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The effects of the seed treatment with two commercial combinations containing fulvic acid and humic acid on wheat germination stress index

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Abstract

The purpose of this study was to investigate the effect of two commercial compounds namely, Humax and Humica consist of Fulvic and Humic acids on germination index of five genotypes of bread wheat under drought stress induced by polyethylene glycol in lab. According to scientific publications, seed treatments with Fulvic and humic acids may increase the rate of germination indices and thus, boost establishment of wheat seedling at drought stress condition.The effect of the two acids and control treatment on germination of five bread wheat genotypes were compared. Positive effect of seed treatments with the compound were observed on different aspects of seed germination.

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Introduction

Wheat is one of the most important crops and enhancement of its seed germination and seedling establishment is a key factor under drought condition (Kafy and Damghani, 2003). Since about 65% of the the arable land in Iran is covered by rain-fed wheat and barley, it is necessary to cope with drought stress impact utilizing optimum agronomic operations and adapted cultivars. At drought stress condition strong seedling establishment at early stage of crop growth is essential to tolerate drought in later stages. Scientific resources shows that seed treatment with organic compounds containing humic acid stimulate germination ability and indices which have positive effects on plant establishment in field (Wua *et al.*, 2005). Humic acid as a resouce of phosphate and carbon can stimulate microorganism population in soil around the seeds (Galston *et al.*, 1997). Humic acid increases membrane permeability and reinforces the enzymatic systems of plants (Mackowiak *et al.*, 2001) and enhance nutritional elements by seedling. It also increases the soil permeability capacity for absorbing more nitrogen and potassium, calcium, magnesium and phosphorus by plants and as a consequence improves germination ability and plant root and stem growth (Piccolo, 1997, Valentinuz, 2006).

(Lobartini *et al.*, 1997) reported that Humic acid is effective in plant growth and mineral uptake and also on germination and seedling growth and root development, shoot and absorption of some macronutrients such as N, P, K, Ca and micronutrients such as Fe, Zn, B, Mn. Also it has been reported that Humic acid treatment could improve germination percentage of two barley cultivars under drought stress conditions and significantly increase the speed of seed germination (Aysu *et al.*, 1996). The purpose of this experiment was to determine the efficacy of Humax and Humic, two commercial combinations containing Humic and fulvic acids, on germination indices and drought tolerance in five wheat genotypes.

Materials and methods

Experimental site and design

To study the effect of seed treatment with two commercial organic comounds containing humic and fulvic acid on wheat germination ability at drought conditions a factorial experiment based on randomized complete block design were conducted with three replications in physiology laboratory of Dryland Agricultural research Sub-Institute in cropping season 2011-2012.

Experimental factors

The first factor was consisted of three different seed treatments with two commercial organic comounds containing, i.e. Humax and Humica containing humic and fulvic acid, and control treatment (without seed treatment) in two different conditions, i.e. induced drought stress by polyethylene glycol (PEG -.0.8 Mpa) and non-stress condition (Distilled water). To make PEG solution with -0.8 Mpa osmotic potential, 251 grams PEG solved in one liter distilled water (Smith *et al.*, 1990).The second factor included four improved bread wheat cultivars , i.e. Rijaw, Owhadi, Karim, Azar2 and one advance genotype called Booma2.

Seed treated

The seed of this cultivar were disinfected utilizing 2.5 % sodium hypochlorite solution for 1 minute, then rinsing them once with distill water and then placed in 0.1% Benomyl solutions for 30 seconds and then rinsed thrice in distilled water and the seeds treated with 10% Humax and Humic solution. 100 treated seeds and control seed of each genotype placed in petri dishes and 10 ml of the prepared PEG solution were added to the certain petri dishes to induce drought stress and 10 ml of distill water to the ones with non-stress (Blum, 1980). Afterward the petri dished were placed in a growth chamber with 20^{o}C and 70% humidity. The seeds were considered germinated if coleorhiza length of germinated seeds were approximately 3 mm.

Initial promptness index (PI) or germination speed and germination stress index (GSI) analysis

Germinated seeds were counted every other day for 10 days (five counts) for measuring promptness index (PI) or germination speed. Following traits were measured and recorded:

1 –Average length of five root and shoot were measured 10 days after starting the experiment.

2- Promptness index was calculated based on the following equation (Buslama and Schapaugh, 1984) for stressed (PIs) and control (PIc) conditions :

$$PI = nd_2\ (1.0) + nd_4\ (0.8) + nd_6\ (0.6) + nd_8\ (0.4) + nd_{10}\ (0.2)$$

Were n is the number of germinated seed at day d.

3- Germination stress index (GSI) calculated based on the following equation:

$$GSI = \frac{PI_s}{PI_c} \times 100$$

Initial root length stress index (RLSI) and shoot length index (SLSI) analysis

Shoot and root length and plant dry weights were recorded after drying at 70°C to a constant weight. From these measurements the root length stress tolerance index (RLSI) and shoot length index (SLSI) were calculated using the following formulae given by (Ashraf *et al*., 2006).

I. RLSI = (Root length stressed plant / Root length of control plants) x 100

II. SLSI = (Shoot length of stressed plant / Shoot length of control plants) x 100

Statistical analysis

Collected data subjected to analysis of variance and Duncan's ***multiple*** range tests at 95% confidence level and MSTAT-C statistical software was used for this purpose.

Results and discussion

Initial PI and shoot length and root length analysis

Analysis of variance showed highly significant (p <0.01) differences among the testing genotypes for PI and shoot length but non-significant difference was observed for root length. Among seed treatments high significant differences were observed for all the traits (Table 1).

Table 1. Analysis of variance based on germination test factorial experiment.

Sources of variation	df	Promptness Index	Root Length	Shoot length
Genotype (G)	4	23.62^{**}	0.36^{ns}	14.50^{**}
Seed treatments (ST)	5	66.96^{**}	7.19^{**}	8.26^{**}
G X ST	20	69.47^{ns}	0.39^{ns}	1.61^{ns}
Error	58	37.42	0.57	0.87
CV (%)	_	16.67	25.16	19.57

* and ** significantly at p < 0.05 and < 0.01, respectively.

Table 2. The mean effect of organic matter on the different characteristics of the germination test.

Seed treatment	Promptness Index	root length (mm)	Shoot length (mm)
Control	40.37 b*	2.6 bc	4.8 ab
Control+PEG	27.47 e	2.7 bc	5.5 a
Humica	34.88 cd	2.9 bc	4.8 ab
Humica+ PEG	31.0 de	4.2 a	5.2 ab
Humax	50.34 a	3.1 b	4.5 b
Humax+ PEG	36.16 bc	2.2 c	3.5 c

*Means with the same letter, are not significantly different according to Duncan's multiple range test (P≤0.05).

Effects of seed treatments on genotype by seed treatments interaction

Genotype by seed treatments interaction was not significant for the traits. Mean comparison of the seed treatments is presented in (Table 2).

Effects of seed treatments on promptness index (PI)

For PI the highest and lowest amount were observed in seed treatments with Humax and PEG, respectively, with significant difference between them. PI in control was significantly higher than Control+PEG, and in the case of Humax vs. Humax+PEG, it was significantly higher in Humax treatment, but it was not significantly different in comparing Humica vs. Humica +PEG, But higher in Humica (Table 2).

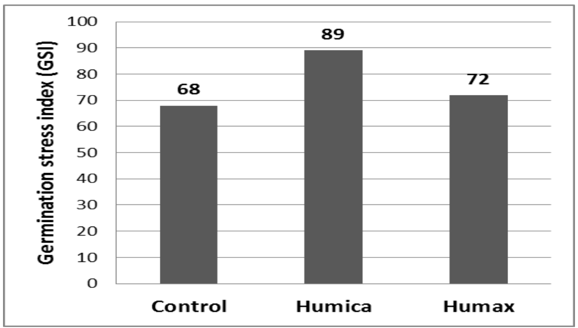

Fig. 1. Effect of seed treatments on germination stress index (Example: $GSI_{Humica}=(PI_{Humica+PEG}/PI_{Humica}) \times 100$).

Effects of seed treatments on shoot length (SL) and root length (RL)

Comparison of these three pair of treatments for shoot length (SL) showed just significant difference between humax and Humax+PEG with higher SL for Humax. A similar result was observed for root length also, but in RL case, difference between Humica and Humica+PEG was significant (Table 2).

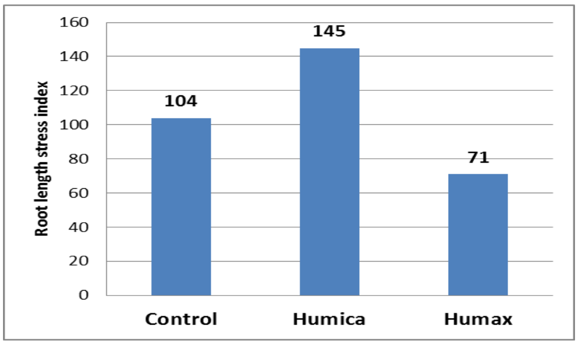

Fig. 2. Root length stress index

Seed treatments efficacy in germination stress index (GSI)

In (Fig 1) the effect of seed treatments on GSI is shown. To calculate GSI for control, Humica and Humax in (Fig 1) in GSI equation, we used PI for each of these treatments in PEG and non-PEG conditions. GSI for Humica was the highest and for control was the lowest (Fig 1). But PI data for Humax in (Table 2) is better in compare with Humica, indicating that GSI presented in (Fig 1) is not a good indicator for comparing Humica vs. Humax. To compare these two compounds, one way is to compare the ratio of each of them to Control+PEG multiplies by 100. This ratio for Humax and Humica were 132 and 113, respectively, indicating that Humax improved PI more than Humica. At the initiation of this research, we hypothesis that compounds containing fulvic and humic acid improve germination indices at drought stress condition. Thus, we expected that in drought stress treatments induced by PEG, the treatments with humica and Humax would show better PI or germination speed than Control+PEG treatment.

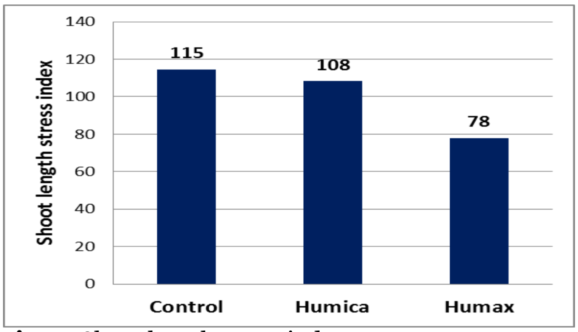

Fig. 3. Shoot length stress index.

Seed treatments efficacy in root length Stress index (RLSI) and Shoot length stress index (SLSI)

Root length Stress index (RLSI) was the highest for Humica (145) and the lowest for Humax (71) and 104 for control (Fig 2). The ratio of the both compounds +PEG to control +PEG multiply by 100 can be considered as root length index (RLI) to compare the effect of Humica and Humax on root length (Table 2). This index for humica was 155.6 and for Humax was 81.5 showed that Humica treatment induced root length more than Humax and this result was similar to the one presented in (Fig 2). Shoot length stress index (SLSI) for the three treatments presented in (Fig 3), showed that for SLSI control was the highest and Humax was the lowest. Shoot length index (SLI) was calculated similar to RLI index mentioned above. SLI for Humica+PEG and Humax+PEG were 94.5 and 63.6, respectively, indicating that seed treatment with the both compounds reduced shoot length in compare to Cotrol+PEG treatment but with no significant difference with Humica+PEG and the amount of reduction in Humax+PEG was much more than Humica+PEG.

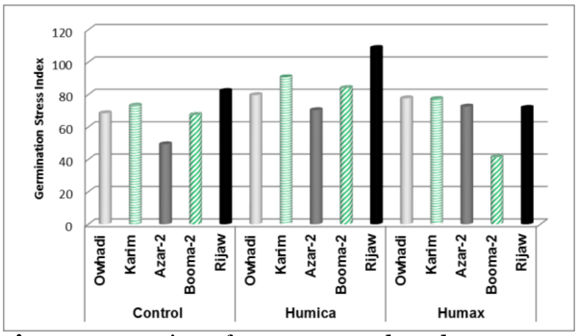

Fig. 4. Interaction of genotypes and seed treatments on GSI.

Interaction of genotypes and seed treatments on GSI

In (Fig.4) the effect of the seed treatments on GSI of the testing genotypes is presented. For control and Humica treatments, rank of genotypes for GSI were similar to each other but all were higher in Humica. In the both, Rijaw had the highest GSI and Azar 2 had the lowest. In Humax, Azar-2 had the lowest GSI and owhadi and Karim had the highest, respectively.

Conclusion

In general it can be concluded that the both commercial compounds containing Humic and Fluvic acid enhance promptness index (speed of seed germination), and also root length growth at stress condition simulated by PEG. Germination stress index and root length stress was higher in Humica+PEG treatment in compare to Humax+PEG and control +PEG, but shoot length was higher in control+PEG that the two other treatments but with no significant difference with Humica+PEG. Interaction of genotypes by seed treatments for GSI was not significant. So, the seed treatments especially with Humica enhance promptness index. Practically, seed treatments with these two commercial compounds enhance vigor growth of seedlings to improve plant establishment at drought condition in seed companies by spraying the solution of these compounds in mixture with fungicide seed treatment at the same time.

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