Research Article

Wheat response to different levels of humic acid and brassinolide

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Abstract

A field experiment was conducted to investigate wheat response to different levels of humic acid and brassinolide at New Developmental Farm of Khyber Pakhtunkhwa Agricultural University Peshawar during Rabi season 2009 -10. The wheat variety Saleem 2000 was sown. The design used was Randomized Complete Block Design (RCBD) with four replications in split plot arrangement. Humic acid was assigned to main plot whereas brassinolide was allotted to sub plot. Plot size of 3 x 3.5 m having 10 rows, 30 cm apart were used. Four levels of humic acid (0, 1, 2 and 4 kg ha⁻¹) were applied at sowing times. Whereas 5 levels of brassinolide (0, 0.5, 1, 1.5 and 2 mg L⁻¹) were sprayed in foliar form at the tillering stage. Increasing humic acid levels up to 2 kg ha⁻¹ increased crop productivity in term of yield and yield components. Higher spikes m⁻² (206.38), grains spike⁻¹ (63.19), thousand grains weight (39.57g), biological yield (11918 kg ha⁻¹), grain yield (4163kg ha⁻¹) and harvest index (34.87%) were obtained from 2 kg HA ha⁻¹ treated plots. Increasing brassinolide concentrations up to 2 mg L⁻¹ increased crop productivity in term of yield and yield components. Higher spikes m⁻² (205.94), grains spike⁻¹ (66), thousand grains weight (39.89g), biological yield (11584 kg ha⁻¹), grain yield (4397kg ha⁻¹) and harvest index (37.87%) were obtained from 2 mg B L⁻¹ treated plots. Increasing brassinolide concentration up to 2 mg L⁻¹ as combined with 2 kg HA ha⁻¹ increased spikes m⁻², grains spike⁻¹, thousand grains weight and grain yield. Application of 2 kg HA ha⁻¹ enhanced wheat crop productivity and thus is recommended for wheat cultivation in the agro climatic conditions of Peshawar for improved yield. Application of 2 mg B L⁻¹ improved wheat crop productivity and thus is recommended for wheat cultivation in the agro climatic conditions of Peshawar for better yield.

Keywords: Wheat (*Triticum aestivum* L.); Humic acid; Brassinolide; Yield and yield components

Introduction

Wheat plays a key role in human economic and social development in addition to food. It ranks first among the cereals on production basis. Production of wheat in Pakistan has increased manifold over the past few decades, yet we faced food shortage. There are two ways to increase the yield, by bringing more area under cultivation or to increase yield per unit area. As the land is fixed, so it is important to use the available land more efficiently.

An area of 9096(000) ha was cultivated for wheat which produced an average yield 2657 kg ha⁻¹ [1].

Humic acid and brassinolide are important compounds that help to increase agricultural production. Humic acid has positive effects on enzyme activity, plant nutrients, growth stimulant and can be considered as "plant food". Humic acid is most responsive in high carbonate crops like potato, carrot, maize, rice, wheat [2]. Many other studies have demonstrated the

practical importance of humic acid application in crop yield of wheat, maize, cotton, sugar beet and groundnut and improvement in soil physio-chemical conditions [3- 6]. In many studies, humic and fulvic acids preparations were reported to increase the uptake of mineral elements to promote the root length [7] and to increase the fresh and dry weights of crop plants [8].

Humic acid (HA) is produced naturally by extracting from our lignite coal [9]. Humic acid gives organic carbon 51-57%, N (4-6%), P (0.2-1%) and has the capability to enhance crops yields through provision of nitrogen and phosphorus to the plants. But the quantity of humic acid applied in the form of fertilizer is up to 2 kg ha⁻¹ which can provide 0.04 to 0.08 kg nitrogen ha⁻¹ and 0.004 kg phosphorus ha⁻¹ to the soil which is very less as compared to the nutritional need of crops [10].

Brassinolide is a steroid compound that play role in cell division and growth discovered in 1973 [11]. Brassinolide provide protection to plants during chilling and drought stress. Brassinolide has been reported to work with auxin to promote cell expansion and elongation. Brassinolide also increases uptake of plant nutrients [12].

Appliance of brassinolide to crops helps to beat environmental stress such as cold, salt tolerance, and herbicidal injury. Brassinolide have been evaluated for use in improving crop yield, quality, stress tolerance, and disease resistance and promising results have been reported with several plants [13].

Brassinolide also increases uptake of plant nutrients. Various carbohydrate fractions of many plants increases due to Brassinosteroids application [14].

Genetic manipulation of brassinolide activity has indeed led to increase in crop yield of 20-60% confirming the value of further research to increase productivity [15].

Homobrassinolide stimulates wheat yield which is manifested by an increase in the

no of grains spike⁻¹, spikes m⁻² and thousand grains weight [16].

Brassinolide application improves the leaf water economy and CO₂ assimilation and enables plant to withstand drought. Root application of homobrassinolide results in enhanced total grain yield and thousand grain weight of both salt stressed cultivars [17].

28 homobrassinolide significantly increased the yield of wheat up to 6.7 t ha⁻¹ as compared to the controlled field which produced 5.7 t ha⁻¹ [18].

Homobrassinolide increases photosynthesis physiologically and thus increases chlorophyll content [16].

Keeping in view the role of humic acid and brassinolide, the present study was carried out to minimize the gap between actual and potential yield of wheat.

Materials and methods

In order to study "wheat response to different levels of humic acid and brassinolide" a field experiment was conducted during Rabi season 2009-2010 at New Developmental Farm Agricultural University Peshawar. The different levels of humic acid and brassinolide were planned as Main plot included Humic acid levels HA₀ (0 kg ha⁻¹), HA₁ (1 kg ha⁻¹), HA₂ (2 kg ha⁻¹), HA₃ (4 kg ha⁻¹) while subplot included Brassinolide Concentrations levels B₀ (0 mg L⁻¹), B₁ (0.5 mg L⁻¹), B₂ (1.0 mg L⁻¹), B₃ (1.5 mg L⁻¹) and B₄ (2.0 mg L⁻¹). The study was carried out in randomized complete block design with split plot arrangement having four replications. Wheat variety Saleem 2000 was sown at a seed rate of 100 kg ha⁻¹. Nitrogen and Phosphorus was applied at the rate 120 kg ha⁻¹ and 60 kg ha⁻¹ respectively. Half of nitrogen and all phosphorus were applied at the time of seed bed preparation while the remaining nitrogen was applied with first irrigation. A plot size of 3m x 3.5m was maintained with row to row distance of 30 cm having ten rows. Humic acid was applied at time of sowing while brassinolide was applied at start of tillering stage as a foliar spray.

Before sowing the field was prepared and was ploughed with normal cultivator. All agronomic practices were carried out uniformly. Data was subscribed on the parameters spikes m^{-2} , grains spike $^{-1}$, thousand grains weight, biological yield, grain yield and harvest index. Data regarding spikes m^{-2} was recorded by counting the number of spikes in one meter long row at three randomly selected places in each sub plots and then converted into spikes m^{-2} . Data regarding number of grains spike $^{-1}$ were recorded by counting the number of grains from ten randomly selected spikes in each sub plot and averaged. For thousand grains weight data after threshing thousand grains from each sub plot and plot were counted weighed with an electronic balance. For biological yield (kg ha $^{-1}$) four central rows from each sub plot were harvested, weighted and converted into kg ha $^{-1}$. Data regarding grain yield (kg ha $^{-1}$) was recorded by harvesting the central three rows from each plot. The harvested rows were then dried, threshed, cleaned weighed and converted into kg ha $^{-1}$. Harvest index was calculated by taking the ratio of grain yield to biological yield and then multiplied with hundred. Data were analyzed according to Randomized Complete Block Design (RCBD) with split plot arrangement. LSD test was also carried out to test the significant differences among the means of different treatments [19].

Results

Spikes m^{-2}

Table 1. Spikes m^{-2} of wheat as affected by humic acid and brassinolide

Brassinolids (mg L ⁻¹)	Humic acid (kg ha ⁻¹)				Mean
	0	1	2	4	
0	173.00	196.25	218.75	199.25	196.81 d
0.5	195.25	200.50	205.00	201.50	200.56 c
1	192.25	212.75	207.25	200.50	203.19 b
1.5	199.50	199.25	200.50	212.75	203.00 b
2	208.25	203.50	212.75	199.25	205.94 a
Mean	193.65 c	204.00 ab	206.38 a	203.50 b	
LSD value at P≤ 0.05 for Humic acid			2.5571		
LSD value at P≤ 0.05 for Brassinolide			1.608		
LSD value at P< 0.05 for Humic acid x Brassinolide			3.216		

Data regarding spikes m^{-2} of wheat as affected by humic acid and brassinolide is shown in Table 1. Analysis of the data indicated that spikes m^{-2} was significantly affected by humic acid and brassinolide. Similarly, the interactive effect of humic acid and brassinolide was also significant. More (206.38) spike m^{-2} were produced in plots where 2 kg HA ha $^{-1}$ was applied as compared to controlled sub plots which produced minimum (193.65) spikes m^{-2} . Regarding brassinolide maximum (205.94) spikes m^{-2} were produced in sub plots treated with 2 mg BL $^{-1}$ as compared with lower (196.81) spikes m^{-2} from controlled plots. Regarding the interactive effect of humic acid and brassinolide with increasing brassinolide concentration spikes m^{-2} increased with increasing humic acid levels up to 2 kg HA ha $^{-1}$ and then decreased by 4 kg HA ha $^{-1}$. Spikes m^{-2} was significantly affected by humic acid and brassinolide. Maximum spike m^{-2} were produced in plots where 2 kg HA ha $^{-1}$ was applied as compared to controlled plots which produced minimum spikes m^{-2} . Plants tolerance to stress and improved growth is observed with nutrients uptake in humic acid applied soil [20]. Regarding brassinolide, maximum spikes m^{-2} were produced in plots treated with 2 mg B L $^{-1}$ as compared with lower spikes m^{-2} from controlled plots. Homobrassinolide stimulates wheat yield which is manifested by an increase in the no of grains spike $^{-1}$, spikes m^{-2} and thousand grains weight [16].

Grains spike⁻¹

Data concerning number of grains spike⁻¹ of wheat as affected by humic acid and brassinolide is presented in Table 2. Analysis of the data revealed that the no of grains spike⁻¹ was significantly affected by humic acid and brassinolide. Interactive effect of humic acid and brassinolide was also found significant. Higher (63.19) grains spike⁻¹ were attained from sub plots where 2 kg HA ha⁻¹ was applied as compared with lower (57.15) grains spike⁻¹ in 0 kg HA ha⁻¹ treated sub plots. Similarly maximum grains spike⁻¹ (66.00) were produced in sub plots treated with 2 mg BL⁻¹ as compared with lower (54.38) grains spike⁻¹ with application of 0 mg B L⁻¹. The interactive response of humic acid and brassinolide revealed that with increasing concentration of brassinolide number of grains spike⁻¹ increases with

increasing level of humic acid up to 2 kg HA ha⁻¹ and then decreases by 4 kg HA ha⁻¹. The no of grains spike⁻¹ was significantly affected by humic acid and brassinolide. Higher grains spike⁻¹ were attained from sub plots where 2 kg HA ha⁻¹ was applied as compared with lower grains spike⁻¹ in 0 kg HA ha⁻¹ treated sub plots. Humic acid increases grains spike⁻¹ [21] due to its capability of decomposing residues and leading to slow release of Nitrogen to plant and soil [10]. Similarly maximum grains spike⁻¹ were produced in sub plots treated with 2 mg BL⁻¹ as compared with lower grains spike⁻¹ with application no brassinolide. Homobrassinolide enhances wheat yield which can be credited by an increase in the number of grains spike⁻¹, spikes m⁻² and thousand grains weight [16].

Table 2. Grains spike⁻¹ of wheat as affected by humic acid and brassinolide

Brassinolide (mg L ⁻¹)	Humic acid (kg ha ⁻¹)				Mean
	0	1	2	4	
0	50.50	55.00	57.00	55.00	54.38 e
0.5	56.00	57.00	58.00	57.00	57.00 d
1	57.00	58.00	60.00	58.00	58.25 c
1.5	58.00	59.00	64.75	59.00	60.19 b
2	64.25	65.50	70.00	64.25	66.00 a
Mean	57.15 c	59.88 b	63.19 a	59.56 b	
LSD value at P≤ 0.05 for Humic acid			0.5847		
LSD value at P≤ 0.05 for Brassinolide			0.9969		
LSD value at P≤ 0.05 for Humic acid x Brassinolide			1.9938		

Thousand grains weight

Data regarding thousand grains weight of wheat as affected by humic acid and brassinolide is depicted in Table 3. Analysis of the data indicated that humic acid and brassinolide had significant effect on thousand grains weight. The interactive response of humic acid and brassinolide was also found significant. Heavier (39.57) thousand grains weight was produced by the plots where 2 kg HA ha⁻¹ was applied as compared with lower thousand grain weight (37.22) in 0 kg HA ha⁻¹. Maximum thousand grain weight (39.89) was recorded for 2 mg BL⁻¹ as compared while lower (36.54) in 1.5 mg B L⁻¹ treated plots.

Considering interactive response of humic acid and brassinolide, with increasing brassinolide concentration number of grains spike⁻¹ increased when humic acid level was increased up to 2 kg HA ha⁻¹ and then decreased with 4 kg HA ha⁻¹. Humic acid and brassinolide had a significant effect on thousand grains weight. Heavier thousand grains weight was produced by the plots where 2 kg HA ha⁻¹ was applied as compared with lower thousand grain weight in 0 kg HA ha⁻¹. Humic acid increases grains spike⁻¹ [21] because of its ability of decomposing residues and resulting in slow release of Nitrogen to plant and soil which in turn

increasing grains weight [16]. Maximum thousand grain weight was recorded from 2 mg BL⁻¹ as compared with lower in 1.5 mg B L⁻¹ treated plots. Application of

homobrassinolide resulted in enhanced total grain yield and 100 grain weight of both salt stressed cultivars [17].

Table 3. Thousand grains weight (g) of wheat as affected by humic acid and brassinolide

Brassinolide (mg L ⁻¹)	Humic acid (kg ha ⁻¹)				Mean
	0	1	2	4	
0	34.15	37.13	37.60	37.30	36.54 e
0.5	37.08	38.08	38.10	37.98	37.81 d
1	37.60	38.60	38.65	38.55	38.35 c
1.5	38.43	39.28	40.30	39.30	39.33 b
2	38.83	39.83	41.23	39.70	39.89 a
Mean	37.22 c	38.94 b	39.57 a	38.88 b	
LSD value at P≤ 0.05 for Humic acid				0.2271	
LSD value at P≤ 0.05 for Brassinolide				0.1808	
LSD value at P≤ 0.05 for Humic acid x Brassinolide				0.3616	

Biological yield

Biological yield data of wheat as affected by humic acid and brassinolide is depicted in Table 4. Statistical analysis of the data revealed that humic acid and brassinolide had a significant effect on biological yield. Whereas interactive effect of humic acid and brassinolide was non significant. Higher biological yield (11918) kg ha⁻¹ was attained by plots where 2 kg HA ha⁻¹ was applied as compared with controlled plots which produced minimum (9902) kg ha⁻¹ biological yield. Maximum (11584) kg ha⁻¹ biological yield was recorded from 2 mg B L⁻¹ as compared with controlled plots which produced (9132) kg ha⁻¹ biological yield. Humic acid and brassinolide had a significant effect on biological yield. Higher biological yield was attained by plots where 2 kg HA ha⁻¹ was applied as compared with controlled

plots which produced minimum biological yield. The convenient availability of nutrients to crops [22], decomposition of residues [23], and better soil physiochemical properties [24] could be the possible reasons for the improved yield. Maximum biological yield was recorded from 2 mg B L⁻¹ as compared with controlled plots which produced biological yield. Homobrassinolide stimulates wheat yield which is manifested by an increase in the number of grains spike⁻¹, spikes m⁻² and thousand grains weight [16]. 28 homobrassinolide significantly increased the yield of wheat up to 6.7 t ha⁻¹ as compared to the controlled field which produced 5.7 t ha⁻¹ [18]. Homobrassinolide increases photosynthesis physiologically and thus increases chlorophyll content [16].

Table 4. Biological yield of wheat as affected by humic acid and brassinolide

Brassinolide (mg L ⁻¹)	Humic acid (kg ha ⁻¹)				Mean
	0	1	2	4	
0	7929	9313	9993	9295	9132 d
0.5	9733	10151	10830	10133	10212 c
1	10572	10991	11668	10971	11051 b
1.5	10276	11093	12358	11073	11200 b
2	11002	11269	12817	11249	11584 a
Mean	9902 c	10876 b	11918 a	10857 b	
LSD value at P≤ 0.05 for Humic acid		506.87			
LSD value at P≤ 0.05 for Brassinolide		379.68			

Grain yield

Data pertaining to grain yield of wheat as affected by humic acid and brassinolide is shown in Table 5. Mediation of the data indicated that humic acid and brassinolide had a significant effect on grain yield. The interactive response of humic acid and brassinolide was also found significant. Highest grain yield (4163 kg ha⁻¹) was attained from the plots where 2 kg HA ha⁻¹ was applied as compared with lower grain yield (3004 kg ha⁻¹) which was produced from controlled plots. Regarding brassinolide maximum grain yield (4397 kg ha⁻¹) was recorded from the plots treated with 2 mg B L⁻¹ concentration as compared with controlled plots which produce minimum (3088 kg ha⁻¹) grain yield. The interactive response of humic acid and brassinolide revealed that with increasing concentration of brassinolide

grain yield increased till 2 kg HA ha⁻¹ and then decreased by 4 kg HA ha⁻¹. Humic acid and brassinolide had a significant effect on grain yield. Highest grain yield was attained from the plots where 2 kg HA ha⁻¹ was applied as compared with lower grain yield which was produced from controlled plots. These results have similarity to those of [10]. Humic acid enhances microbial activity [25] and also the ability of humic acid to release the nutrients slowly could be the possible reasons for improved crop yield. Regarding brassinolide, maximum grain yield was recorded from the plots treated with 2 mg B L⁻¹ concentration as compared with controlled plots which produced minimum grain yield. Root application of homobrassinolide resulted in enhanced total grain yield and 100 grain weight of salt stressed cultivars [17].

Table 5. Grain yield of wheat as affected by humic acid and brassinolide

Brassinolide (mg L ⁻¹)	Humic acid (kg ha ⁻¹)				Mean
	0	1	2	4	
0	2327	3250	3533	3241	3088 e
0.5	2724	3400	3648	3391	3290 d
1	2875	3552	3798	3540	3441 c
1.5	3370	3731	4100	3720	3730 b
2	3723	4384	5106	4376	4397 a
Mean	3004 c	3767 b	4163 a	3757 b	
LSD value at P≤ 0.05 for Humic acid			123.53		
LSD value at P≤ 0.05 for Brassinolide			71.586		
LSD value at P≤ 0.05 for Humic acid x Brassinolide			143.17		

Harvest index

Data regarding harvest index of wheat is shown in Table 6. Mediation of the data revealed that humic acid and brassinolide had a significant effect on harvest index. The interactive response of humic acid and brassinolide was found non significant. Maximum harvest index (34.87 %) was obtained from the plots where 2 kg HA ha⁻¹ was applied which is statistically at par with 1 and 4 kg HA ha⁻¹. In case of brassinolide higher harvest index (37.87 %) was recorded by 2 mg BL⁻¹ as compared with lower (31.11 %) lower harvest index which was obtained from plots where 1 mg B L⁻¹ was applied.

Humic acid and brassinolide had a significant effect on harvest index. Maximum harvest index was attained from the plots where 2 kg HA ha⁻¹ was applied which was statistically at par with 1 and 4 kg HA ha⁻¹. Humic acid play an important role on the decomposition of residues [26] and provided high nitrogen content from mungbean residues which resulted in high grain yield, biological yield and harvest index [25]. In case of brassinolide higher harvest index was recorded from 2 mg B L⁻¹ as compared with lower harvest index which was obtained from plots where 1 mg B L⁻¹ was applied. Humic acid play an important role in the decomposition of

residues [26] and provided high nitrogen content which resulted in high grain yield,

biological yield and harvest index [25].

Table 6. Harvest index of wheat as affected by humic acid and brassinolide

Brassinolide (mg L ⁻¹)	Humic acid (kg ha ⁻¹)				Mean
	0	1	2	4	
0	29.65	34.93	35.41	34.92	33.73 b
0.5	28.04	33.52	33.71	33.51	32.19 c
1	27.23	32.33	32.60	32.29	31.11 c
1.5	33.30	33.63	33.23	33.59	33.44 bc
2	33.84	38.94	39.95	38.74	37.87 a
Mean	30.41 b	34.60 a	34.87 a	34.53 a	

LSD value at P≤ 0.05 for Humic acid

1.8294

LSD value at P≤ 0.05 for Brassinolide

1.4072

Conclusion and recommendation

It was concluded from the results of this experiment that humic acid level at the rate of 2 kg ha⁻¹ enhanced yield and yield components. Considering brassinolide effect, the application of brassinolide at the rate of 2 mg L⁻¹ performed better as compared to rest. Based on results and conclusion, it is recommended that Humic acid at the rate of 2 kg ha⁻¹ and brassinolide at 2 mg L⁻¹ can be used for higher yield of wheat in Peshawar valley.

Authors' contributions

Conceived and designed the experiments: S Anwar & I Khan, Performed the experiments: I Khan & B Iqbal, Analyzed the data: S Anwar & I Khan, Contributed reagents/ materials/ analysis tools: S Hussain, MM Anjum, A Hussain, MO Iqbal & N Ali, Wrote the paper: S Anwar & B Iqbal.

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