_{30}K_{20}$ kg/ha and $N_{70}P_{40}K_{25}$ kg/ha was at par in this regard. The pooled data showed that the significant increase in GDD values at physiological maturity through the use of $N_{70}P_{40}K_{25}$ kg/ha was 4.48 and 6.65% over application of $N_{60}3_{40}K_{20}$ kg/ha and $N_{50}P_{25}K_{15}$ kg/ha, separately.

A thorough analysis of the data indicated that, applying liquid biofertilizers to malt barley seeds did not significantly influenced GDD value at boot leaf stage (Table 1 and 2). At heading, 50% anthesis and 7 days after 50% anthesis, malt barley seed with *Azotobacter* + PSB + KMB exhibited highest GDD values significantly surpassing the control but comparable to single inoculation of *Azotobacter*, PSB and KMB during experimentation. On the basis of pooled analysis, seed inoculated with *Azotobacter* + PSB + KMB pointedly enhanced GDD value by 4.94, 5.69 and 10.18% at heading, 4.98, 5.61 and 9.96% at 50% anthesis and 5.10, 5.92 and 10.39% at 7 days after 50% anthesis over inoculation with PSB, KMB and control, respectively but at par with single inoculation with *Azotobacter*. At these respective crop stages, the magnitude of increase in GDD value with the inoculation of *Azotobacter* was 6.75, 6.68 and 6.92% over control.

At 14 and 21 days after 50% anthesis, application of liquid biofertilizers to malt barley seeds consisting of the combination of *Azotobacter* + PSB + KMB recorded highest GDD value which was significantly higher over control comparable to seed inoculation of *Azotobacter,* PSB and KMB separately, in the year of 2020–21 and 2021–22. The combined data indicated that seed inoculation using *Azotobacter* + PSB + KMB notably elevated GDD value by 5.29, 6.07 and 10.49% at 14 days after 50% anthesis and by 5.38, 6.18 and 10.77% at 21 days after 50% anthesis over inoculation with PSB, KMB and control, respectively but at par with inoculation with *Azotobacter* only. At both the respective stages, the magnitude of increase in GDD value with the inoculation of *Azotobacter* and PSB was 6.96 and 4.93% and 7.15 and 5.11%, over control, respectively. The inoculation of malt barley seed with liquid biofertilizers did not significantly influenced GDD value of crop at physiological maturity over both years of the research and in the combined analysis.

Normalized difference vegetation index (NDVI): The data reflected that the NDVI values of the malt barley crop at all growth stages showed a significant increase with the rise in fertility levels in 2020–21 and 2021–22 and in combined analysis (Table 2). The fertilization with $N_{70}P_{40}K_{25}$ kg/ha recorded highest NDVI value at 50% anthesis, 7, 14 and 21 days after 50% anthesis which showed significant increase over application of $N_{60}P_{30}K_{20}$ kg/ha and $N_{50}P_{25}K_{15}$ kg/ha during both years.

Therefore, in comparison to fertilization of $N_{60}P_{30}K_{20}$ kg/ha and $N_{50}P_{25}K_{15}$ kg/ha, use of $N_{70}P_{40}K_{25}$ kg/ha significantly increased mean NDVI value at 50% anthesis by 12.28, 32.71, 7 days after 50% anthesis by 11.85, 31.88, 14 days after 50% anthesis by 11.84, 29.25 and 21 days after 50% anthesis by 11.89, 28.99%, respectively. The data indicated that seed inoculation with biofertilizers failed to bring about significant variation on NDVI value of malt barley crop in either year of the study.

Table 2 Effect of fertility levels and biofertilizers on periodic normalized difference vegetation index and chlorophyll content of malt barley

Treatment	NDVI				Chlorophyll content (mg/g of fresh leaf)	
	Pooled					
	At 50% anthesis	7 days after 50% anthesis	14 days after 50% anthesis	21 days after 50% anthesis	50 DAS	75 DAS
Fertility levels						
$N_{50}P_{25}K_{15}$	0.491	0.508	0.540	0.569	1.162	1.928
$N_{60}P_{30}K_{20}$	0.578	0.599	0.624	0.656	1.253	2.022
$N_{70}P_{40}K_{25}$	0.649	0.670	0.698	0.734	1.313	2.087
SEM±	0.007	0.008	0.007	0.007	0.008	0.015
CD (*P*=0.05)	0.019	0.021	0.020	0.020	0.024	0.042
Liquid biofertilizers						
Control	0.558	0.577	0.606	0.639	1.164	1.901
Azotobacter	0.578	0.599	0.626	0.659	1.263	2.033
PSB	0.572	0.588	0.612	0.645	1.243	2.010
KMB	0.568	0.590	0.620	0.650	1.234	1.999
Azotobacter + PSB + KMB	0.588	0.610	0.639	0.673	1.310	2.117
SEM±	0.009	0.010	0.009	0.009	0.011	0.019
CD (*P*=0.05)	NS	NS	NS	NS	0.030	0.054

NDVI, Normalized difference vegetation index; DAS, Days after sowing. Treatment details are given under Materials and Methods.

Chlorophyll content: Various fertility levels exhibited significant effect on chlorophyll content at 50 and 75 DAS throughout the years and in combined analysis (Table 2). Consequently, when contrasted with the application of $N_{60}P_{30}K_{20}$ kg/ha and $N_{50}P_{25}K_{15}$ kg/ha, the malt barley crop under the influence of $N_{70}P_{40}K_{25}$ kg/ha synthesized more chlorophyll content in leaves at 50 and 75 DAS during both years.

The aggregated data indicated that the magnitude of increase in chlorophyll content by applying $N_{70}P_{40}K_{25}$ kg/ha was of the order of 4.78 and 12.99% at 50 DAS and 3.21 and 8.24% at 75 DAS over application of $N_{60}P_{30}K_{20}$ kg/ha and $N_{50}P_{25}K_{15}$ kg/ha, respectively. The chlorophyll content of malt barley is significantly impacted by the inoculation of seeds with liquid biofertilizers at 50 and 75 DAS in both cultivation years. Based on the pooled results, the significant improvement in chlorophyll content at 50 DAS with seed inoculation of *Azotobacter,* PSB, KMB separately and conjoint inoculation of *Azotobacter* + PSB + KMB was 8.50, 6.78, 6.01 and 12.54% at 50 DAS and 6.94, 5.73, 5.15 and 11.36% at 75 DAS over control, individually. The combined seed inoculation with *Azotobacter* + PSB + KMB made highest chlorophyll content of leaves at 50 DAS outperforming individual inoculations of *Azotobacter*, PSB and KMB during both years. In the pooled analysis, seed inoculation with the combination of *Azotobacter* + PSB + KMB suggestively improved chlorophyll content estimated at 50 DAS by 3.72, 5.39 and 6.15% and at 75 DAS by 4.13, 5.32 and 5.90% over individual seed inoculation of *Azotobacter*, PSB and KMB, respectively.

The findings showed that application of $N_{70}P_{40}K_{25}$ kg/ha + coating seed with *Azotobacter* + PSB + KMB increased duration to heading, 50% anthesis and physiological maturity because more application of nutrients delays maturity. Hence, the aforesaid fertility level also recorded higher GDD and NDVI value of malt barley crop as compared to lower fertility levels. Increasing fertility levels, in combination with seed inoculation using biofertilizers, significantly boosts nutrient availability to the crop. This enhanced nutrient uptake improves the physiological status of the plants, supporting better metabolic functioning and sustained growth. As a consequence, key phenological stages such as heading, 50% anthesis, and physiological maturity are delayed. This delay in the crop's development suggested that the improved nutrient environment allows for more prolonged vegetative and reproductive growth, facilitating optimal resource allocation and overall plant health. The extension of these growth stages can be attributed to the biofertilizers' ability to stimulate microbial activity in the rhizosphere, which in turn enhances nutrient solubilization and uptake efficiency. Consequently, this prolonged availability of nutrients appears to extend the crop's growth cycle by preventing premature senescence and sustaining plant vigour for a longer period. The findings of the investigation are aligned with Kumar (2005) and Shirinzadeh *et al.* (2013). Under present investigation, the application of nitrogen enhanced vegetative growth by boosting photosynthetic activity, allowing the leaves to remain active for an extended duration. This improvement in leaf function led to greater plant height and increased dry matter accumulation, ultimately resulting in a delayed ear emergence in malt barley. Increasing fertility levels extended days to maturity thus improved growing degree day values (Chhabra *et al.* 2018). This happened because of delayed progression of phenological stages leads to greater utilization of accumulated heat units due to high temperature lasts for extended growth period. So, the requirement of degree day values increased for various growth stages with extended period (Pandey *et al.* 2010). The NDVI is a widely recognized and frequently used remote sensing tool for assessing the health, vigour, and greenness of vegetation. NDVI operates by analyzing the difference between the reflectance of near-infrared (NIR) light, which healthy, green vegetation reflects strongly, and red light, which is absorbed by chlorophyll during photosynthesis. This contrast between NIR and red light reflectance provides an index that reflects the overall photosynthetic activity and biomass of the plant canopy, making NDVI a valuable indicator of plant health, growth stage, and productivity.

Likewise, NDVI is directly linked to the chlorophyll content of plant leaves, as higher chlorophyll levels enhance the plant's ability to absorb red light for photosynthesis while reflecting more NIR. When plants are well-nourished, especially with key nutrients like nitrogen, the chlorophyll content increases, leading to higher NDVI values (Williams and Johnson 2019). Enhanced fertilization, particularly with nitrogen-based fertilizers, promotes more robust vegetative growth by ensuring that plants have the nutrients necessary to support photosynthetic processes. As a result, crops exhibit denser, greener canopies, and this is reflected in increased NDVI readings (Prakash *et al.* 2015, Chopra *et al.* 2016, Aechra *et al.* 2020, Smith and Green 2020). Amongst plant nutrition, nitrogen increases greenness of plant thus higher NDVI value. The lesser the amount of nitrogen provided to the crops, the diminished the chlorophyll content and reduced the absorption of radiation in the visible spectrum, subsequently causing a decline in the NDVI value of the crops. Consequently, insufficient nitrogen resulted in early yellowing of the plants and a hindered vegetative growth. The positive effect of increasing fertility levels on NDVI value of crop was also observed by Singh *et al.* (2013), Tigre *et al.* (2014) and Terefe *et al.* (2018).

The highest growing degree day values, NDVI and chlorophyll content was obtained when malt barley was fertilized with $N_{70}P_{40}K_{25}$ kg/ha which was considerably greater than the fertilization of $N_{60}3_{30}K_{20}$ kg/ha and $N_{50}P_{25}K_{15}$ kg/ha. The combine inoculation of *Azotobacter* + PSB + KMB demonstrated most effective as it recorded highest chlorophyll content in leaves and took highest GDD values at heading stage, 50% anthesis, 7, 14 and 21 days after 50% anthesis as compared to inoculation with PSB, KMB and control but remained at par with *Azotobacter* only.

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