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Effect of bioaugmented Linz-Donawitz slag and biochar on physiological and yield attributes of wheat (*Triticum aestivum*)

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ABSTRACT

Industrial wastes and agricultural by-products are increasingly used in crop production as supplements along with fertilizers. An experiment was conducted during the winter (*rabi*) seasons of 2021 and 2022 at the research farm of the ICAR-Indian Agricultural Research Institute, New Delhi to determine the individual and combined effects of bioaugmented Linz-Donawitz (LD) slag and biochar on physiological and yield attributes of wheat (*Triticum aestivum* L.) variety HD 2967. Bioaugmented products with cow-dung, LD slag and biochar in different combinations were prepared in laboratory scale and applied in crates. All the treatments were applied with 100%RDF except control. Growth parameters such as total leaf area, chlorophyll content, NDVI; and leaf photosynthetic attributes such as net photosynthetic rate, stomatal conductance and transpiration rate were affected by bio-augmentation. Our study reported an increase in total leaf area (18.2–21.3%), chlorophyll content (26.5–31.0%), net photosynthetic rate (93.2%), stomatal conductance (61.3%), transpiration rate (24.7%) in bioaugmented treatments with LD slag and biochar over 100%RDF. A yield increase of 25.6 and 27.1% was found in bioaugmented treatments with a combination of LD slag (2 t/ha) and biochar (1 t/ha) over 100%RDF during 2021 and 2022, respectively. No. of spikes, grains per spike and dry biomass weight were positively influenced by bioaugmentation. The bioaugmented treatments with a combination of LD slag and biochar gave significantly higher grain yield, followed by bioaugmented LD slag compared to bioaugmented biochar alone in the respective application rate of treatments.

Keywords: Biochar, Bioaugmentation, Cow-dung, Linz-Donawitz slag, Wheat yield

India is the second-largest steel producer, with a production capacity of 157.5 million tonnes annually (Ministry of Steel, AR 2022–23). The cement industry uses most of the slag produced as a by-product during the production of steel, known as blast furnace slag. The Linz-Donawitz (LD) slag produced during steel production presents a challenge (Velayudhan *et al.* 2023). Around 180 kg of LD slag is produced from each tonne of crude steel, resulting in annual production of 10–12 million tonnes of LD slag, which is anticipated to double by 2030 (Singh *et al.* 2021). Heavy metals are present in traces in slag however, the levels may not be high enough to cause environmental hazards. Slags can be utilized in agriculture as fertilizers and for remediation of soil acidity. Utilization of LD slag helps to enhance agricultural yield while reducing the amount of waste that builds up in the environment. It predominantly contains oxides of silicon, iron, calcium, phosphorous, magnesium and manganese (Cota *et al.* 2023). Some studies revealed that applying biochar along with organic or inorganic fertilizers may improve soil characteristics and promote plant development. Biochar has the potential to alter the characteristics of agricultural soils by slowing carbon and nitrogen release (Pereira *et al.* 2015). Bioaugmentation of slag and biochar by using indigenously available active microbes in cow-dung for accelerating the process of biogas digestion systems is a new arena of research. The slurry produced in a biogas system with slag and biochar can be used for supplementing crop nutrition. Thus, in combination, organic and inorganic amendments are valuable tools for protecting and boosting soil fertility and crop productivity. Several studies have been conducted on steel slag and biochar utilization for improving agricultural production in rice (*Oryza sativa* L.) crop. The accelerated effect of bioaugmented LD slag and biochar in combination or alone in wheat (*Triticum aestivum* L.) crop has not been explored yet. Therefore, this investigation aimed to study the influence of bioaugmented LD slag and biochar on physiological traits and yield attributes of wheat.

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MATERIALS AND METHODS

Laboratory preparation of Bioaugmented LD slag: Fresh cow-dung from livestock farm along with LD slag

(TATA steel limited) and biochar (Merck Life Science Pvt. limited) were used as experimental substrates. Laboratory scale experiments were conducted using a 1 litre capacity conical flask. The inverted gas collection jar was filled with water and connected to the flask with a rubber tube. The substrates were mixed in different proportions for bioaugmentation, and water was added to dilute the substrates. The digesters were maintained at normal temperatures. Anaerobic digestion of substrates was done for 21 days. After bioaugmentation, these well-digested products were applied in crates 1 week before sowing in wheat. The quantity of cow-dung, LD slag and biochar used in bioaugmentation were calculated at 4 different rates: Cow-dung-0.75 t/ha, 2.25 t/ha, 4.5 t/ha and 6 t/ha; LD slag-0.25 t/ha, 0.75 t/ha, 1.5 t/ha and 2 t/ha; Biochar-0.125 t/ha, 0.375 t/ha, 0.75 t/ha and 1 t/ha. The ratio of cow-dung (CD), slag (LD) and biochar (BC) used in bioaugmentation were as follows: T_1, CD, LD, BC (0.75: 0.25: 0.125); T_2, CD, LD (0.75: 0.25); T_3, CD, BC (0.75: 0.125); T_4, CD, LD, BC (2.25: 0.75: 0.375); T_5, CD, LD (2.25: 0.75); T_6, CD, BC (2.25: 0.375); T_7, CD, LD, BC (4.5: 1.5: 0.75); T_8, CD, LD (4.5: 1.5); T_9, CD, BC (4.5: 0.75); T_{10}, CD, LD, BC (6: 2: 1); T_{11}, CD, LD (6: 2); T_{12}, CD, BC (6: 1); T_{13}, CD alone (0.75:0:0).

Experimental site: A crate experiment was conducted during winter (*rabi*) seasons of 2021 and 2022 at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi [28º63' N; 77º15' E; 228.6 amsl], under Indo-Gangetic plains region. The experimental site is in a semi-arid region with Typic Ustochrepts sandy loam alluvial soil. The minimum and maximum temperatures were 7.1 and 39ºC during 2021 and 33 and 5.5ºC during 2022. The experimental soil had a *p*H of 8.23 and soil organic carbon of 0.41%. The available nitrogen (N), phosphorus (P) and potassium (K) contents were 156.1, 13.8 and 275.4 kg/ha, respectively.

Treatments: The experiment was conducted with 16 treatments and control. All the treatments were provided with 100%RDF except control. The experiment was planned in a completely randomized design (CRD) with three replications of wheat variety HD 2967. T_1 to T_{13} represent bioaugmented products in laboratory experiments. These products were applied in crate experiments. The ratio of CD, LD and BC in bioaugmentation and its application rate in crates (t/ha) were same. The unamended treatments included T_{14}, LD Slag-2 t/ha; T_{15}, Biochar 1 t/ha; T_{16}, 100% RDF and control (0% RDF).

Growth and leaf photosynthetic parameters: Total leaf area was measured using a leaf scanner (Biovis PSM, Expert Vision Labs Pvt Ltd, Mumbai, India), plant's chlorophyll content (Konica-Minolta SPAD-502 chlorophyll meter) and density of greenness; and crop health (NDVI) measured using a handheld crop sensor, Trimble Green Seeker R (Trimble, Sunnyvale, CA, USA). A portable photosynthetic equipment, an infrared gas analyser (IRGA) LI-6400 XT (Li-COR, Lincoln, NE, USA) was used to measure the leaf's photosynthetic properties on the plant's uppermost fully expanded leaf between the hours of 9:00 and 11:00 on a clear, sunny day. The chamber's airflow rate was 500 mol/s, the CO_2 concentration was ambient, and the relative humidity was between 70 and 75%. Three plants per crate were chosen at random, and measurements of net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) were recorded.

Yield parameters: Wheat plant samples were collected during the time of harvest. Yield attributes accounted were number of spikes, grain per spike, dry biomass yield, grain yield and 1000-grain weight.

Statistical analysis: The data collected for treatments were statistically assessed using the analysis of variance (ANOVA) method using SAS 9.3 software (SAS Institute, Cary, NC, USA) for a completely randomized design. The significant difference between the mean values of treatments was determined by using the least significant difference ($P<0.05$) at a 95% confidence level.

RESULTS AND DISCUSSION

Growth and leaf photosynthetic parameters

Total leaf area: The total leaf area ranged from 475.9 to 735.4 $cm^{2/}$tiller during the flag leaf stage in 2021 and 495.0 to 772.6 cm^2/tiller in 2022 (Table 1). The significantly higher total leaf area was measured with bioaugmented treatments (T_{10} and T_8) in both years. The bioaugmentation of LD+BC and LD performed superior as compared to bioaugmented biochar and unamended treatments. There was an increase in total leaf area of about 18.2 and 21.3% compared to 100%RDF in 2021 and 2022, respectively. Kumar *et al.* (2021) opined that leaf area serves as a basis for assessing productivity. The higher total leaf area may be due to the accumulation of sugars and starch, which causes leaf expansion (Zhu *et al.* 2021). In bioaugmentation, the synergetic effect of cow-dung, slag and biochar may have accumulated more photo-assimilates in the leaf, contributing to a higher total leaf area.

Chlorophyll content: The chlorophyll content (SPAD values) ranged from 35.13 to 55.3 in 2021 and 34.63 to 54.57 in 2022 (Table 1). SPAD recorded significantly higher chlorophyll content for bioaugmented treatments at higher application rates, T_7, 55.30; T_{12}, 54.80 and T_8, 53.96 in 2021. During 2022, treatment T_9 showed significantly higher chlorophyll content (54.57). Chlorophyll content in bio-augmented treatments showed an increase of 26.5 and 31.0% over 100% RDF during 2021 and 2022, respectively. Higher chlorophyll and N contents in plants enhance photosynthesis (Ookawa *et al.* 2004), resulting in greater leaf area and accumulation of dry matter (source), large sizes of yield attributes (sink) and finally, yield. The synergetic effect of bioaugmentation of LD slag and biochar at higher application rates improved nutrient bioavailability, lowered water stress, superior soil microbial activity and favourable soil microclimate for nutrient uptake resulted in higher SPAD values. These results are in line with the previous research of Harish *et al.* (2022).

Normalized Difference Vegetation Index (NDVI): NDVI ranged between 0.33 to 0.70 during the tillering stage in 2021 and 0.32 to 0.68 in 2022 (Table 1). NDVI value was significantly higher in bioaugmented treatments T_{11} and T_8 during 2021 and T_{11} and T_{10} during 2022. Higher application rates of slag and biochar favoured higher leaf reflectance measured through NDVI, gave statistically significant values indicating greater leaf area expansion in different bio-augmented treatments. Bio-augmented treatments with a combination of LD slag and biochar, and that with LD slag alone gave significantly higher NDVI values compared to bio-augmented treatments with biochar alone. The higher the NDVI, the better the plant water conditions; hence, the crop canopy was greener and thicker/denser.

Leaf photosynthetic parameters: Bioaugmentation with LD slag and biochar influenced Pn, Gs and TR in wheat. The average net photosynthetic (Pn) rate ranged from 2.50 to 16.93 (μ mol CO_2 /m^2/ s) during flowering stage in 2021 and 2.53 to 17.16 (μ mol CO_2 /m^2/s) during 2022 (Table 1). T_4 and T_7 showed significantly ($P<0.05$) higher values during both the years. The stomatal conductance (Gs) ranged from 0.031 to 0.307 mol H_2O/m^2/sec during the flowering stage (Table 2). Treatment T_4 (0.304 mol H_2O/m^2/sec) recorded ~61.29 and 61.50% higher Gs than 100% RDF during 2021 and 2022, respectively. Treatment T_4 and T_7 recorded higher net photosynthetic rates, and T_4 recorded significantly higher stomatal conductance during both the years. The transpiration rate ranged from 1.18 to 8.81 m mol H_2O/m^2/sec in 2021 and 1.19 to 8.93 m mol H_2O/m^2/sec in 2022 (Table 1). Average transpiration rate (TR) was significantly higher ($P<0.05$) in T_{10} (8.87 m mol H_2O/m^2/sec) and T_{11} (8.45m mol H_2O/ m^2/sec). The lowest Pn, Gs and TR were recorded with the absolute control. The utilization and uptake of crop nutrients significantly influenced physiological processes, including photosynthesis and yield production (Choudhary *et al.* 2022). Slag contains a remarkable amount of Ca and Si; hence, silicic acid helps leaves to stand straight, increasing their capacity to absorb light and promoting photosynthesis (Luyckx *et al.* 2017). Consequently, biochar and cow-dung increase plant nutrient availability and improve physiological characteristics. However, in our study synergistic effect of LD slag, biochar and cow dung slurry improved the photosynthetic parameters of the crop.

Yield attributes

No. of spikes and grains per spike: The number of spikes/m^2 ranged from 320 to 425 in 2021 and 310 to 413 in 2022. The grains per spike varied from 20–50 nos. (Table 2). No. of spikes/m^2 was significantly higher for T_7, T_8, T_{10}, and T_{11}compared to other treatments for both the years. Bioaugmented treatments with LD slag and biochar at higher application rates (T_7 and T_{10}) and bioaugmented treatments with LD slag alone (T_8 and T_{11}) recorded higher spikes compared to bioaugmented treatments with biochar

Table 1 Effect of bio-augmented LD slag and biochar on growth and leaf photosynthetic parameters of wheat

Treatment	Total leaf area (cm^2/tiller)		Chlorophyll content (SPAD values)		Normalized deviation vegetation index (NDVI)		Net photosynthetic rate (μ mol $CO_2/m^2/s$)		Stomatal conductance (mol H_2O/m^2/sec)		Transpiration rate (m mol H_2O m^2/ sec)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
T_1	673.0	692.1	48.5	46.6	0.61	0.58	12.1	12.2	0.208	0.212	5.07	5.14
T_2	684.8	688.5	45.9	46.6	0.59	0.61	10.0	10.2	0.184	0.187	4.61	4.67
T_3	635.7	665.2	46.6	45.8	0.57	0.58	12.6	12.7	0.230	0.235	5.62	5.69
T_4	715.9	713.6	49.8	49.0	0.64	0.63	16.9	17.1	0.301	0.307	7.06	7.16
T_5	681.8	702.7	49.5	49.2	0.59	0.58	13.5	13.7	0.137	0.139	4.39	4.45
T_6	661.2	675.7	50.3	49.6	0.61	0.60	11.6	11.8	0.188	0.192	6.51	6.60
T_7	709.7	730.3	55.3	53.1	0.69	0.66	16.8	17.0	0.160	0.163	6.31	6.39
T_8	721.7	772.6	53.9	53.7	0.69	0.67	11.6	11.8	0.151	0.154	4.88	4.95
T_9	667.9	681.8	52.7	54.5	0.64	0.65	11.3	11.4	0.193	0.197	6.19	6.27
T_{10}	735.4	724.1	51.9	53.2	0.68	0.68	13.2	13.4	0.200	0.205	8.81	8.93
T_{11}	719.5	770.2	51.8	51.0	0.70	0.68	12.5	12.6	0.239	0.244	8.40	8.51
T_{12}	707.3	729.2	54.8	53.5	0.68	0.66	14.0	14.2	0.202	0.206	7.15	7.34
T_{13}	633.2	636.9	46.5	47.1	0.63	0.62	7.62	7.72	0.111	0.113	3.31	3.35
T_{14}	662.7	667.9	46.1	45.6	0.59	0.58	11.6	11.6	0.159	0.162	6.04	6.12
T_{15}	621.9	687.5	43.7	42.7	0.59	0.59	5.35	5.40	0.083	0.084	3.24	3.28
T_{16}	638.7	699.6	43.7	41.6	0.61	0.59	8.76	8.88	0.187	0.190	7.75	7.86
Control	475.9	495.0	35.1	34.6	0.33	0.32	2.50	2.53	0.032	0.032	1.18	1.19
LSD (P=0.05)	61.4	83.7	3.61	3.56	0.044	0.047	0.82	0.85	0.027	0.027	0.56	0.55

Treatment details are given under Materials and Methods.

alone at respective application rates (T_3, T_6, T_9, T_{12}). When bioaugmented, higher doses of the combination of LD slag:1.5 t/ha and 2 t/ha and biochar: 0.75 t/ha and 1 t/ha increased the no. of spikes. The significantly lowest values were recorded with control in both seasons. Bioaugmented treatments with a combination of slag and biochar and slag alone recorded significantly higher no. of spikes and grains per spike when compared to bioaugmented treatments with biochar alone of respective application rate.

Dry biomass yield (t/ha) and test weight: The average dry biomass yield ranged from 5.35 to 10.6 t/ha during 2021 and 5.54 to 10.6 t/ha during 2022 (Table 2). Treatment T_{10} recorded significantly higher dry biomass yield for both years followed by T_{11}. Biomass yield increased up to 22.2% in bioaugmented treatments compared to 100% RDF. However, the lowest average dry biomass yield was recorded with the control. The yield attributes of wheat crops, viz. no. of spikes, grains per spike, and dry biomass weight were significantly affected by bioaugmentation. The average test weight of grains ranged from 28.3 to 33.4 g during 2021 and 28.2 to 34.8 g during 2022 (Table 2). T_{10} recorded significantly higher test weight during both the years. There was no significant difference between T_4, T_8 and T_9 in 2021 and T_{11} in 2022. In both the years, control recorded significantly lower test weight. Bioaugmented treatments with LD slag @2 t/ha (T_{10}, T_{11}) were superior in all yield attributes. However, bioaugmented treatments with LD @1.5 t/ha (T_7, T_8) were performed on par with parameters such as no. of spikes and grains per spike.

Grain yield: The seasonal grain yield varied from 2.17 to 4.75 t/ha during 2021 and 2.13 to 4.78 t/ha during 2022 (Table 2). Treatment T_{10} (4.75 and 4.78 t/ha) recorded significantly higher yields than the other treatments during 2021 and 2022, respectively. This was followed by T_{11} (10.3 and 10.4 t/ha). From our studies, it is clear that bioaugmented treatments with a combination of LD slag and biochar at increasing application rates recorded higher yields (T_1, T_4, T_7 and T_{10} recorded 3.96 t/ha, 4.14 t/ha, 4.50 t/ha and 4.76 t/ha, respectively) and were followed by bioaugmented LD slag alone at respective application rates. Whereas the yield was significantly lower in treatments bioaugmented with biochar alone at respective application rates compared to combination treatments. Bioaugmented treatments significantly gave higher yields than non-bioaugmented treatments at respective rates. A yield increase of 25.6% and 27.1% were found in bioaugmented treatments with LD slag: 2 t/ha + biochar: 1 t/ha over 100%RDF during 2021 and 2022, respectively. In both the seasons, control gave significantly lowest grain yield.

The fertilization of LD slag at higher application rates increases soil nutrients and enriches soil microorganisms that have a beneficial role in nutrient mobilization. Microorganisms play a vital role in the microbial decomposition of organic matter and nutrient mobilization, which in turn regulates the increase in crop yield. Gown *et al.* (2018) reported that grain yield increased by 10.3 to 15.2% higher with the LD slag amendment (2 t/ha) compared to the unamended control. This is due to the enhanced rate

Table 2 Effect of bioaugmented LD slag and biochar on yield attributes of wheat

Treatment	Spikes/m^2		Grains/spike		Dry biomass yield (t/ha)		Grain yield (t/ha)		1000-grain weight (g)	
	2021	2022	2021	2022	2021	2022	2021	2022	2021	2022
T_1	336	334	42	42	9.00	9.02	3.96	3.97	32.7	33.6
T_2	325	321	43	43	8.95	9.08	3.94	3.99	30.9	31.7
T_3	322	319	41	40	8.85	8.78	3.90	3.85	30.9	33.0
T_4	396	386	49	48	9.34	9.60	4.10	4.19	33.2	33.8
T_5	358	359	49	48	9.22	9.62	4.08	4.22	32.3	32.2
T_6	343	360	41	42	9.12	8.98	3.97	3.97	32.8	33.3
T_7	418	407	49	49	10.1	10.1	4.48	4.53	32.5	32.9
T_8	421	412	50	50	9.74	10.0	4.39	4.45	33.6	33.7
T_9	357	371	45	46	9.28	9.46	4.10	4.15	33.4	33.7
T_{10}	425	411	50	50	10.6	10.6	4.75	4.78	33.2	34.7
T_{11}	415	413	49	50	10.3	10.4	4.62	4.63	31.2	34.8
T_{12}	367	380	47	47	9.47	9.70	4.18	4.31	32.4	33.0
T_{13}	333	346	41	41	8.83	8.81	3.90	3.84	30.4	32.1
T_{14}	387	393	43	45	9.68	9.99	4.31	4.43	31.2	32.4
T_{15}	331	349	41	44	9.22	9.35	4.08	4.13	31.3	33.4
T_{16}	329	337	42	43	8.66	8.68	3.78	3.76	31.0	32.1
Control	320	310	21	20	5.35	5.54	2.17	2.13	28.3	28.2
LSD (P=0.05)	18.07	19.95	3.1	3.3	0.213	0.327	0.123	0.173	1.77	2.15

Treatment details are given under Materials and Method.

of photosynthesis and adequate nutrient availability to the rice plant. Also, slag silicate fertilizer (4 t/ha) increased rice yield by 13 and 18% in the no-tillage and tillage rice cropping systems, respectively (Ali *et al.* 2009). Likewise, biochar and cow-dung also influenced the growth and yield attributes in our study but not to the extent of LD slag. If biochar is used in conjunction with inorganic or organic fertilizers, crop yields, especially on tropical soils, can be boosted. The porous structure of biochar, along with its high internal surface area and ability to adsorb soluble organic matter, gases, and inorganic nutrients are likely to provide a highly suitable habitat for the microbes to colonize, grow, and reproduce. Thus, biochar improves the soil environment and increases plant nutrient availability, indirectly leading to increased plant development (Steiner *et al.* 2008). In our study, the synergetic effect of bioaugmented LD slag and biochar at increasing application rates (2 t/ha) is credited for improving the growth and yield of wheat.

On the basis of the two-year study, it can be concluded that the combined use of bioaugmented LD slag and biochar improved the conditions for the growth, development and yield of wheat by increasing the nutrient content in the soil and offering better nutrient accessibility for the wheat crop. Bioaugmented treatments with a combination of LD slag @2 t/ha and biochar @1 t/ha recorded significantly higher yield attributes, followed by bioaugmented slag compared to bioaugmented treatments with biochar alone. Applying biochar along with organic or inorganic fertilizers may improve the soil's physical, chemical, and biological characteristics and promote plant development. No. of spikes, grains per ear, biomass production, and grain yield were higher in bioaugmented treatments when compared to the 100% RDF and control. Utilizing bioaugmented slag instead of direct slag could be a sustainable management strategy however, it needs further evaluation under various soil and climatic conditions.

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