

The Effect Of different Concentrations Of humic Acid on seed Germination Behavior and Vigor of barley

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Abstract: To identify the effect of humic acid on the seed germination and early seedling growth of barley, an experiment was conducted at the Biotech Research Center of the University of Zabol, Zabol, I.R. Iran. The experimental design for this study was a split-plot randomized design with four replicates. Main plot treatments were five different concentrations of acid humic (0, 250, 500, 750 and 1000 mg l⁻¹) and subplot treatments consisted five priming duration (0, 6, 12, 18 and 24 h). Statistically significant differences were found to exist between interaction of different concentration of humic acid and priming duration on seedling dry weight, seedling vigor index, dynamic weight of seed reserves and efficient use of resources. The greatest seedling growth parameters was observed in seeds primed at concentration of 750 mg l⁻¹ for 12 h. Increasing priming duration up to 12 h, increased and then decreased the parameters. In addition to, concentration increasing up to 500 and 750 mg l⁻¹ had the most effective impact on seedling growth parameters. In conclusion, seed priming with humic acid through the influence of heterotrophic growth can produce stronger seedlings.

Keywords: Seed priming, humic acid, seed vigor, barley.

INTRODUCTION

Seed priming is nowadays being extensively used to improve seed germination and seedling emergence in a wide range of crop species (Parera and Cantliffe, 1994) and is basically a physiological process in which the seeds are presoaked before planting which, by itself, allows partial imbibition though preventing the germination (Nascimento and Aragão, 2004). During the priming, several processes including storage, material handling, activation and synthesis of a number of enzymes and nucleic acids, repair and build up, ATP synthesis, and the cytoplasmic membrane repair in treated seeds will all start to develop (Parera and Cantliffe, 1994).

Humic acid is a principal component of humic substances, which are the major organic constituents of soil (humus), peat and coal. It is produced by biodegradation of dead organic matter. It is not a single acid; rather, it is a complex mixture of many different acids containing carboxyl and phenolate groups so that the mixture behaves functionally as a dibasic acid or, occasionally, as a tribasic acid (Akinci, *et al.*, 2009; Canellas, *et al.*, 2002).

Humic acid (HA), a decomposition product of organic matter, are characterized as a heterogeneous natural resource, ranging in colour from yellow to black, having high molecular weight, and resistance to decay. Humic acid, as a commercial product contains 44-58% C, 42-46% O, 6-8% H and 0.5-4% N, as well as many other elements (Larcher, 2003; Lee and Bartlett, 1976). It improves soil fertility and increases the availability of nutrient elements by holding them on mineral surfaces. The humic substances are mostly used to remove or decrease the negative effects of chemical fertilizers from the soil and have a major effect on plant growth, as shown by many scientists (Ghabbour and Davies, 2001). The auxin like activity of these humic substances in plants influences the cell division and cell elongation (Mato, *et al.*, 1971; Schnitzer and Weightman, 1974).

Research conducted using humic acid on seed germination, seedling vigor, yield and quality etc, has proved that humic acid is a miracle organic crop energizer (Lulakis and Petsas, 1995; Malik and Azam, 1985). Humic substances are known to stimulate the germination of several varieties of agricultural seeds (Lulakis and Petsas, 1995; Malik and Azam, 1985, David, *et al.*, 1994; Dursun, *et al.*, 1999). The immersion of seeds in a sodium humate solution was reported to increase germination, water absorption, and respiration (David *et al.*, 1994), the length of roots and shoots (Rauthan and Schnitzer, 1981; Malik and Azam, 1985) and the fresh and dry mass of roots and shoots (Lulakis and Petsas). The aim of the present study was to evaluate the effects of incubation period on seed germination and seedling growth of treating barley seeds with different concentration of humic acid.

MATERIALS AND METHODS

The experiment was carried out in June 2011 at the Biotech Research Center of the University of Zabol, Zabol, I.R. Iran. Seeds of barley (*Hordeum vulgare*) were obtained locally, and before being used for bioassays, their germination potentials was examined at $25 \pm 1^\circ\text{C}$ in darkness and germination over 95% also guaranteed the viability of the seeds (Asgharipour and Armin, 2010). The seeds were sterilized with 10:1 water/bleach (commercial NaOCl) solution for 5 minutes and subsequently washed with diluted water.

The seeds were then soaked in increasing concentrations (0, 250, 500, 750 and 1000 mg L⁻¹) of humic acid at four different duration (6, 12, 18 and 24 h). The experiment was laid out as a split-plot design with humic concentration as the main plot and four duration of soaking as the sub-plot, together with four replicates. Twenty-five seeds were then germinated in sterilized Petri dishes, 100mm in diameter, on Whatmann filter-paper moistened with 10 mL of double-distilled water. Petri dishes were subsequently kept in the dark, at 25°C , for a span of 7 days. The solutions were renewed after 3 days. After 7 days, 10 seedlings of each petri were sampled with an aim to measure the root length and shoot height using a ruler (against a black background). Dry weight was also evaluated after drying the specimens (10 seeds) for 72 hours at 76°C . During the experiment, germinated seeds were counted on a daily. Seed viability was assessed by the final cumulative percentage of germination at the end of the tests. Here, germination was considered only when the radicles were longer than 2 mm. Seedling Vigor Index (SVI) was calculated based on (Soltani, *et al.*, 2006):

$$\text{SVI} = (\text{plumule length} + \text{radicle length}) \times \text{seed viability}$$

The dynamic reserve of seeds was obtained by subtracting the initial dry seed weight and seed residue (Parera and Cantliffe, 1994). The ratio of normal seedling dry weight to the weight of the dynamic storage was considered as the seed storage use efficiency.

The experiments were repeated twice and the pooled mean values were separated on the basis of Duncan Multiple Range Test (DMRT) at a probability level of 0.01.

RESULTS AND DISCUSSION

Two-way ANOVA exhibited that interaction effects of humic acid concentration and soaking duration was significant on seedling dry weight, seedling vigor index, dynamic reserve of seeds and seed storage use efficiency. No statistically significant differences have been found to exist between the different concentrations and soaking period of humic acid on final germination percentage. Seedling dry weight significantly changed between different treatments (Fig. 1). Soaking of seeds in concentration of 750 mg L⁻¹ of humic acid for 12 h exhibited the greatest seedling dry weight, while priming seeds at concentration of 750 mg L⁻¹ of humic acid for 0 h had the least dry weight of seedlings. (Atiyeh, *et al.*, 2002) reported that different humic substances can be positively affect the growth of tomato seedlings grown in different environments.

Different humic acid concentrations and duration had different effects on seedling vigor index. Trend of seedling vigor index across different treatments revealed that the greatest seedling vigor index in seeds occurred when seeds primed with concentrations of 500 and 750 mg L⁻¹ of humic acid for 12 h (with values of 2370 and 2207, respectively), while in seed priming with control (non-application of humic acid) caused the highest radicle length.

The effect of seed priming on germination indicated that germination increased in primed seeds due to some metabolic and biochemical changes during priming. For example, in these seeds part of the protein and carbohydrate are broken down due to enzyme activity and the hydrolysis reaction. This process resulted in rapid germination and hence seedling emergence can be improved (Andoh, and Kobata, 2002).

Increasing concentration of humic acid and duration of priming significantly increased dynamic reserve of seeds. This is indicating better transport of storage materials of seed to vegetative organs. Priming with concentration of 250 mg L⁻¹ humic acid for 12 h and exhibited the greatest dynamic reserve of seeds (5.11 mg seed⁻¹), while priming of seeds without humic acid for 0 h had the least dynamic reserve of seeds (Fig. 3).

Such positive effects of humic acid on plant growth is a concentration-dependence phenomenon and may be due to hormone-like activity of humic acid on cellular respiration, photosynthesis, membrane permeability of root cells, protein synthesis and various enzymatic reactions (Canellas, *et al.*, 2002). The experiment revealed that different concentration levels of humic acid and duration of priming had a significant effect on seed storage use efficiency.

The greatest storage use efficiency of seeds was observed in primed seeds in concentration of 750 mg L⁻¹ of humic acid for 12 h (Table 2). Priming of seeds with concentration of 1000 mg L⁻¹ for 0 h had the lowest storage use efficiency.

Over all results showed that application of humic acid has a positive effect on germination and early seedling growth of barley seeds. Such beneficial effects depend on both the concentration levels of humic acid and duration of priming. The positive effects of different solutions of humic acid on germination and growth of seedlings can be due to better water absorption and transport of the stored materials to the root and shoot growth has well as hormone-like activity of this substance. Therefore, humic acid applications increase the successful establishment of plants and ensure maximum performance under the environmental conditions.

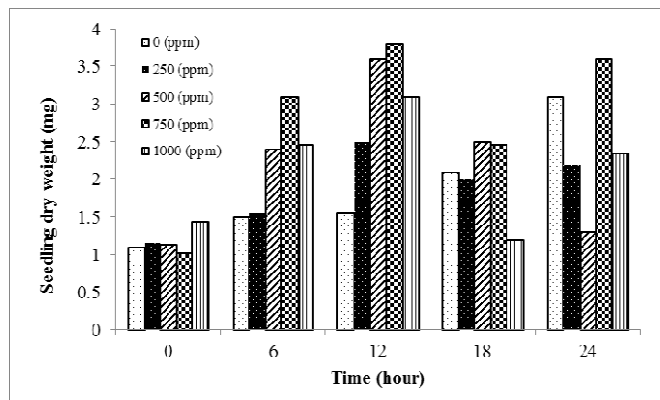


Fig. 1: Influence of various concentrations of humic acid and duration of priming on seedling dry weight (mg) of barley.

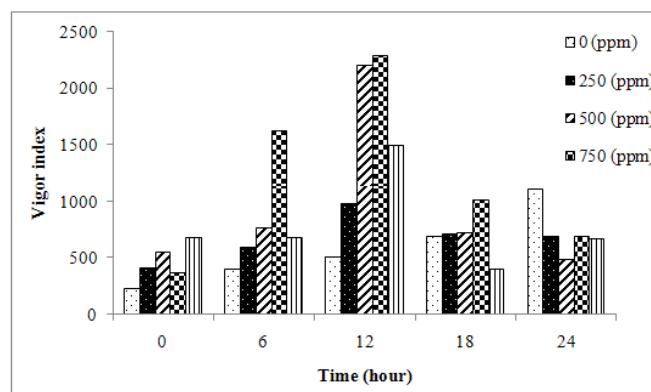


Fig. 2: Influence of various concentrations of humic acid and duration of priming on vigor index of barley seedlings.

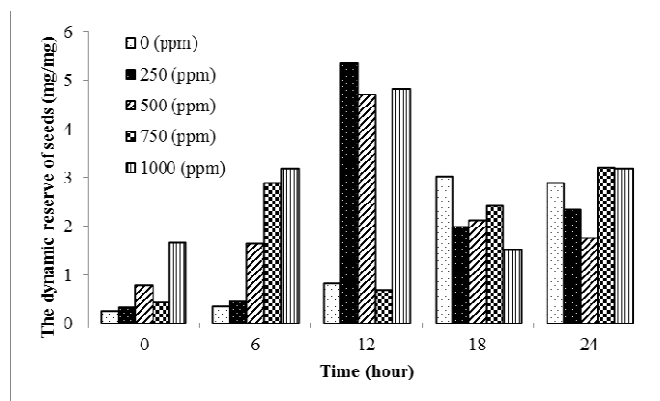


Fig. 3: Influence of various concentrations of humic acid and duration of priming on dynamic reserve of seeds of barley.

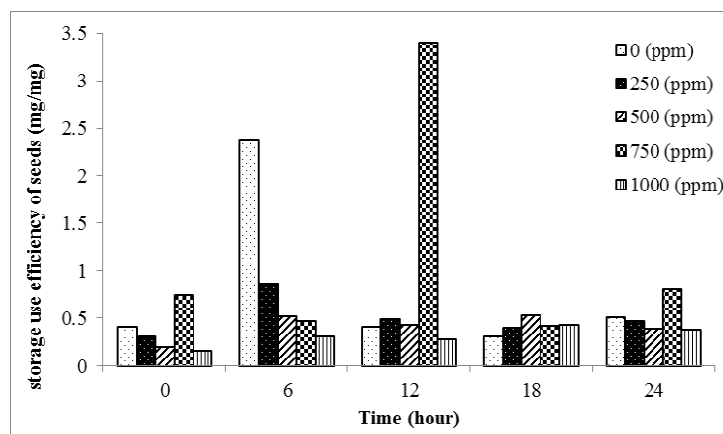


Fig. 4: Influence of various concentrations of humic acid and duration of priming on storage use efficiency of seeds of barley.

REFERENCES

- Akinci, Ş., T. Büyükeskin, A. Eroglu and B.E. Ferdogan, 2009. The Effect of Humic Acid on Nutrient Composition in Broad Bean (*Vicia faba* L.) Roots. Not Sci. Biol., 1(1): 81-87.
- Andoh, H. and T. Kobata, 2002. Effect of seed hardening on the seedling emergence and alpha-amylase activity in the grains of wheat and rice sown in dry soil. Japan. J. Crop. Sci., 71: 220-225.
- Asgharipour, M.R. and M. Armin, 2010. Inhibitory effects of *Sorghum Halepense* root and leaf extracts on germination and early seedling growth of widely used medicinal plants. Advances in Environmental Biology, 4(2): 316-324.
- Atiyeh, R.M., S. Lee, C.A. Edwards, N.Q. Arancon and J.D. Metzger, 2002. The influence of humic acids derived from earthworm processed organic wastes on plant growth. Bio. Res. Tech., 84: 7-14.
- Canellas, L.P., F.L. Olivares, A.L. Facanha-Okorokova and A.R. Facanha, 2002. Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H^+ -ATPase activity in maize roots. Plant Physiology, 30: 1951-1957.
- David, P.P., P.V. Nelson and D.C. Sanders, 1994. A humic acid improves growth of tomato seedling in solution culture, Journal of Plant Nutrition, 17: 173-184.
- Dursun, A., İ. Güvenç and M. Turan, 1999. Macro and micro nutrient contents of tomato and eggplant seedlings and their effects on seedling growth in relation to humic acid application, Improved Crop Quality by Nutrient Management, Anaç, D., Martin-Prevel, P. Editors., Kluwer Academic Publishers, Dordrecht, Boston, London.
- Ghabbour, E.A. and G. Davies, 2001. Humic substances: Structures, models and functions, Royal Society of Chemistry, England.
- Larcher, W., 2003. Physiological Plant Ecology: Ecophysiology and stress physiology of functional groups, 4th. Edition, Springer, New York.
- Lee, Y.S. and R.J. Bartlett, 1976. Stimulation of plant growth by humic substances. Soil Science Society of America Journal, 40: 876-879.
- Lulakis, M.D. and S.I. Petsas, 1995. Effects of humic substances from vine-canes mature compost on tomato seedling growth. Bioresource Technology, 54: 179-182.
- Malik, K.A. and F. Azam, 1985. Effects of humic acids on wheat seedling growth. Environmental and Experimental Botany, 25: 245-252.
- Mato, M.C., R. Fabregas and J. Mendez, 1971. Inhibitory effect of soil humic acids on indole acetic acid oxidase. Soil Biol. Biochem., 3: 285-288.
- Nascimento, W.M. and F.A.S. Aragão, 2004. Muskmelon seed priming in relation to seed vigor. Scientia Agricola, 61(1): 114-117.
- Parera, C.A., D.J. Cantliffe, 1994. Pre-sowing seed priming. Hortic. Rev., 16: 109-141.
- Schnitzer, E.A. and F. Wightman, 1974. Metabolism of auxin in higher plants. Ann. Rev. Plant Physiol., 25: 487-513.
- Soltani, A., M. Gholipour, E. Zeinali, 2006. Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. Environmental and Experimental Botany, 45: 195-200.