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# The Role of Hydroponics Technique as a Standard Methodology in Various Aspects of Plant Biology Researches

Masoud Torabi, Aliakbar Mokhtarzadeh and Mehrdad Mahlooji  
*Seed and Plant Improvement Institute (SPII)*  
Iran

## 1. Introduction

It is simple-mindedness if you think that soilless culture included modern practical knowledge in the world, so that the evidence indicate that from many centuries ago, scientists tried to grow plant in containers aboveground. Whatever it is considerable, the hydroponics system took place in various places and ages in historical context. The paintings in the temple of *Deir el Bahari* show that in 4000 year ago the Egyptians attempted to transfer and grow trees in big pots in media culture (Raviv & Lieth, 2007). The written proofs have shown that *John Woodward* carried out the early hydroponic systems upon plant nutrition researches in 1699, when he attempted to realize whether plants got their nutrients from soil or water. Water culture used as a method, so that the soil gradually added to water and at the same time, the plant growth and development monitored. The results showed that soil and water provide nutrients for plant growth and development (Jones, 1982). In the nineteenth century, French and German scientists attempted to investigate in terms of plant nutritional requirements and then it has developed by American and English scientists during the first half of the twentieth century (Cooper, 1975; Graves, 1983). Nine elements determined as essential elements for plant growth and developments, and then from 1859 to 1965, *Julius von Sachs* and *Wilhelm Knop* suggested a standard technique of research called solution culture, which nowadays has been applied widespread (Douglas, 1984; Jones, 1982). Except ancient reports of hydroponic activities related to 4000 years ago, the rest of hydroponics activities up to 1929 only covered plant biology researches. After 1929 commercial aspects of crop production via nutrition solution began by *William Frederick Gericke* from the University of California at *Berkeley*, and the other hydroponic systems developed subsequently (Hoagland and Arnon, 1950; Hershey 2008).

Obviously due to temperament and various methods of hydroponics or soilless techniques, these methods applied for plant biology researches (Le Bot et al., 1997; Hershey, 1992; Raven et al. 2004; Sonneveld & Voogt, 2009; Hershey 2008). Regarding to hydroponics efficiency, capability of modification and possibility of its development, the use of hydroponic systems has been unavoidable for plant biology researchers.

Soilless is a methodology to use for plant cultivation in nutrient solutions (water containing fertilizers) with or without the use of an organic or inorganic medium (sand, clay-expanded,

gravel, vermiculite, rockwool, perlite, peatmoss, coir, coco-peat and sawdust) to provide mechanical support. Water culture system has no organic or inorganic supporting media for plant roots (Jensen, 1999). Additionally soilless methods are classified to open (i.e., once the nutrient solution is delivered to the plant roots, it is not reused) or closed (i.e., surplus solution is recovered, replenished, and recycled) (Raviv and Lieth, 2007). Hydroponic or soilless techniques have been used in many aspects of plant biology researches such as plant nutrition, heavy metals toxicity, identification of elements deficiency, screening for abiotic stresses, screening for aluminum toxicity, root functions, root anatomy and so on (Jones, 1999; Jones, 1982). Choosing a proper soilless method based on research purposes, lead us to achieve credible and informative data.

Based on temperament of soilless culture, many advantages make soilless culture a specified method for plant biology researches. In soilless culture, the researchers are able to manage plant nutrients, control PH and EC in media, monitor the mutual elements interactions, control the micro and macro nutrient concentrations, and investigate the anions and cations absorption (Sonneveld and Voogt, 2009).

Hershey (2008) said, "Growing plants in solution culture is often easier than soil culture because there is no need for dirty soil, there are no soil-borne diseases or pests, irrigation is less frequent in solution culture than in soil culture, solution culture irrigation can be easily automated, roots are visible, and the root zone environment is easily monitored and controlled. Strength of hydroponics is its fascinating history"

This chapter will lead researchers and students to identify those aspects of plant biology that competent to run in soilless methods with emphasis on those experiments carried out by soilless methods.

## 2. Plant nutrition

Plant nutrition is not only involved with 20 essential elements in a complex process but also some other elements that are either beneficial or harmful to plant metabolism are effective on plant nutrition. The plant nutrition individually influenced by response of the crops due to genome of crop, cultural practices and environmental conditions. In plant nutrition both deficit and excess of elements influence on quantity and quality of yield, so that it seems monitoring of nutrients concentration in crop can be a way of crop management. It is not fulfill that researcher drop all additional factors in rhizosphere and just consider root and nutrient interactions in soil (Harold et al., 2007; Sonneveld and Voogt, 2009). From middle of twenty century, the use of soilless methodology in plant nutrition researches has grown up exponentially. Many researches carried out in hydroponics and the results of those experiments based on the nutrition solution where the plants grown and solutions considered as soil (Sonneveld, and Voogt, 2009). The proper conditions in terms of PH, EC and presence or absence of some elements is monitoring nutrients uptake during the plant nutrition researches (Sonneveld and Voogt, 1990).

Hydroponics provided proper conditions to identify the individual effects of elements on yield quality and quantity, because in hydroponic systems there are fewer interactions relevant to soil properties. In this case, there are many reports in terms of influences of potassium on yield qualitative attributes (Lester et al 2010; da Costa Araujo et al. 2006; Flores et al. 2004; Chapagain and Wiesman 2004; Chapagain et al. 2003).

Results obtained based on hydroponics have shown that existence of sulfate in root zone caused growth and development increase in *Gomphrena globosa L.* due to elevated micro and macro elements absorption. Most of the elements in nutrient solutions formula are in sulfate form, that cause increases the availability of other nutrients and subsequent amelioration in plant growth Figure 1 confirms the obtained results (Wang and Zahng 2009).

Assessment of micronutrients and new recommended nutrients in plant tissues and rhizosphere without the use of hydroponics seems to be so difficult. In order to determine of optimal level of silicon ( $\text{Na}_2\text{SiO}_3$ ) for maize growth in nutrient solution under nutrition solution conditions two trials conducted. In the first trial, plants were subjected to two levels of Si; control (0 mM Si) and +Si (3 mM Si). In the second trial, plants were subjected to a wide range of Si concentrations in nutrient solution with seven treatments (0, 0.4, 0.8, 1.2, 1.6, 2.0 and 3.0 mM Si). In the first trial, there was no significant difference between levels of Si accumulation in shoot and root dry matter. In the second trial, plants subjected to 0.8 and 1.2 mM Si were superior in terms of fresh and dry matter production, plant height and leaf area. The results showed that 1 mM Si is an optimum concentration for maize growth (Hafiz Faiq et al. 2009).

Tarja et al. (2009) studied influence of silicon on silver birch (*Betula pendula*) seedling grown as a deciduous forest tree in hydroponics. They reported that there was a 6% increase on leaf growth when the plants fed with silicon. The relative advantage in this experiment was that peat soil that used as a hydroponics medium expected has very low silicon levels since the management of silicon and other nutrient were on the hands of researchers.

Absorption of nutrients occurs in a specific range of PH and soil reaction. Determining the nutrients absorption where subjected to limitative materials like lime preferred by hydroponics method. In this case, Sandra and Juan (2009) conducted a trial to study the efficiency of the application of Fe, Mn, Zn and Cu chelates to correct the deficiencies in soybean in nutrition solution in the presence of lime. They reported that the best treatment for all appliances of Mn and Zn was o,pEDDHA. Chemical and commercial forms of fertilizers effect on quality and quantity of crop yields, so proper understanding of suitable forms of nutrients in physiological studies provided clean and adequate yield. In order to examine the influence of various N types on shoot growth, Naoko and Jun, (2010) performed a nutrient solution trial on barley, the results showed when urea was used, shoot biomass production, and particularly tillering declined.

Due to heterogeneity of the materials and organisms in the soils and interaction between them, soilless culture as a simplified experimental method developed and utilized in a broad range of researches (Naoko and Jun 2010). However Naoko and Jun (2010) mentioned that whereas the hydroponic methods contributed to the plant nutrition advancements, but for investigating the interaction between elements and organisms it seems that soil experiments are necessary.

Axton, (2009) through a hydroponic trial has shown that how certain nutrient deficiencies can affect the growth of pumpkin individually. The seedlings of pumpkin grown in four types of water culture (complete nutrition, potassium deficient, nitrogen deficient and iron deficient) solutions compared with controlled nutrient deficiencies. The results showed that there was no significant difference between plants grown in complete nutrition and potassium deficient solutions and the growth rate of seedling in both type of nutrition

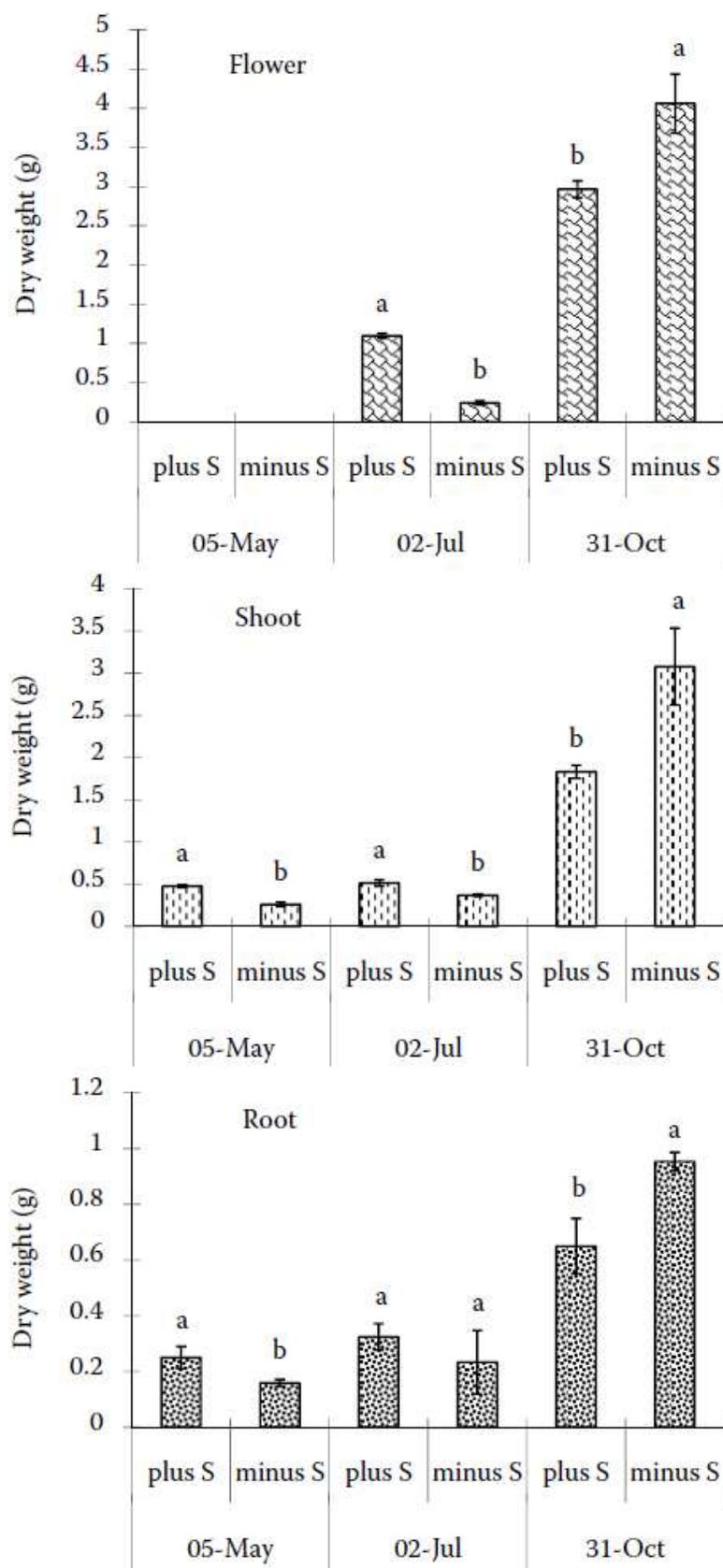


Fig. 1. Biomass of globe amaranth with and without root sulfate added (Wang and Zahng 2009)

solutions were the highest. The rate of growth for those seedlings grown in iron deficient was least, and the nitrogen deficient plant grew slightly more than the iron deficient plant.

Beatty et al. (2010) investigated low and high levels of nitrogen uptake and nitrogen utilization at two environments in barley to compare the morphological characteristics and yield of spring barley genotypes grown in the field and in compost or hydroponics growth chambers. They reported that the results obtained from hydroponics system could identify phenotypic and genetic characteristics for improving nitrogen use efficiency in spring barley. Regarding to results observed from hydroponics system they mentioned that there was a significant difference in terms of amino acids among the genotypes of spring barley due to nitrogen utilization.

A hydroponically experiment conducted to find out the effects of various zinc concentrations on sugar beet. The results obtained from controlled hydroponics environment (nutrition solution: 50, 100 and 300  $\mu\text{m}$ ) indicated that when the roots of sugar beet were subjected to high concentration of Zn sulphate, the root and shoot fresh and dry masses decreased, and root/shoot ratios increased. Furthermore, plants grown with excess Zn had inward-rolled leaf edges, a damaged and brownish root system, and short lateral roots. The hydroponics system provided the possibility of investigating the interactions between Zn and other elements in terms of absorption. The researchers found that in the high Zn concentration, the concentrations of N, Mg, K and Mn decreased in all plant parts, adversely the concentrations of P and Ca increased. Additionally with increasing of Zn concentration, the chlorophyll and carotenoid concentrations decreased, and carotenoid/chlorophyll and chlorophyll *a/b* ratios increased (Sagardoy et al., 2009a).

An experiment conducted in hydroponics system in order to investigate the effects of Zn concentrations on growth and photosynthetic parameters of sugar beet. The obtained results have shown that toxicity of Zn caused a large reduction in photosynthetic rate and subsequently a declination in accumulation of dry matter. The exploration in cause details of net assimilation rate reduction indicated that Zn did not affect leaf photochemistry, but it was due mostly to marked decreases in stomatal conductance (Sagardoy et al., 2009b).

Nowadays has been marked that root discharges affects absorption of nutrients, thus without hydroponics system the identification of these activities is impossible. Hydroponics system provides special condition to monitor root activities, where through an experiment in hydroponics system acid phosphatase produced by roots of white lupin (*Lupinus albus L.*) monitored. Acid phosphatase has a main role in inorganic phosphate (Pi) absorption. The results cleared that APase behavior in the rhizosphere caused release of Pi and provide it to white lupin plants grown under P deficient (Wasaki et al., 2008).

For recognizing the relationship (antagonism and synergism) between nutrients, the hydroponics system provides a desired and suitable condition for more understanding of these issues. In this case Liu et al. (2004) designed a hydroponics trial (nutrient solution) to examine the influence of phosphorus (P) nutrition and iron tablet on root surfaces in arsenate uptake, and its inside translocation in seedlings of three cultivars of rice (*Oryza sativa*). The obtained results have shown that however tablet of arsenate had no significant influence on dry matter of shoots and roots but the rate of arsenate concentrations elevated in tissues, mostly in roots. In absence of P, rice roots appeared reddish after 24 h, showing the creation of iron plaque. The tissues arsenic concentrations in iron tablet were

significantly higher in absence of P than when P was included in the nutrition solution. This has shown that iron tablet might sequester arsenic, and consequently reduce the translocation of arsenic from roots to shoots. Arsenic significantly declined the concentrations of iron (Fe) in roots and shoots and somewhat P concentrations in shoots.

Tracing of nutrients in plants and comparison between plants in terms of nutrient absorption has used since last decade by radiocesium in hydroponics system. Because of interaction between the nutrients in rhizosphere, the use of hydroponics seems necessitous. Tang and Wang (2001) studied the interaction between copper and radiocesium in Indian mustard and sunflower grown in the hydroponic solution. They reported that there was a significant difference between Indian mustard and sunflower in terms of absorption of copper, so that uptake and translocation of copper by Indian mustard was more than sunflower. In terms of copper immobilization sunflower showed higher copper immobilization significantly.

### 3. Allelopathy in rhizosphere

Allelopathy is the straight effects of chemicals released from one plant on the growth and development of another plant. The influence has known from many years but newly accepted as a rightful area of research considerably. Researches indicated that allelopathy plays a considerable role in natural ecosystems and has a potential to become an important issue in agro-ecosystems. Interference between plants can be due to allelopathy and competition for nutrients, space and natural resources. It is impossible to distinguish allelopathy in the field, thus determining the interference of allelopathy is so difficult. Explaining the allelopathy needed to identify chemical procedures, biologically active substances and the phytotoxic potential (Olofsdotter 1998).

It seems that identifying all mentioned cases such as chemical materials, activities, interactions and chemical procedures needed to study in some substances except soils, because in the soil researcher cannot distinguish the effects of allelopathic chemical materials and interaction between them. Hydroponics is a proper method to identify quantitative and qualitative determination of allelopathic materials and procedure of interaction between allelopathic materials with other chemical compound.

Tang and Yong (1982) introduced new method of hydroponics to collect the allelopathic chemicals from the undisturbed plant roots. They modified and developed new nutrition solution and sand culture for continuous extraction of quantities of extracellular chemicals from donor plant (*Hemarthria altissima*) as forage grass (Fig. 2). The hydroponics system included a sand culture that always nutrition solution circulated continuously through the root system and a column containing XAD4 resin. The resin selectively adsorbed extracellular hydrophobic metabolites, while nutrients circulated to maintain plant growth. Seeds of lettuce used as bioassays for collecting the root exudates. After determining by chromatography, they reported that inhibitors were mainly phenolic compounds. They emphasized that this trapping system should be useful for a wide range of allelopathic studies.

Jose and Gillespie (1998) carried out a trial to assess the influence of juglone (5-hydroxy-1, 4-naphthoquinone) on the growth and development of corn (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) seedlings in hydroponics system. The treatments were included of three

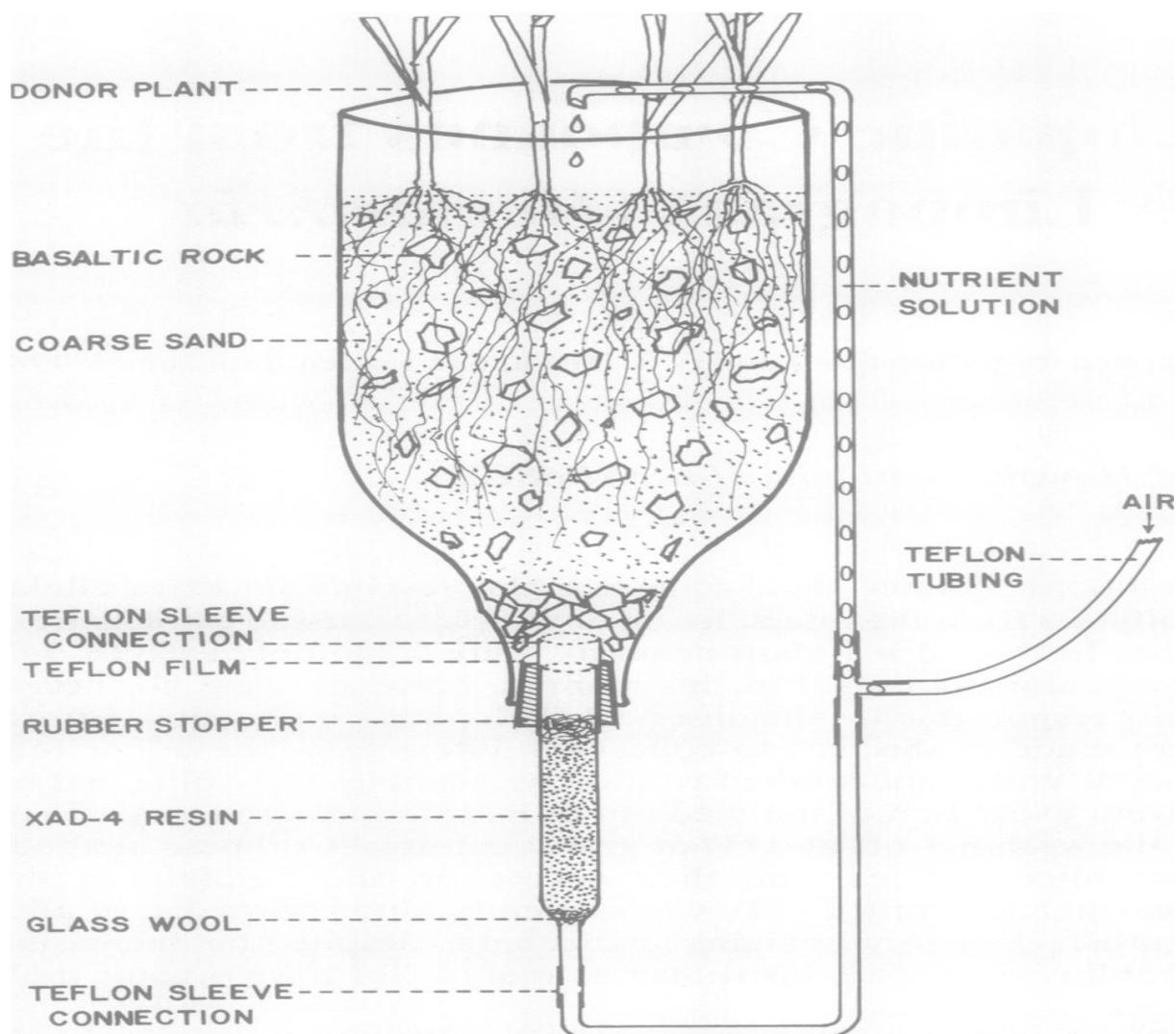


Fig. 2. The hydrophobic trapping root exudates in hydroponic system (by Tang and Yong 1982)

concentrations of juglone ( $10^{-6}$  M,  $10^{-5}$  M, and  $10^{-4}$  M). They reported that after the three days the juglone adversely affected on various aspects of growth such as shoot and root relative growth rates, leaf photosynthesis, transpiration, stomatal conductance, and leaf and root respiration. They indicated that soybean was more sensitive than corn relative to use of juglone. Leaf photosynthesis more influenced by toxicity of juglone in compare to other physiological parameters. Overall, the results have shown that both corn and soybean are sensitive to juglone so that growth reductions in corn and soybean in black walnut alley cropping may partly be due to juglone phytotoxicity.

In order to study the allelopathic effects of wheat straw on transgenic potato seedlings an experiment conducted by Zuo et al., (2010). Potato seedlings were cultured on normal medium (normal treatment) and nutrient-deficient medium (acclimated treatment) and then transferred to MS medium, which contained wheat straw powder, where wheat straw powder inhibited potato seedling growth in both treatments. They have shown that the greatest inhibition was for plant fresh weight and least for plant height. The inhibitive effects of wheat straw were greater for seedling roots compared to shoots. Growth inhibition

of potato seedlings from the normal treatment increased as the amount of wheat straw powder in the culture medium increased. Calculations indicated that the presence of wheat straw would lead to a 55% reduction in the total biomass of normal potato seedlings compared to a 39% reduction for acclimated seedlings. Parameters such as net photosynthesis rate (Pn) changed as the nutrient content of the culture medium increased or decreased, but the changes in the parameters were smaller for acclimated seedlings compared to normal seedlings. In summary, the results show that previous exposure to pressures such as nutrient deficiency may increase the allelopathic pressure resistance of transgenic potato seedlings (Fig 3).

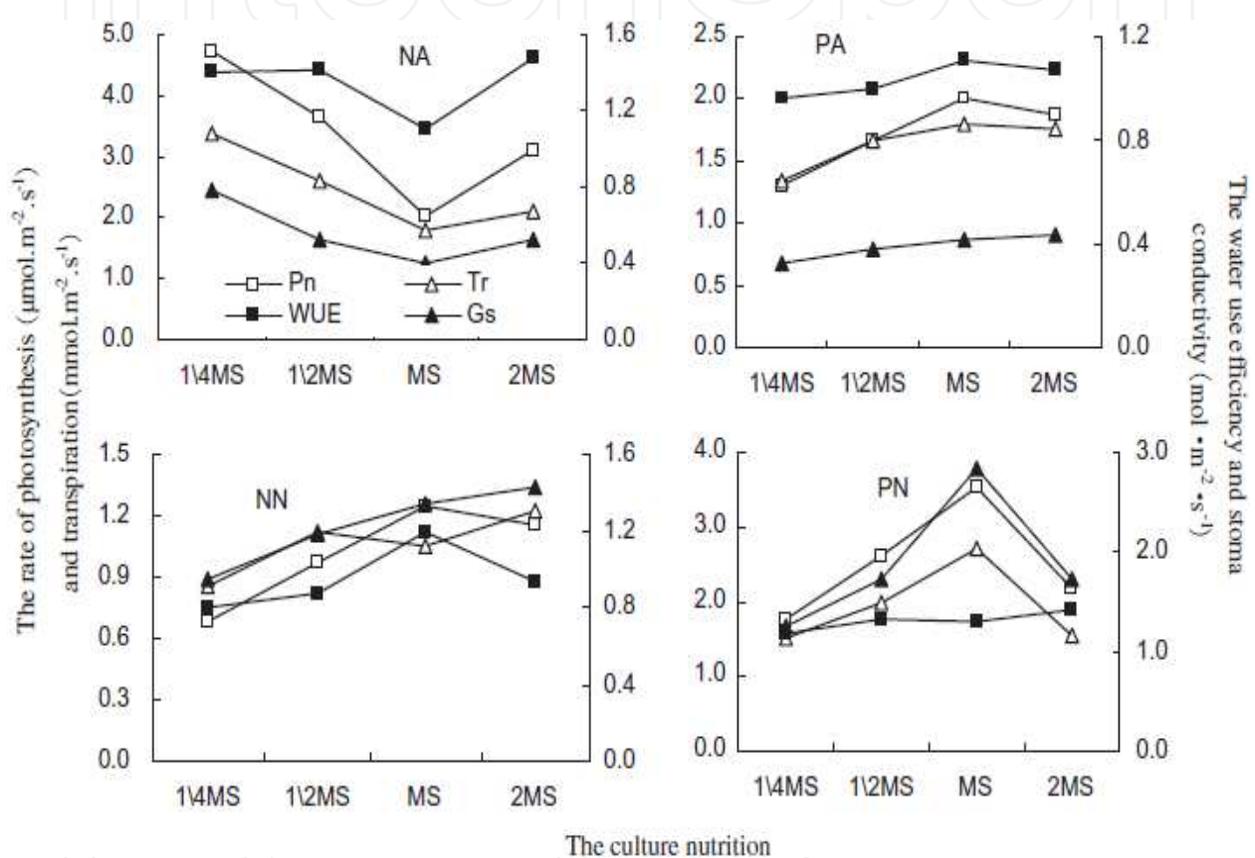


Fig. 3. The effect of wheat stubble on photosynthesis of two kinds of transgenic potato. NA: normal seedling under 0.01 mg/ml wheat straw; PA: acclimated seedling under 0.01 mg/ml wheat straw; NN: normal seedling without wheat straw; PN: acclimated seedling without wheat straw ( Zeho et al., 2010)

Belz and Hurlle (2004) in order to identify the allelopathy materials and screening the grain crops developed a fast and consistent bioassay method. The bioassay carried out in hydroponics culture, and a range of experiments with 2-(3H)-benzoxazolinone, an allelochemical of several grain crops, was carried out to define the basic protocol. The -(3H)-benzoxazolinone influenced on growth, enzyme activities, ascorbate peroxidase, catalase, glutathione S-transferase, peroxidase, phenylalanine ammonia-lyase, and chlorophyll fluorescence, whereby root length was the most reliable response parameter of *Sinapis alba* L. and it was selected as the recipient species. They found that *Secale cereale* L. and *Triticum aestivum* L. as donor species caused optimize the protocol.

#### 4. Abiotic stresses

For investigation the abiotic stresses, we need to apply modified conditions that are similar to arid conditions. The hydroponics system can provide modified conditions those capable researchers to elucidate the mechanisms involved in the response of plants to abiotic stresses. Actually, hydroponics can help to understand abiotic stresses tolerant mechanisms; also defines conditions to identify abiotic stress-inducible genes in the tolerant plants (Mohsenzadeh et al. 2009). The abiotic stresses associated with osmotic issues, thus the use of hydroponics system for studying the abiotic stresses such as salinity and drought stresses is unavoidable. In study on abiotic stresses, the adjustment of osmotic pressure in soil due to few factors such as interference of soil chemical and physical properties is difficult or somehow impossible, so that methodology of hydroponics system is unique in these cases (Szira et al. 2008).

For screening the tolerant plants in face to abiotic stresses the hydroponic methods provide proper conditions to achieve right data relevant to physiological and biochemical responses such as proline concentration, K/Na ratio, chlorophyll contents, and photosynthesis rate (Khan and Shirazi, 2009).

Using of nutrient solution to study on abiotic stresses is suitable for marsh plants or for understanding the effects of salt on plant growth, but a hydroponics solution is not representative of the natural habitat of most agricultural plants in seedling and growth stages. Studying on abiotic stresses in most of plants by using inorganic media culture such as sand, perlite and vermiculate culture can be appropriate (peel et al. 2004). In particularly, salinity stress studies involved with various salts that every salt has special effects on plants physiology, so that in order to distinguish individual influences the use of hydroponics system is necessary.

From last decades the researchers were looking to find a proper method for cultivating plants, imposed to a controlled water deficit. Testing genotypes for water deficit in the soil seems so hard or sometimes impossible. maintainig a uniform pattern, and constant water potential through the whole soil profile is difficult (Munns et al. 2010). Enhancing of drain quickly, perlite or vermiculite as inorganic substrates can play perfect role in water deficit testing in compare with soil (Passioura, 2006).

In a water deficit test in order to reduce water potential, mannitol used as non-ionic osmotic. High-molecular-weight polyethylene glycol (PEG, MW 6000) used to control water potential of imposing plants to water deficit (Munns et al. 2010).

Using of dry soil to simulate a drought or soil water deficit causes reduced access to nutrients by plants, thus plants may suffer from N or P deficiency while in hydroponics system this problem has been solved (Munns et al. 2010).

Hydroponics system is the most appropriate and profitable method for investigation of abiotic stresses on plants. Bouslama (1984) evaluted 20 soybean genotypes for drought tolerance by three screening methods. The methods were included: seed germinating in polyethylene glycol-600 (PEG) at  $-0.6$  MPa osmotic pressure, subjecting seedlings to PEG-600 at  $-0.6$  MPa osmotic pressure in hydroponic solution for 14 days, and a heat tolerance test based on the cellular membrane thermo-stability. Based on obtained results they reported that there is a high significant correlation between two tested methods including

the hydroponic seedlings and heat tolerance tests where the tolerant cultivars in both methods were same. They demonstrated that evaluation of plants for abiotic stresses in germination stage could not be reliable to identify abiotic stresses. Finally, they indicated that among the three tested methods the hydroponics as a consistent methodology is proper and reliable to assess the genotypes and selecting the tolerant genotypes.

Baranova et al. (2007) investigated utilization of storage starch in the cells of cotyledon mesophyll and root meristem in the course of alfalfa seedling on the nutrient solutions with different concentrations of NaCl, Na<sub>2</sub>SO<sub>4</sub>, and mannitol. They showed that salt stress inhibit early development of seedlings because of reduction in the mobilization of reserves. In order to analyze the dynamics of starch mobilization in the seedlings of alfalfa at osmotic stress, they evaluated the effect of two salts and an osmoticum on the cells of cotyledon mesophyll and root meristem. They assumed that injurious effect of salts depends on the osmotic component of their action.

Peng et al. (2008) in their experiment on eco-physiological response of alfalfa seedling to salts ((NaCl, Na<sub>2</sub>SO<sub>4</sub>, NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>), and 30 salt-alkaline combinations demonstrated that salt and salt-alkali decreased total biomass and biomass components of seedling definitely and furthermore the interaction between salinity and alkalinity cause changes the root activities due to salinity gradient. They showed that the physiological responses such as leaf electrolyte, leakage rate and proline content varied with different levels of salinity and alkalinity.

Al-Khateeb (2006) studied growth and ions concentration of alfalfa seedlings in response to different concentrations of Na<sup>+</sup> and Ca<sup>2+</sup>. They indicated that with increasing Na<sup>+</sup>, total dry matter including root, stem, and leaves declined, but increasing Ca<sup>2+</sup> alleviated the detrimental effects of Na<sup>+</sup>.

Assessment of barley germplasms in face to drought stress carried out by using 47 Tibet annual wild barley genotypes in hydroponics system. The observations included chlorophyll content, plant height, and biomass of shoot/root were significantly reduced (Zhao 2010).

Lafitte et al. (2007) in a study evaluated genotypes of rice in face of drought stress in different stages by various methods such as soil and hydroponic culture. Effects of drought stress on rice genotypes in hydroponic method was more clearly visible where in hydroponics system the level of abscisic acid as index of drought stress was high in plants in compare with plants cultivated by other methods.

## 5. Plant roots

The root is located underground in most plants which anchor the plant, store nutritious products, absorb water and nutrient elements from soil and conduct to other parts of the plant. Roots make and secrete lots of exudates and help to produce organic matters in the soil. Roots also alter the chemical and physical properties of the soil. Hydroponics can make easier the study of the root anatomy, morphology and root/shoot ratio in plants under controlled conditions. Hydroponics can be used to study root and liquid interactions, symptoms of nutrient deficiencies in different plants without interference of limiting factors in the soil, the enzymatic activities of the roots, the role of root exudates in availability of

essential nutrients for plants and microorganisms activities in the rhizosphere and the effects of toxic elements on roots, efficiently. Investigating the architecture of root system is necessary because crop productivity is influenced by the availability of water and nutrients that are distributed in the soil heterogeneously (Cichy et al 2009; Gregory et al. 2009). Changes in soil water exploitation by plants due to changes in root system architecture influence the accumulation of crop biomass (Hammer et al., 2009). Several non soil-filled techniques are available for measuring and visualizing the root architecture and finding its relationship to plant productivity (Gregory et al. 2009). Hydroponics is an ideal method for observing the hidden parts of the plant and study root growth and development over time in different conditions. This article refers to some studies that reflect the different aspects of hydroponic applications in studying the plant roots.

Chen et al. (2011) evaluated the reliability and efficiency of a semi-hydroponic bin system in screening root traits of 20 wild genotypes of lupin (*Lupinus angustifolius* L.) and found significant differences in both architectural and morphological traits among genotypes. They concluded that this system is a desirable tool for evaluating root architecture of deep root systems and a large number of plants in a small space. Herminde Reinoso et al. (2005) studied the effects of increasing concentrations of  $\text{Na}_2\text{SO}_4$  (0-531 mmol L<sup>-1</sup>) on anatomical changes in the roots of *Prosopis strobilifera* seedlings were grown hydroponically in Hoagland's solution with addition of 19 mmol L<sup>-1</sup>  $\text{Na}_2\text{SO}_4$  every 48h until final salt concentration of 531 mmol L<sup>-1</sup> were reached. The anatomical changes were noticeable and different with other treatments at 531 mmol L<sup>-1</sup> including more cortical layers in primary root and earlier development of endodermis in younger root zones and decreased hypocotyls cambial activity in older root zones. Anderson et al. (1991) compared the effects of  $\text{NH}_4$  nutrition with  $\text{NO}_3$  nutrition on root morphology of corn (B73×LH51) that was cultured in complete nutrient solutions containing  $\text{NO}_3$  or  $\text{NH}_4$ -N. They demonstrated thicker roots and better growth of plants in  $\text{NH}_4$  nutrition than  $\text{NO}_3$  nutrition. Swenen et al. (1986) reported large differences in root development of different genotypes of juvenile *Musa* spp plants grown under hydroponic conditions. Bacilio-Jimenez et al. (2003) collected the root exudates from rice plantlets cultured under hydroponic conditions. Then, they compared the root exudates effects on the chemotaxis of two strains of endophytic bacteria collected from the rice rhizosphere with chemotactic effects of two strains, one collected from the corn rhizosphere and another one isolated from the rice rhizosphere. Their results showed that rice exudates caused a higher chemotactic response for endophytic bacteria than for other strains of bacteria presented in the rice rhizosphere. McQuattie et al. (1990) studied the effects of excess aluminum on changes in root anatomy of one year red spruce (*Picea rubens* Sarg.) cultured in a nutrient solution containing 0, 50, 100 or 200 mg/L aluminum. They reported well developed aluminum toxicity symptoms in the roots including decreased root length but increased root diameter and the number of cell layers in the root cap. The root tips examination by light and electron microscope showed a great cellular changes in roots, premature vacuolation, phenolic material accumulation, intercellular spaces formation, cellular membrane disruption and cytoplasm injury. Ergul Cetin (2009) studied the effects of Boron (B) excess and deficiency on anatomical structure of alfalfa (*Medicago Sativa* L.) in -B, control, +B hydroponic cultures. The results showed that B deficiency (-B) affected the root cortex parenchyma. In +B treatment there was a significant decrease in the diameter of roots. The degree of xeromorphy calculation showed that *M. Sativa* L. developed xeromorphic structure after +B treatment.

## 6. Heavy metals

The soils naturally contaminated by heavy metals are found in areas where metal ores come to the surface and deteriorate due to weathering. These kinds of soils are found in some regions around the world (Malaisse et al., 1999; Proctor, 2003; Rajakaruna et al., 2009). In regions with originally low heavy metal, human activities such as ore-mining and ore-refining industry nearby, are the main reasons of soil and water contamination (Barcan, 2002; Mandal et al., 2002). But in many areas the main reason of heavy metal contamination of soil and ground water is applying metals in agriculture. Examples are the application of copper and zinc-based pesticides, copper in vineyard, mineral fertilizers and sewage sludge in crop production (Komarek et al., 2010; He et al., 2005).

Molecular mass  $>5.0 \text{ g cm}^{-3}$  of heavy metals is distinctly higher than the average particle density of soils ( $2.65 \text{ g cm}^{-3}$ ). Certain heavy metals such as Manganese (Mn), Zinc (Zn), Iron (Fe), Copper (Cu), Cobalt (Co), or Molybdenum (Mo) are necessary for the growth of organisms. Others have a single role in some organisms such as Vanadium (V) in some peroxidases and in V-nitrogenases and Nickel (Ni) in hydrogenases. The rest of the heavy metals such as Cadmium (Cd), Lead (Pb), Uranium (U), Thallium (Tl), Chromium (Cr), Silver (Ag), and Mercury (Hg) are always toxic to organisms. Arsenic (As) and Selenium (Se) are not heavy metals but they are often called "metalloids". With the exception of Iron, all heavy metals with the concentration above 0.1% in the soil are toxic to plants.

Plants depend on soil for up taking water and nutrients. Thus, the soils contain excess levels of heavy metals decrease crop productivity under certain conditions. The heavy metals pollution of soil, ground water and surface water is a serious threat to the environment and human health. Therefore, heavy metal toxicity, screening and selecting heavy metals tolerant and remediative plants and mechanisms involved in their detoxification have been subjects of many researches in the entire world.

Many researchers reported the advantages and limitations of hydroponics in heavy metal studies. Meharg (2005) reported that uptake the heavy metals by plants and any subsequent phytotoxic reaction should be analyzed under controlled conditions and suggested hydroponics as a suitable way to screen and identify the heavy metal tolerant and remediative plants before field studies. For example, Kupper et al. (2000) found that *Arabidopsis halleri* (Brassicaceae) and *Sedum alfredii* (Crassulaceae) can accumulate Cd under hydroponics condition. Hongli and Wei (2009) investigated 23 Amaranth cultivars from different regions in hydroponics' culture and observed that *Amaranthus* cv. Tianxigmi is a Cd hyperaccumulator. Duskenhov et al. (1995) demonstrated that *Brassica juncea* can accumulate a remarkable amounts of Cd in its tissues under hydroponic conditions. Varad and Freitas (2003) reported that Sun flower can remove cesium, strontium, uranium and lead from hydroponic solution. Yang et al. (2002) reported that *Elsholtzia splendens* can be used as a phytoremediation for Cu-contaminated soils. Moreno-Jimenez et al. (2009) investigated Hg and As tolerance and bioaccumulation in *Pistacia lentiscus* and *Tamarix gallica* plants under hydroponic conditions and showed that *T. gallica* accumulated more Hg and As and had higher tolerance to both Hg and As than *Pistacia lentiscus*.

Whilst hydroponics is useful for screening and selecting heavy metals tolerant and remediative plants but there is evidence that plants react in a different way when they are

grown under hydroponic conditions or soils (Zabtudowska et al.;2009). Therefore extrapolating results from hydroponics to field conditions are too optimistic and unrealistic without considering these limitations (Dickinson et al.:2009).For example, Moreno-Jimenez et al. (2010) compared phytoavailability of Arsenic(As) in mine soils and hydroponics and reported that As had a low solubility in mine soils and the extrapolation of data obtained in hydroponics to mine soils were unrealistic and proposed that hydroponics data only in As levels in the range 0-10 $\mu$ M that plants are exposed in soils can be extrapolated to soil conditions confidently.Qing Cao et al.(2007) investigated toxicity of Arsenic(As) and Cadmium(Cd) individually and in combination on root elongation of wheat seedlings (*Triticum aestivum*.L.) in hydroponics and in soils. In their hydroponic and soil experiments As and Cd showed synergetic and antagonistic effects on wheat root elongation, respectively. They concluded that phytoability of Cd is increased by forming uptake-facilitating Chloride complexes in a soil with high level of Chloride, also, soil characteristics (PH, organic matter, absorption capacity and moisture) affect the availability and toxicity of Cd in soil matrix. On the contrary, hydroponics can be used to evaluate suitable plants extract contaminants from groundwater, surface water, wastewater, and retain within their roots by the way of rhizofiltration, confidently. For example, Jing et al.( 2007) suggested rhizofiltration a suitable way for extracting Pb, Cd, Cu, Ni, Zn, and Cr,from contaminant aqueous sources. Dushenkov et al. (1995) found out that hydroponically grown plants such as Indian mustard (*B. juncea* L.) and Sunflower (*H. annuus* L.) can extract the toxic metals like, Pb, Cu, Cr, Cd, Zn,and Ni, from aqueous solutions efficiently. Karkhanis et al.(2005) in an experiment in green house, used pistia, duckweed,and water hyacinth(*Eichornia rassipes*) to remove heavy metals from aquatic environment polluted by coal ash containing heavy metals.

Coiso et al(2006) and hernandez-Allica et al(2007) suggested hydroponics the only way for eliminating mass transfer limitations and elucidating free metal ion and uptake and translocation metal-chelate within the plant. For example, January et al.(2007) conducted experiments to find out the effectiveness of Sunflower (*Helianthus annuus*) for the phytoremediation of mixed heavy metals including : Cd, Cr, Ni,As and Fe. The experiments were conducted under hydroponic conditions in order to eliminate mass transfer problems that would be present in soil experiments. The results showed that heavy metal uptake preference was Cd = Cr > Ni,Cr > Cd >Ni >As.

Hydroponics can be used to identify and characterize the mechanisms of tolerance to excess heavy metals in plants. For example: Wei et al.(2008) compared a more tolerant plant species (*sorghum sudanense*) with a less tolerant plant species (*Chrysanthemum coronarium*) were grown in hydroponic culture in the presence of Cu excess(up to 50 mM) to find out the possible role of their cell walls in relation to tolerance. The authors observed that, total Cu concentrations in *C. coronarium* shoots and cell walls were much higher than in *S. sudanense*.However, the percentage of cell wall bound Cu in *C. coronarium* was much lower,but the percentage of water soluble Cu was higher than *S. sudanense*.In contrast,the amount of Cu in the roots and the percentage of cell wall bound Cu in *S.sudanense* were substantially higher than *C.coronarium*. As a result, the amount of Cu load on protoplast of *S. sudanense* was much lower than in *C. coronarium*.

Hydroponics can be used to study interesting properties from a physiological point of view efficiently. For example, in a study conducted in hydroponic cultures Shi (2008) investigated

the role of root exudates of *Elsholtzia splendens* on activating Cu in soils and reported that the amount of root exudates in Cu dissolution from contaminated soil was higher than the amount dissolved in deionized water significantly. Thus, concluded that root exudates of *Elsholtzia splendens* can activate Cu in the soil strongly. Lou et al. (2004) studied the seed germination of *Elsholtzia splendens* in Hydroponic cultures containing excessive Cu and found out that lower than 250 mmol L<sup>-1</sup> of Cu in culture solution stimulate seed germination, but at higher concentrations (750 mmol L<sup>-1</sup>) of Cu, seed germination decreased.

## 7. Aluminum toxicity

Metals are found naturally in soils that may be useful or toxic for plants or environment. Many researchers reported metal toxicity in plants (Brown et al., 1975; Chaney et al., 1977; Foy et al., 1978). Aluminum (Al) is not an essential element for plant growth, but low concentrations can increase plant growth or cause other desirable effects (Foy, 1983; Foy et al., 1978). Aluminum toxicity is the most common limiting factor for plants grown in acid soils. Excess Al interrupts cell division in roots, increases the cell wall rigidity, fixes phosphorous in soils, reduces root respiration, interferes with uptake, transport and use of several essential elements such as Ca, Mg, K, Fe and P (Foy, 1992).

Improving aluminum tolerant plants and identification the mechanisms involved in tolerance of plants are the most suitable ways to decrease destructive effects of aluminum toxicity. Plants greatly differ in tolerance to Al, available literature data showed a great differences in the experimental conditions that determine the Al toxicity and plant reactions as follows: The concentration of Al and Al form in the medium, PH of substrate and PH stability, exposure time, ionic strength of the culture medium, soil type, availability of essential nutrients, plant age and plant variety (Poschenrider et al.; 2008). Since tolerance is genetically controlled, screening and selecting of Al tolerant genotypes under hydroponics condition can lead to improve tolerant cultivars to excess aluminum because hydroponics provides easy access to root system, exact control over available nutrients and PH and non-destructive measurements of tolerance (Carver and Ownby, 1995). Additionally, studies have shown that the primary response to Al stress occurs in the roots, therefore root growth measurements under Al stress can be used in several Al tolerance screening techniques (Kim et al., 2001). Hydroponics is a very suitable technique for measuring the root growth under Al stress conditions. For example Makau et al (2011) hydroponically investigated maize (*Zea mays*), garden pea (*Pisum sativum*), bean (*Phaseolus vulgaris* L.) and cucumber (*Cucumis sativus*) tolerance to excess Al in the rhizosphere during the early plant growth stages and introduced the root elongation as a single most significant index in ranking the genotypes for Al tolerance. Hydroponics has been used to evaluate a large number of soybean genotypes for aluminum tolerance quickly (Silvia et al., 2001). Foy et al. (1965) compared an aluminum tolerant cultivar with an aluminum sensitive cultivar of *Triticum aestivum* in nutrient solutions with or without aluminum and found out that aluminum tolerant cultivar was able to increase PH in nutrient solutions. Wood et al. (1984) reported that the concentration of salts or ionic strength in nutrient medium related to the critical level for tolerance to aluminum. Speher (1994) with studies under hydroponics condition selected tolerant soybean genotypes to excess aluminum. Ma et al. (1997) studied 600 barley lines with a rapid hydroponic screening way and reported that ninety lines had medium tolerance to excess aluminum. Krizec et al. (1997) conducted an experiment for evaluating two

cultivars of *Coleus blumei* in nutrient media containing 0 to 24 mg/l aluminum and on an acid Al-toxic. Sivaguru and Paliwal(1993) tested twenty two rice cultivars for studying their tolerance in nutrient solution containing aluminum in toxicity level at PH 4.1. They reported six tolerant cultivars on the basis of root and shoot tolerance indices. They also demonstrate the mechanism of tolerance to toxic levels of aluminum on the basis of mineral uptake and mineral utilization. The tolerant plants in the presence of aluminum took up and utilized Ca and P efficiently, but the aluminum sensitive cultivars took up and utilized less Ca and P (Sivaguru et al.,1993).

## 8. Conclusion

Many advantages make hydroponics a specific technique for plant biology researches. In plant nutrition researches hydroponics allows researchers manage nutrients and control PH, EC, macro and microelements concentrations in nutrients media, monitor mutual-element interactions and investigate the anions and cations absorption. Hydroponics provides proper conditions to determine the individual effects of nutrient elements on quality and quantity of crop yields. Absorption of plant nutrients occurs in a specific range of PH and soil reaction, thus determining the nutrients absorption where subjected to limitative materials like lime preferred by hydroponics method. However, for investigating the interaction between nutrient elements and organisms it seems that soil experiments are necessary. In allelopathy researches, interference between plants can be due to allelopathy, competition for nutrients, space and natural resources. Therefore, separation the individual effects of allelopathy from the other interferences is easier in hydroponics. Research in terms of influence of water stress on plants in the soil involve by deficit of nutrients while in hydroponics method not only the effect is due to water deficit but also it is possible to adjust water potential. Some of researchers believe that study in terms of salt stress except hydroponics system, will be associate with many problems in the results because not only regulation of osmotic potential in rhizosphere is complicate but also the separation the effects of various salt is impossible. In plant roots studies hydroponics can make easier the study of the root anatomy, morphology and root/shoot ratio in plants under controlled conditions. Hydroponics can be used to study root and liquid interactions, symptoms of nutrient deficiencies in different plants without interference of limiting factors in the soil, the enzymatic activities of the roots, the role of root exudates in availability of essential nutrients for plants and microorganisms activities in the rhizosphere, the effects of toxic elements on roots and root architecture efficiently. Many researchers reported the advantages and limitations of hydroponics in heavy metal studies. Meharg(2005) reported that uptake the heavy metals by plants and any subsequent phytotoxic reaction should be analyzed under controlled conditions and suggested hydroponics as a suitable way to screen and identify the heavy metal tolerant and remediative plants before field studies. Whilst hydroponics is useful for screening and selecting heavy metals tolerant and remediative plants but there is evidence that plants react in a different way when they are grown under hydroponic conditions or soils (Zabtudowska et al.;2009). Therefore extrapolating results from hydroponics to field conditions are too optimistic and unrealistic without considering these limitations (Dickinson et al.:2009). Improving aluminum tolerant plants and identification the mechanisms involved in tolerance of plants are the most suitable ways to decrease destructive effects of aluminum toxicity. Since tolerance is genetically controlled, screening and selecting of Al tolerant genotypes under hydroponics

condition can lead to improve tolerant cultivars to excess aluminum because hydroponics provides easy access to root system, exact control over available nutrients and PH and non-destructive measurements of tolerance (Carver and Ownby, 1995). Additionally, studies have shown that the primary response to Al stress occurs in the roots, therefore root growth measurements under Al stress can be used in several Al tolerance screening techniques (Kim et al., 2001). Hydroponics is a very suitable technique for measuring the root growth under Al stress conditions.

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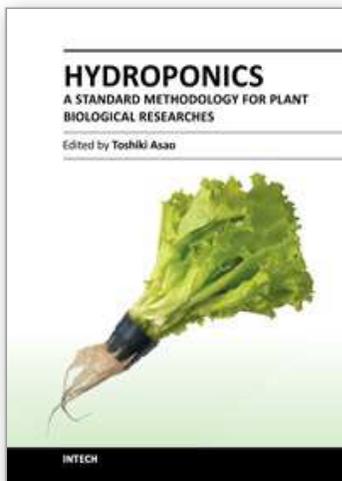
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## **Hydroponics - A Standard Methodology for Plant Biological Researches**

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Hydroponics-A standard methodology for plant biological researches provides useful information on the requirements and techniques needs to be considered in order to grow crops successfully in hydroponics. The main focuses of this book are preparation of hydroponic nutrient solution, use of this technique for studying biological aspects and environmental controls, and production of vegetables and ornamentals hydroponically. The first chapter of this book takes a general description of nutrient solution used for hydroponics followed by an outline of in vitro hydroponic culture system for vegetables. Detailed descriptions on use of hydroponics in the context of scientific research into plants responses and tolerance to abiotic stresses and on the problems associated with the reuse of culture solution and means to overcome it are included. Some chapters provides information on the role of hydroponic technique in studying plant-microbe-environment interaction and in various aspects of plant biological research, and also understanding of root uptake of nutrients and thereof role of hydroponics in environmental clean-up of toxic and polluting agents. The last two chapters outlined the hydroponic production of cactus and fruit tree seedlings. Leading research works from around the world are brought together in this book to produce a valuable source of reference for teachers, researcher, and advanced students of biological science and crop production.

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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
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