The Effect of Humic Acid on Nutrient Composition in Broad Bean (*Vicia faba L.*) Roots

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Abstract

Humic acids promote the conversion of mineral nutrients into forms available to plants. It also stimulates seed germination and viability, and its main effect usually being more prominent in the roots. The objective of this study was to determine the influence of humic acid on broad bean (*Vicia faba L.*) cultivar ‘Eresen 87’ on root growth and development as well as nutrient uptake, during investigation in a pot experiment. Treatment with leonardite, as humic acid source positively affected both germination and harvesting, enhancing root length and biomass. Humic acid (HA) caused significant increase of fresh (RFW) and dry (RDW) weights by 30.1% and 56.6% of broad bean roots, respectively. Flame photometer and atomic absorption spectrophotometry analyses revealed that K content was major nutrient among the tested elements. Humic acid increased the contents of Na and K significantly. The content of Ca and Fe was not significantly increased whereas Cu, Mn and Zn content decreased under HA treatment.

Keywords: Humic acid, nutrients, *Vicia faba L.* broad bean, roots.

Introduction

Broad bean is nutritionally important vegetable all over the world, containing 20-36% protein for human and animal consumption. While in Turkey 47.000 ton dry broad bean has been produced, total production is up to 4 438 510 ton in the world (Anon., 2005).

Humic acids 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 Bartlette, 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 (Linchan, 1978; Ghabbour and Davies, 2001; Pal and Sengupta, 1985).

Humic acid has an essential role in agricultural processes. It increases cation exchange capacity and enhances soil fertility, converting the mineral elements into forms available to plants (Stevenson, 1994). Humic substances lead to a greater uptake of nutrients into the plant root and through the cell membrane (Yılmaz, 2007; Tipping, 2002; Kulikova et al., 2005). Humic acids show a sponge-like tampon character in the wide pH scale, its activity may be changed by various pH levels but neutralizes soil pH, so many trace elements become available to the plant (Yilmaz, 2007). Humic substances can break the bonds between phosphate and the iron ions in between acid soils and in calcium and iron ions in alkaline soils (Stevenson, 1994).

The available studies have revealed correlations between the root growth and development and the uptake of some nutrients. For instance, humic acid caused increases in length and dry weight of maize plant roots, and enhanced the uptake of nitrogen, phosphorus, K⁺, Ca²⁺, Cu²⁺, Mn²⁺, Zn²⁺ and Fe³⁺ (Eyheraguibel et al., 2008). Humic substances increased root length in *Helianthus annuus* L. (Kolsarci et al., 2005), in maize roots, and uptake of micronutrients such as Zn²⁺, Fe³⁺, Mn²⁺ and Cu²⁺ (Sharif et al., 2002), as well as root dry weight in tomato and cucumber (Atiyeh et al., 2002); in ryegrass, humic substances stimulated root development and enhanced nitrogen, K⁺, Cu²⁺ and Mn²⁺ content (Bidegain et al., 2000); and increased root fresh and dry weight (Dursun et al., 1999). According to Adani et al. (1998) commercial humic acid affected tomato root fresh and dry weights of tomato as well as iron content, depending on the source of the humic acid. The two concentrations (20 and 50 mg/L) of humic acid, resourced from fertiliser, caused iron to increase to 113%, and 123% whereas humic substance derived from leonardite increased iron content to 135% and 161% in tomato roots. David et al. (1994) reported that more K⁺
and Ca\(^{2+}\) accumulation occurred in tomato roots grown under greenhouse conditions.

This investigation was undertaken to examine the effects of humic acid on the absorption of Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\), Cu\(^{2+}\), Fe\(^{3+}\), Mn\(^{2+}\) and Zn\(^{2+}\) by roots of broad bean seedlings grown in the pots experiment. The hypothesis was that the nutrients absorbed from the soil in the presence of humic acid, which can regulate soil pH, should enhance the uptake of beneficial elements in the rhizosphere of broad bean.

**Materials and methods**

**Design of experiment and germination of broad bean seeds**

The experiments were conducted with broad bean cultivar “Eresen 87” in growth room conditions of the Department of Botany, Marmara University, during the period from November 2007 to 2009. The seeds were obtained from the Aegean Agricultural Research Institute, Izmir. Humic acid as the commercial product “Black Gold”, sourced from leonardite was kindly provided by Hektas LTD., Istanbul.

The cultivar seeds after imbibition in distilled water, were germinated in separate petri dishes, prepared by placing disks of filter paper in the base of each, arranged for randomised treatment as control HO (Hoagland) and experimental (HA). The HO and HA (humic acid) treatments were applied to the seeds as full strength Hoagland solution (Hoagland-Arnon, 1950) and humic acid (10 ml/l) added to Hoagland solutions respectively. The solutions used in the experiment are as formulated in Tab. 1 and 2. After germination the seeds were transferred to single pots (10 cm in diameter) containing 280g sterilised (Gardol) compost media after pH was adjusted to 4.5 by adding H\(_2\)SO\(_4\) and mixed thoroughly.

The pots were set up in a completely randomized block (Mead and Curnow, 1983) at 23\(\pm\)2 °C (Eriş and Şeniz, 1997), 55\(\pm\)5% moisture and exposed to 4000–4200 lux light intensity for 14/10 h day and night periods respectively. For a period of two months the control and experimental sets of replicate plants, were given a three day interval, with 30 ml Hoagland (HO) or of Hoagland + 10 ml/l humic acid (HA) solutions respectively.

**Nutrient analyses**

For the determination of element composition of the roots, at the harvesting time, the roots of HO and HA treated plants were excised and their fresh and dry weights determined by the following methods (Roberts et al., 1993; Beadle, 1993, Mackey and Neal, 1993).

The nutrient analyses samples were prepared by wet ashing method described by Kaçar, (1972). The dried samples were crushed using mortar and pestle. Each powdered sample was transferred to an erlenmeyer flask, to which was added 6 ml nitric acid + perchloric acid solutions. The samples were kept for 30 minutes at 40 oC in water bath for digestion and the solution removed by heating at 150-180 °C until reduced to 1 ml extract. This residue was dissolved with distilled water and made up to 100 ml in standard flasks. The samples for determination of Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\) and Cu\(^{2+}\), Fe\(^{3+}\), Mn\(^{2+}\) and Zn\(^{2+}\) were analysed using flame photometers (FP) (Jenway) and flame atomic absorption spectrophotometers (FAAS) Varian Liberty Series II atomic emission spectrophotometers (ICP-AES) with air-C\(_2\)H\(_2\) respectively.

**Results and discussion**

The data obtained from the experiments with 6 replicates set up in a randomised arrangement, were subjected to NCSS (2004) analysis for two-samples for T test range test at 5% level to determine significance of differences between means. Means are indicated with standard error (± s.e.).

**Statistical analyses**

The statistically evaluated results for root parameters of broad bean in both Hoagland (HO) and Hoagland + Humic acid (HA) treatments were presented in Tab. 3.

Among plant samples, significant differences regarding effect of humic acid concentration and origin are mostly associated with crop root growth and development. Lu-lakis and Petras (1995) stated that water uptake increases nutrient absorbance by the roots in the presence of humic acid, which enhances the development of lateral roots.

In fact, different concentrations of humic acids originating from fertilisers and leonardite have variously increased root fresh weights in tomato (David et al., 1994). These results are supported by our observations of the significant increasing of 30.1% (p=0.000187 < a=0.05) in fresh root...
Tab. 3. The effect of humic acid on broad bean (Vicia faba L.) roots

| Parameters          | HO       | HA       |
|---------------------|----------|----------|
| Root fresh weight (g) | 3.06 ± 0.13 | 3.98 ± 0.13* |
| Root dry weight (g)  | 0.30 ± 0.02  | 0.47 ± 0.02* |

Means of 6 replicates, ±: standard error, HO: Hoagland treatment (control), HA: Humic acid treatment *: Significantly different from control (HO).

Some reports stated that increasing of root density resembles the hormonal activity of plant auxine which also cause increasing root formation and weight (O’Donnell, 1973; Muscolo et al., 1999; Canellas et al., 2002). According to Zandonadi et al., (2007), in maize, IAA and humic acid can enhance the lateral root development by activating cell membrane and the H+ pump in the tonoplast of plant cells.

In terms of RDW humic acid in broad bean showed a significant increase of 56.6% compared to control treatment in broad bean roots. The results were supported by studies on roots of maize (Erdal et al., 2000; Sharif et al., 2002; Eyheraguibel et al., 2008), in marigold, pepper, tomato and strawberry (Arancon et al., 2003), egg plant and tomato (Dursun et al., 1999), tomato (David et al., 1994; Adani et al., 1998), tomato and cucumber (Atiyeh et al., 2002), and in wheat (Malik and Azam, 1985). Some reports explain these positive effects in terms of ability of humic acid to hold the nutrients in rhizosphere. Humic acids enhance the absorbance capacity of nutrients of the roots by having carboxyllic and phenolic groups and increasing H+-ATPase activity in the root cells (Canellas et al., 2002)

**Effect of humic acids on nutrient contents of the roots**

The concentration of different nutrients, namely, potassium, copper, sodium, calcium, iron, zinc and manganese, were determined in broad bean plants grown under both Hoagland (HO) and Hoagland+Humic acid (HA) solutions. The results obtained at the harvesting stage of growth were evaluated as gram in dry weight. The nutrient concentrations among the HO and HA treatments are presented in Fig. 3.

**Potassium/Sodium**

K+ is an major cationic nutrient which is essential to all plant life mostly in terrestrial plants. It also activates crucial enzymatic reactions and contributes to the osmotic pressure of the vacuole, which helps to maintain structural rigidity.

Potassium content was clearly higher than that of Na+ found in roots (Fig. 3. B). The highest concentrations of K+ accumulated in roots in the presence of humic acid. The increase of K+ in HA differed significantly (111.4%; p=0.002 < α=0.05) from control seedlings. The findings supported the study on tomato root by David et al., (1994). According to Samson and Visser (1989) the reason for the increase of K+ is humic acid, which stimulates the permeability of cell membranes. We suggest that compared to HO treatment of broad bean root, the significant increase of K+ content was related to K+ ingredients being released freely and easily absorbed by plants roots.

As a factor contributing to salinity, sodium has an important negative effect on salt sensitive plants such as broad bean. However, in our experiment the ratio of sodium was increased 1.86 times in plants treated with humic acid, where K+ increased 2.11 times compared to control values. The ratios of K+:Na+ remained almost unchanged in the roots of broad bean that found as 4.30 and 4.96 in HO and HA values respectively. These results support suggestions that tolerance to sodium accumulation may be more related to the K+:Na+ ratio in the cell than to the absolute Na+ concentration (Benzyl and Reuveni, 1994; Qian et al., 2001) so, this gradual increase in Na+ may not have caused any injuries, but, more probably maintains, Na+ balance between cytoplasm and vacuole (Subbarao et al., 2001) and protects cytoplasmic membranes under saline soil (Hare et al., 1998; Rehman et al., 2002; Sakamoto and Murata, 2002).

Although sodium ions are not essential for the growth of most land plants which do not seem to have transport
systems specifically for Na\(^+\) uptake, Na\(^+\) can still enter plant cells via several routes (Fig. 3, A). Current evidence suggests that Na\(^+\) enters root cells mainly through various cation channels. These channels could be voltage-dependent cation channels or voltage-independent cation channels (VIC). Among them, VIC channels are considered the major route for Na\(^+\) entry into plant cells (Amtmann and Sanders, 1999; Schachtman and Liu, 1999; Türeman and Skerrett, 1999; White, 1999).

The Na\(^+\) content of underground parts of broad bean plants was found as 86.4% which increased significantly \(p=0.002 < \alpha=0.05\). Since the concentration of Na\(^+\) in the experiment derived mostly from the salt of Na\(_2\)MoO\(_4\) in both treatments (HO and HA), a much higher Na\(^+\) absorption than that in the cytosol of root cells, Na\(^+\) movement into root cells is passive (Valdrigh et al., 1996). The increase of Na\(^+\) may be related to humic acid causing greater root permeability by increasing lateral root development and total root bio-mass.

**Calcium**

Humic acid increased Ca\(^{2+}\) content 32% however this did not significantly differ from HO (Fig. 3, C). The available research revealed that Ca\(^{2+}\) content in increased in tomato (David et al., 1994). The reason of Ca\(^{2+}\) content relatively lower than K\(^+\) was considered to be directly related to antagonistic effects of Ca\(^{2+}\) on K\(^+\) (Mathers, 2002), and Na\(^+\) (Türeman, 1988). According to Türeman, (1988), Ca\(^{2+}\) absorption can be relatively preferred to K\(^+\). In the experiment its content was found to be 10 fold higher than that of Ca\(^{2+}\) and excessive K\(^+\) content can inhibit the absorption of Mg\(^{2+}\) and Ca\(^{2+}\) from the roots.

**Iron/Zinc**

The Fe\(^{3+}\) content in HA did not show any significant differences compared to HO treatment of broad bean root (Fig. 3, D). Lee and Bartlett (1976) found that, in maize roots, Fe\(^{3+}\) concentration was decreased after applying HA. In tomato plants grown in greenhouse conditions, applying humic acid increased the Fe\(^{3+}\) content in its roots (David et al., 1994). Our results support this increase
however, without any significant differences, related with the reduction from Fe\(^{3+}\) to Fe\(^{2+}\) and humic can chelate Fe\(^{3+}\) to change its form to be absorbed.

There appears to be no information relating to zinc accumulation in broad bean root, however Zn\(^{2+}\) absorption greatly varied in both plant species and growth media (Kabata-Pendias and Pendias, 2001). Despite it being stated that absorption is closely related with nutrient concentrations, particularly the presence of Ca\(^{2+}\) is of great importance. Contrary to this, in our experiment the Zn\(^{2+}\) content has decreased while Ca\(^{2+}\) increased in HA treated plants. The Zn\(^{2+}\) content decreased in HA treatment by 26% but did not show any significant differences from controls (Fig. 3. F). Some reports state that the antagonism between Fe\(^{3+}\) - Zn\(^{2+}\), and Zn\(^{2+}\) interfered more with the absorption and translocation of Fe\(^{3+}\) rather than it did with Cu\(^{2+}\) and Mn\(^{2+}\). On the other hand, Zn\(^{2+}\) decreasing in broad bean root may be related with the Fe\(^{3+}\) causing the absorption of Zn\(^{2+}\) and its toxicity (Olsen, 1972).

### Manganese

Mn\(^{2+}\) is known to show remarkable variation in its effects among plant species, stage of growth, different organs as well as ecosystem differences. Mn\(^{2+}\) content always increases with the increase of soil acidity. The broad bean grown under pH 6.5 was not in suitable condition for its absorption in higher levels. Mn\(^{2+}\) content decreased as 26% in HA treatment compared to HO but did not differ significantly (Fig. 3. E). The result is also seems to be related to the antagonistic effect of Ca\(^{2+}\) and Mg\(^{2+}\) on Mn\(^{2+}\) uptake (Bozcuk, 2000). The other reason seems to be a toxic effect of Mn\(^{2+}\) on broad bean roots, since it shows toxicity in acidic soil and causes difficulties of Fe\(^{3+}\) uptake (Ergene, 1987).

### Copper

Copper content in *Vicia faba* L. treated with HA decreased 27% but did differed significantly from controls (Fig. 3. G). Eyheraguibel et al., (2008) found that the Cu\(^{3+}\) increased significantly in HA treated maize plant roots by 14% compared to control. According to Mackowiak et al., (2001), in wheat plants grown with HEDTA, Cu\(^{3+}\) can not freely enter its root since HEDTA can make a stronger bond with Cu\(^{2+}\) compared to humic acid Cu\(^{2+}\) bonds. David et al., (1994) stated that in tomato plants grown under low nutrient media, addition of humic acid causes increase of Cu\(^{2+}\) in its roots while Cu\(^{2+}\) concentration increased in tomato stems, under high nutrient treatment.

### Conclusions

The results revealed that the plants grown with HA were significantly affected in both fresh and dry weights and development of roots, compared to plants grown under only HO. HA-treated roots were clearly affected, both, during germination (Fig. 1) and harvesting time (Fig. 2) as well in bio-mass.

These results confirm the data from the findings of various plants grown in different of humic acid media (resourced from leonardite, peat, fertilisers etc.) as well as various treatments (hydroponics, pot or field trials) that allowed us to understand the effectiveness of humic acid progress of root growth in *Vicia faba* L. Both fresh and dry weights increased respectively by 30.1% and 56.6% and differed significantly. The major contribution to the development of the roots seem to be increasing of potassium and sodium concentrations. The latter is supposed to be active in membrane potential hence may allow other possible nutrients to be absorbed which are not tested in this experiment.

Finally, from all these results we can conclude that humic acid application on broad bean affected the nutrient composition of roots. Humic acid increased Na\(^{+}\) and K\(^{+}\) content significantly in broad bean to 86.4% and 111.4% respectively whereas Ca\(^{2+}\) content increased only by 32.5%, but this was not significantly different at the 0.005 level (Fig. 3). We concluded that humic acid may increase the membrane permeability of root cells and cause uptake and transport of Na\(^{+}\), K\(^{+}\), Ca\(^{2+}\), Fe\(^{3+}\) and Mn\(^{2+}\). Many investigators have proposed the effect of humic substances on cell membranes to be by increasing permeability to some ions and decreasing others. Certain nutrient uptake increase in this membrane support the findings of significant increases in plasma membrane H\(^{+}\)ATPase activity found in maize root (Pinton and Cesco, 1999).

Since humic substances have very complex structures, further study is still necessary to determine the relationship between the single compounds of humic acid a well as their biological activity in nutrient uptake. Our findings can be summarized as follows:

1. Humic acids have increased root development and growth in broad bean.
2. Humic acid increased steadily the content of K\(^{+}\), Na\(^{+}\) and Ca\(^{2+}\) in tissues but without the changes of the ratios of they have shown in HO treatment.

The nutrient analyses collected from broad bean roots needs more attention and further study with a better chemical identification and subsequent testing of pure compounds in relation to the chelating mechanisms, which may improve the uptake of unavailable nutrients present in soils.

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