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Wheat response to plant growth promoting rhizobacteria, humic acid and sn-brassinolide

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Abstract

In order to study the effect of non-chemical sources of nutrients and phytohormone like substances on wheat growth, yield and yield components, this experiment was conducted in 2013 at the research field of Islamic Azad University, Karaj branch, Iran. The experiment was conducted in factorial in the form of a randomized complete block design with four replications and three treatments: (1) humic acid in two levels including 0 and 7L/ha, (2) plant growth promoting rhizobacteria application in two levels including without inoculation and inoculation of a bacterial mixture (*Azotobacter chroococcum* + *Azospirillum lipoferum* + *Pseudomonas putida*) with seed, and (3) Brassinolide application in four levels including 0, 30, 50 and 70 ml/ha. Analysis of variance showed that Brassinolide, humic acid and PGPR had significant effect on all measured traits including plant height, grain weight, dry weight, the number of grains in spike, spike length, 1000 grain weight, the number of tillers and the harvest index. The three-fold interaction of the three factors had only a significant effect on plant height, total dry weight, spike length, 1000 grain weight and the number of tillers. The interaction of 70 ml/ha Brassinolide × humic acid application × PGPR application was the most effective treatment of this experiment which increased plant height by 42%, total dry weight by 68%% and 1000 grain weight by 35% compared with the control.

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Introduction

In recent decades, increased world population has created the need for more and more food production which was possible through the extensive application of chemical fertilizer to field crops. However, application of chemical fertilizers caused vital damages to the natural ecosystems. As the result, today there is an increasing attempt to use non-chemical sources of nutrients instead of the chemical fertilizers. These alternatives include biofertilizers and organic materials (Astaraei and Koochaki, 1996).

Wheat (*Triticum aestivum* L.) is the most important food crop in human nutrition which is cultivated all around the world. Wheat is cultivated in areas with average annual precipitation ranging from 250 to 1300 mm; however, more than 75% of world wheat production occurs in areas with average annual precipitation ranging from 375 to 875 mm. It can also grow in areas 0 to 4750 m above the sea level (Kazemi Arbat, 1999; Nourmohammady *et al.*, 2001). Enhancement of wheat yield is possible through different agronomic practices; one of the most important one is nutrient management. In addition to chemical fertilizers, biofertilizers and other non-chemical sources of nutrients are an important part of integrated nutrient management. Some organic materials are also capable of increasing plants growth and yield such as humic acid and Brassinolides.

Humic substances are organic-mineral materials such as amino acids, peptides, phenols, aldehydes and nucleic acids in band with cations which form a very complicated structure with wide range of functions. Humic acid naturally presents in all soil; forming about 80% of soil organic materials. Humic acid forms when organic materials decompose. Humic acid is a biological stimulator to plants growth. It facilitates nutrients absorption by plant roots and also prevents nutrients leaching from soil (Mackowiak *et al.*, 2001; Samavat and Malakouti, 2005). Humic acid improves soil physico-chemical condition and increases the activity of microorganisms. It also improves plants growth and yield through phytohormonal effects (Sabzevari and Khazaei, 2009). Abdel-Mawgoud *et al.* (2007) reported that foliar application of humic acid increases plants growth through increasing the content of auxin, cytokinin and gibberelin in plants. Chen *et al.* (2004) also reported that application of humic acid increases plants root growth, nutrient uptake, leaf area and photosynthesis rate; consequently increasing plants growth and yield.

Brassinolide is a group of Brassinosteroides with biological activity and may be considered as some types of phytohormones. Brassinosteroides may be found in very low concentration in nearly all plant species; they accumulate mainly in pollens and immature seeds. Brassinosteroides have improving effects on germination, rooting, flowering and maturity; generally increasing plants growth and yield. They also induce resistance to abiotic stresses. They are considered as multi-functional phytohormones (Sakurai and Fujioka, 1993; Sasse, 1997). Maeda (1965) reported that treating plants with Brassinosteroides increased cell elongation and cell turgor pressure; resulting in improved plant growth. Sasse *et al.* (1995) treated *Eucalyptus camaldulensis* seeds with epi-Brassinolide and observed an increased germination rate. Vardhini and Rao (1998) also tested the effect of Brassinolides on groundnut and tomato and reported an increased growth and yield.

In addition to biologically active substances and phytohormones, soil microorganisms may also be beneficial to plants growth and yield. Today, application of biofertilizers instead of high doses of chemical fertilizers is a common agronomic practices especially in developed countries. Biofertilizers not only provide mineral nutrients to plants, but improve soil physico-chemical and biological activity. They are also cost effective and environmentally friendly (Moallem and Eshghizadeh, 2007; Wiedenhoeft, 2006). Biofertilizers are a wide range of soil microorganisms from bacteria to fungi with various beneficial effects on plants life. They provide nutrients to pants, increase root system development, facilitate water absorption and enhance plant resistance to biotic/abiotic stresses (Han and Lee, 2005; Lambers *et al*., 2006; Wiedenhoeft, 2006).

Regarding the represented benefits of biologically active substances, phytohormones and biofertilizers to plants growth and yield, and the complexity of their interactions, this experiment was conducted to evaluate the effects of humic acid, Brassinolide and plant growth promoting rhizobacteria on growth and yield of wheat.

Materials and methods

Location

This experiment was conducted in 2013 at the research field of Islamic Azad University, Karaj branch, Iran (35° 55' N, 50° 54' E, 1313 m above the sea level). Soil at the test site was loamy-sand (clay, 34%; silt, 22%; sand, 44%) with the pH of 7.8 and EC of 3.62 ds/m. Other soil properties are listed in Table 1.

Experimental design and treatments

The experiment was conducted in factorial in the form of a randomized complete block design with four replications and three treatments:

Humic acid

In two levels including 0 and 7 L/ha. The recommended dose of humic acid was 200 g humic acid for 100 kg seed. So, 12 kg wheat seed was mixed with 20 g humic acid, prior to seeding.

Plant growth promoting rhizobacteria

In two levels including without inoculation and inoculation of bacterial mixture (*Azotobacter chroococcum* + *Azospirillum lipoferum* + *Pseudomonas putida*) with seed. Seeds were inoculated with the bacterial mixture 30 minutes before seeding.

Brassinolide

In four levels including 0 (control), 30, 50 and 70 ml/ha.

Agronomic practices

The field was prepared by moldboard plow, disk and leveler. Then seeds were planted manually based on 300 plants/m^2 density. Each plot included six planting rows 50 cm apart and 3.5 m long. During the growth period, weeds were controlled manually and no pesticide was applied in order to prevent the disturbance of the bacteria's function.

Measurements and statistical analysis

At the end of the growth period, sampling was conducted from the two middle rows of each plot to measure the traits. To measure grain weight, 1 m^2 of each plot was harvested; grains were separated from straw and weighted. Twenty spikes were selected from each plot to study the yield components. The measure traits in this experiment included: plant height, grain weight, total dry weight, the number of grains in spike, spike length, 1000 grain weight, the number of tillers and the harvest index.

After collecting all required data, statistical analysis was conducted using SAS software, and means were compared according to the Duncan's multiple range test.

Results and discussion

Plant height

Analysis of variance indicated the significant effect of Brassinolide, humic acid and plant growth promoting rhizobacteria (PGPR) on plant height. Moreover, the interaction of Brassinolide × PGPR and humic acid × PGPR had significant effect on this trait (Table 2). Mean comparison of Brassinolide levels indicated that increasing the application rate of Brassinolide resulted in the enhancement of plant height. In 70 ml/ha Brassinolide level, plant height was 14.5 cm higher than the control (Table 3). Application of humic acid also resulted in 9.59 cm enhancement of plant height compared with the control (Table 4). Inoculating seeds with PGPR increased plant height (8.65 cm) compared with the non-inoculated control (Table 5). Mean comparison of the interactions are shown in Tables 6-9. Among the three-fold interactions (Table 9), 70 L/ha Brassinolide × humic acid × PGPR had the highest plant height (89.5 cm) and the control had the lowest plant height (63 cm).

Organic fertilizers gradually provide a wide range of nutrients to plants and increase their growth and yield (Samavat and Malakouti, 2005). Humic acid may be considered as an organic fertilizer which not only increases the availability of nutrients to plants, but improves soil texture and structure. It also has some phytohormone like effects on plants (Nardi *et al.*, 2002). The increasing effect of Brassinolide, humic acid and PGPR on plant height in our experiment was also observed in other experiments (Albayrak and Camas, 2005; Zaied *et al.*, 2007).

Table 1. Physico-chemical properties of the test site soil.

Dept (cm)	OC (%)	TNV (%)	Total N (%)	P_{ava} (ppm)	K_{ava} (ppm)	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)
0-30	0.91	10	0.092	9.1	201	132	0.94	23

Table 2. Analysis of variance of the effect of treatments on the measured traits.

SOV	df	Mean Squares (MS)							
		Plant height	Grain weight	Total dry weight	The number of grains in spike	Spike length	1000 grain weight	The number of tillers	Harvest index
Rep	3	ns	ns	**	ns	**	**	**	ns
Brassinolide (A)	3	**	**	**	**	**	**	**	**
Humic acid (B)	1	**	**	**	**	**	**	**	**
A × B	3	ns	*	ns	**	ns	ns	ns	**
PGPR (C)	1	**	**	**	**	**	**	**	**
A × C	3	**	**	ns	**	ns	ns	**	ns
B × C	1	ns	ns	**	ns	**	**	**	ns
A × B × C	3	**	ns	**	ns	*	**	**	ns
Error	15	3.13	3687.53	21251.6	3.52	1.49	0.89	72.85	0.01
CV (%)	-	8.36	9.46	11.3	5.25	4.05	12.37	13.52	12.6

ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

Grain weight

Analysis of variance showed that Brassinolide, humic acid and PGPR application had a significant effect on grain weight. Results also showed that the interaction of Brassinolide × humic acid and Brassinolide × PGPR had a significant effect on grain weight. This trait was not affected by the three-fold interaction (Table 2). Mean comparison showed that grain weight increased when the level of Brassinolide application increased. Grain weight was the highest (1451.26 g/m²) in 70 ml/ha Brassinolide and the lowest (932.88 g/m²) in the control (Table 3). Application of humic acid also increased grain weight from 1014.68 g/m² to 1426.81 1014.68 g/m² (Table 4). Mean comparison of plant growth promoting rhizobacteria also showed that inoculating seeds with PGPR increased grain weight from 1029.25 g/m² to 1425.4 g/m² (Table 5).

Table 3. The effect of Brassinolide on the measured traits.

Treatments	Plant height (cm)	Grain weight (g/m²)	Total dry weight (g/m²)	The number of grains in spike	Spike length (cm)	1000 grain weight (g)	The number of tillers / m²	Harvest index
Control	66.12d	932.88d	2313.25d	71.12d	9.41c	41.46c	150.662d	39.8c
50 ml/ha	78.25b	1353.54b	2895.75b	78.00c	10.62b	46.36a	250.75b	49.2b
70 ml/ha	80.62a	1451.26a	3022.75a	87.86a	12.35a	46.80a	292.5a	53.7a
30 ml/ha	74.04c	1304.38c	2548.13c	80.50b	10.82b	43.48b	246.87c	47.6c

Means in a column followed by the same letter are not significantly different at P×0.05.

Humic acid increases the life period of photosynthetic tissues; increasing the grain weigh. Wolf *et al.* (1988) observed a significantly positive correlation between grain dry weight and leaf area durability and reported that the length of leaves life is as important as leaf production, for plant yield and grain weight. Nardi *et* *al.* (2002) also reported that increases plant growth and yield through positive physiological effects such as the effect on plant cell metabolism and enhancement of leaf chlorophyll content. Result of our experiment indicated that in addition to humic acid, the application of PGPR also enhanced plant grain weight; which is in agreement with the observations of other researchers (Mostajeran *et al.*, 2005). Our experiment also showed the improving effect of Brassinolide on grain weight. This may be due to the effect of Brassinolide on grain filling stage through enhancement of translocation of sugars from leaves to grains (Abdel Hamid, 2008).

Table 4. The effect of humic acid on the measured traits.

Treatments	Plant height (cm)	Grain weight (g/m²)	Total dry weight (g/m²)	The number of grains in spike	Spike length (cm)	1000 grain weight (g)	The number of tillers / m²	Harvest index
Control	68.47b	1014.68b	2359.43b	72.25b	10.14b	41.91b	139.25b	40.3b
Humic acid	78.06a	1426.81a	2696.13a	85.0a	10.96a	45.13a	246.12a	53.8a

Means in a column followed by the same letter are not significantly different at P×0.05.

Total dry weight

Results of this experiment showed that Brassinolide, humic acid and PGPR had significant effect on total dry weight. Results also showed that among the interactions, the effect of humic acid × PGPR and the three-fold interaction of Brassinolide × humic acid × PGPR was significant on this trait (Table 2). Mean comparison indicated that increasing the application rate of Brassinolide resulted in the enhancement of total dry weight; total dry weight was 2313.25 g/m² in the control and 3022.75 g/m² in 70 ml/ha treatment (Table 3). Applying humic acid also increased total dry weight from 2359.43 g/m² to 2696.13 g/m² (Table 4). Results of man comparison also indicated that inoculating seeds with PGPR had also an improving effect on this trait; increasing it from 2379.63 g/m² to 2870.31 g/m² (Table 5). Mean comparison of the three-fold interaction showed that total dry weight was the highest (3796.0 g/m²) in 70 ml/ha Brassinolide × humic acid × PGPR and the lowest (2259.5 g/m²) in the control (Table 9).

Table 5. The effect of plant growth promoting rhizobacteria (PGPR) on the measured traits.

Treatments	Plant height (cm)	Grain weight (g/m²)	Total dry weight (g/m²)	The number of grains in spike	Spike length (cm)	1000 grain weight (g)	The number of tillers / m²	Harvest index
Control	69.03b	1029.25b	2379.63b	70.93b	9.83b	41.21b	159.3b	40.8b
PGPR	77.68a	1425.4a	2870.31a	83.31a	11.15a	44.83a	232.46a	51.0a

Means in a column followed by the same letter are not significantly different at P×0.05.

Enhancement of nutrient uptake normally results in the enhancement of plant growth and yield. Various experiments proved that humic acid facilitates nutrient absorption through biochemical and phytohormone like effects. Sharif (2002) applied 50 and 100 mg/l humic acid on maize leaves and observed that the foliar application increased shoot dry weight by 20% and root dry weight by 39% compared with the control. Enhancement of plant dry weight as the result of Brassinolide application in this may be attributed to the effects of Brassinolide on vegetative growth of plants (Abdel Hamid, 2008). Results of this experiment also indicated that PGPR application had also an improving effect on total dry weight, which is in agreement with the findings of Karakurt *et al.* (2009).

The number of grains in spike

Analysis of variance indicated that Brassinolide, humic acid and PGPR significantly affected the number of grains in spike. Moreover, the interaction of Brassinolide × humic acid and Brassinolide × PGPR had significant effect on this trait. The effect of the three-fold interaction of Brassinolide × humic acid × PGPR was not significant on the number of grains in spike (Table 2). Mean comparison of Brassinolide levels indicated that application of higher doses of Brassinolide resulted in the enhancement of the number of grains in spike; it was 71.12 in the control and 87.86 in 70 ml/ha treatment (Table 3). Treating the plants with humic acid also resulted in the enhancement of the number of grains in spike from 72.25 to 85 (Table 4). Inoculating seeds with plant growth promoting rhizobacteria also increased the number of grains in spike from 70.93 to 83.31 (Table 5).

Table 6. The effect of interaction of Brassinolide × humic acid on the measured traits (which were significantly affected by the interaction).

Treatments	Grain weight (g/m²)	The number of grains in spike	Harvest index
a1b1	862.75 f	70.25 e	37.4 g
a1b2	1063.36 e	74.28 d	39.0 fg
a2b1	1275.25 c	75.43 c	47.2 c
a2b2	1428.67 b	84.50 b	49.1 bc
a3b1	1462.50 b	86.75 a	51.7 b
a3b2	1598.53 a	89.63 a	62.5 a
a4b1	1108.25 e	72.61 cd	43.3 e
a4b2	1192.47 d	77.44 c	45.7 d

Means in a column followed by the same letter are not significantly different at P×0.05.

There are various reports about the effect of plant growth promoting rhizobacteria on plants yield, such as wheat (Vessey, 2003). Ram and Prasad (1985) reported that inoculating wheat seed with *Azotobacter chroococcum* and *Azospirillum brasilense* increased grain yield, nitrogen uptake and straw yield by 13.9%, 16% and 12.6%, respectively, compared with the control. Regarding the effect of humic acid on the number of grains in spike, Rezvantalab *et al.* (1998) also reported that application of humic acid increased the number of grains in ears of maize. Results of our experiment also showed that Brassinolide application had also an improving effect on the number of grains in spike; Kerrit (2005) reported the same observations.

Table 7. The effect of interaction of Brassinolide × plant growth promoting rhizobacteria on the measured traits (which were significantly affected by the interaction).

Treatments	Plant height (cm)	Grain weight (g/m²)	The number of grains in spike	The number of tillers / m²
a1c1	63.25 g	867.75 g	68.60 f	142.25 g
a1c2	67.54 f	1039.50 f	72.78 e	180.06 f
a2c1	75.40 cd	1188.77 d	78.65 d	205.25 de
a2c2	81.08 b	1479.65 b	83.50 c	256.19 c
a3c1	77.75 c	1281.12 c	87.20 b	289.31 b
a3c2	83.50 a	1569.34 a	93.75 a	315.75 a
a4c1	75.86 d	1098.67 f	70.50 e	196.45 e
a4c2	72.25 e	1269.25 e	77.25 d	216.80 d

Means in a column followed by the same letter are not significantly different at P×0.05.

Spike length

Analysis of variance indicated the significant effect of Brassinolide, humic acid and PGPR on spike length. Results also indicated that the interaction of humic acid × PGPR and the three-fold interaction of Brassinolide × humic acid × PGPR had significant effect on spike length (Table 2). Mean comparison of Brassinolide levels showed that spike length was the highest (12.35 cm) when 70 ml/ha Brassinolide was applied and the lowest in the control (Table 3). Application of humic acid to plants had also an improving effect on spike length, increasing it from 10.14 cm to 10.96 cm (Table 4). Mean comparison also showed that inoculating wheat seeds with PGPR increased spike length from 9.83 cm to 11.15 cm (Table 5). Moreover, studying the three-fold interactions represented that spike length was the highest (14.35 cm) in 70 ml/ha Brassinolide × humic acid × PGPR and the lowest (8.1 cm) in the control (Table 9).

Table 8. The effect of interaction of humic acid × plant growth promoting rhizobacteria on the measured traits (which were significantly affected by the interaction).

Treatments	Total dry weight (g/m²)	Spike length (cm)	1000 grain weight (g)	The number of tillers / m²
b1c1	2294.37 d	9.65 d	41.27 c	150.37 c
b1c2	2582.75 c	10.65 c	45.88 b	198.12 b
b2c1	2804.87 b	11.03 b	46.56 b	203.47 b
b2c2	3157.56 a	11.47 a	49.80 a	297.25 a

Means in a column followed by the same letter are not significantly different at P×0.05.

Results of this experiment indicated that application of Brassinolide, humic acid and PGPR increased spike length. These treatments increase plant growth, yield and yield components through increasing the availability of nutrients to plants and increasing the hormonal activity of plants. Chen and Aviad (1990) reported that application of humic acid increased plant yield and yield components through increasing the availability of nutrients to plant and plant photosynthesis rate. Kohen *et al.* (1980) also found the enhancement of wheat spike length as the result of inoculation with azospirillum bacteria.

1000 grain weight

Analysis of variance showed that the effect of Brassinolide, humic acid and PGPR was significant on 1000 grain weight. Moreover, the effect of interaction of humic acid × PGPR and the three-fold interaction of Brassinolide × humic acid × PGPR was significant on this trait (Table 2). Mean comparison of Brassinolide levels indicated that 1000 grain weight was the highest (46.80 g) in 70 ml/ha Brassinolide and the lowest (41.46 g) in the control (Table 3). Application of humic acid also increased 1000 grain weight from 41.91 g to 45.13 g (Table 4). Inoculating wheat seeds with plant growth promoting rhizobacteria also resulted in the enhancement of 1000 grain weight from 41.21 g to 44.83 g (Table 5). Mean comparison of the three-fold interactions showed that 1000 grain weight was the highest (52.27 g) in 70 ml/ha Brassinolide × humic acid × PGPR and the lowest (38.65 g) in the control (Table 9).

Results of this experiment indicated that all treatments including Brassinolide, humic acid and PGPR increased 1000 grain weight. There are various reports about the effect of Brassinolide on 1000 grain weight; Abdel Hamid (2008) reported that Brassinolide application increased 1000 grain weight of wheat. Humic acid also increases grain weight by enhancing the translocation of photosynthetic assimilates from leaves to grains. Abou-Aly and Mady (2009) found that humic acid application increased wheat 1000 grain weight. Sharif (2002) tested the effect of humic acid on maize and reported that application of 0.5-1 kg/ha humic acid increased grain yield and straw yield of wheat. Plant growth promoting rhizobacteria such as azospirillum and azotobacter have different beneficial effects on plants growth and yield. Mohammadvarzi (2010) reported that plant growth promoting rhizobacteria increase plant growth and yield through enhancement of nutrients availability to plant roots, production of phytohormones and enhancement of photosynthesis rate. They also increase plant root development rate and facilitate the absorption of water and nutrients.

The number of tillers

Analysis of variance showed that Brassinolide, humic acid and PGPR application had significant effect on the number of tillers. Among the interactions, Brassinolide × PGPR, humic acid × PGPR and the three-fold interaction of Brassinolide × humic acid × PGPR had significant effects on this trait (Table 2). Mean comparison showed that the number of tillers was the highest when the highest dose of Brassinolide was applied; there were 150.66 tillers / m² in the control and 292.5 tiller / m² in 70 ml/ha Brassinolide treatment (Table 3). Application of humic acid also resulted in the enhancement of the number of tillers from 139.25 tillers / m² to 246.12 tillers / m² (Table 4). Inoculating seeds with plant growth promoting rhizobacteria increased the number of tillers from 159.3 tillers / m² to 232.46 tillers / m² (Table 5). Studying the mean comparison of the three-fold interactions showed that the number of tillers was the highest (405 tillers / m²) in 70 ml/ha Brassinolide × humic acid × PGPR and the lowest (151 tillers / m²) in the control (Table 9).

Table 9. The effect of interaction of Brassinolide × humic acid × plant growth promoting rhizobacteria on the measured traits (which were significantly affected by the interaction).

Treatments	Plant height (cm)	Total dry weight (g/m²)	Spike length (cm)	1000 grain weight (g)	The number of tillers / m²
a1b1c1	63 j	2259.5 i	8.1 f	38.65 h	151 m
a1b1c2	66.5 ij	2432 gh	8.75 e	40.10 g	166.2 l
a1b2c1	66 ij	2496 h	9.7 de	41.25 f	168.5 l
a1b2c2	69 hi	2656 g	9.96 d	43.56 d	196 jk
a2b1c1	74.5 efg	2854 f	9.6 de	44.15 d	258.6 h
a2b1c2	75.5 def	3057.5 e	11.5 c	39.53 gh	293.5 g
a2b2c1	76.5 cdef	3134.7 d	8.7 bc	39.45 h	306.4 f
a2b2c2	84 a	3307.8 c	12.7 b	51.7` ab	323.9 e
a3b1c1	72.5 fgh	3292.5 cd	11.75bc	49.35 b	353 d
a3b1c2	80.2 bc	3445.5 b	11.9 bc	44.23 c	364 c
a3b2c1	81.5 ab	3416.4 b	11.4 c	44.20 c	383 b
a3b2c2	89.5 a	3796 a	14.35 a	52.27 a	405 a
a4b1c1	72 gh	2482 h	10.9 cd	40.70 e	232 j
a4b1c2	67 ij	2532.5 gh	8.85 e	41.12 ef	236.7 j
a4b2c1	79.5 bcd	2883.5 g	8.8 ef	42.62 d	248 i
a4b2c2	77.51 cde	3112.7 e	9.75 c	45.36 c	255.5 h

Means in a column followed by the same letter are not significantly different at P×0.05.

Results of this experiment indicated that application of Brassinolide increased the number of tillers. This was observed in the experiments of Kerrit (2005) and Abdel Hamid (2008). The inoculation of PGPRs had also an improving effect on the number of tillers. Cassan *et al*. (2009) tested the effect of *Azotobacter chroococcum* and *Azospirillum brasilense* on rice plants and reported that the inoculation increased the number of tillers.

Harvest index

Analysis of variance indicated the significant effect of Brassinolide, humic acid and PGPR on harvest index. Results also indicated that among the interaction, only the effect of Brassinolide × humic acid was significant on this trait (Table 2). Mean comparison showed that the harvest index was increased when Brassinolide application rate increased. The harvest index was 39.8 in the control and it was increased to 53.7 when 70 ml/ha Brassinolide was applied (Table 3). Application of humic acid increased harvest index from 40.3 to 53.8 (Table 4). Inoculating seeds with PGPR had also an improving effect on this trait; increasing it from 40.8 to 51 (Table 5).

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