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### POTATO MINITUBER PRODUCTION UNDER HYDROPONIC SAND CULTURE

#### Babak DARVISHI

Seed and Plant Certification and Registration Institute, Iran Corresponding author: Bdarvishi\_84@yahoo.com

#### ABSTRACT

Isolation and use of sterile growing media are two important factors in hydroponic production of healthy potato mini-tubers. Sand can be disinfected by solarization, while organic growing media may harbor some pathogenic agents. Under hydroponic condition, number and size of potato tubers are usually controlled by nutritional factors such as nitrogen, phosphorus and pH. The main objective of present study was to find an appropriate combination of N, P and pH (with respect to tuber number) under hydroponic sand culture and to evaluate some physiological traits affected by nutrients and pH. A factorial experiment based on completely randomized design with 4 replications was conducted. The experimental factors included N, P and pH. Some morphological and physiological traits including tuber number, rate of net photosynthesis, concentration of hormones ABA and IAA were studied. Results showed that higher phosphorus concentration for 10 days increased tuber number per plant, but tuberization was not influenced by nitrogen interruption and intermittent reduction of pH. None of N, P and pH affected total nitrogen concentration of potato leaf, stem and tuber. Higher phosphorus concentration increased the level of endogenous ABA and IAA, induced tuberization and thereby increased net photosynthesis rate of potato plants.

Keywords: potato, hydroponic, sand, nutrition, tuberization.

#### **INTRODUCTION**

Adding substrate to hydroponic system is a tool to enhance chemical and physical inertia. As a consequence, labour and energy are saved (Rolot and Seutin, 1999).In developing countries, different organic growing media as peat-moss and coconut fiber have traditionally been used to produce potato mini-tubers. Organic growing media may harbor some pathogenic agents such as *Erwiniaspp*, *Spongospora subterranean* or *Streptomyces scabies* (Rolot and Seutin, 1999). The other short-comings of such systems are relatively high cost of organic substrate as well as low rate of oxygen diffusion, which adversely affects plant growth (Cho et al., 2006). Nowadays, multiplication of potato mini-tubers is mainly done under NFT (Nutrient Film Technique) and aeroponic systems (Farran and Mingo-Castel, 2006). The advantages of multiplication via NFT and aeroponic over conventional methods include precise using of nutrients, high multiplication rate of tubers, better

sanitary control of growing media and also relieved control of weeds. In spite of producing high number of tubers under these conditions, they are costly and laborious (Novella et al., 2008), so using such systems are not practical and commercial in developing countries.

Sand provides an inert nature media with more pore volume (more oxygen transport), stable structure, less water retention and large volume of accessible water to the plants (Dole and Wilkins, 1999). The nature of sand may cause a mechanical resistance and it may affect stolon development (Ewing and Struik, 1992). Insufficient resistance can result in vigorous stolon growth (Cary, 1986) or secondary stolons and many small tubers (Vreugdenhil and Struik, 1989). In this study nutritional management was applied to induce tuberization in potato plantlets. Chang et al. applied nitrogen interruption and increased tuber numbers by 18%. They suggested nutrient interruptions should be conducted after sufficient haulm development to minimize a reduction of tuber set (Chang et al., 2008). Kang et al. reported nitrogen deficiency induced potato tuberization without causing a significant retardation to the plant growth (Kang et al., 1996). Other nutritional element has been used to increase tuber set (Sanderson et al., 2003) and number (Rosen and Bierman, 2008) in potato plants is phosphorus. Rosen and Bierman (2008) reported that petiole P was positively correlated with number of potato tubers per plant. Intermittent reduction of solution pH could be a means to stimulate tuber production under hydroponic conditions. Wan et al. reported tuber initiation was induced in plants subjected to intermittent pH reductions compared to constant pH 5.5 (Wan et al., 1994).

The main objective of present study was 1. to develop a relatively low cost method for potato mini-tuber production and 2. to find the best combination of N, P and pH to produce maximum number of minitubers. Some morphological and physiological traits (including tuber number, rate of net photosynthesis, concentration of hormones ABA and IAA) were measured in order to understand why the best combination of N, P and pH gave higher numbers of minitubers.

# MATERIALS AND METHODS Experimental set up and treatments

The experimental set up was an opened sand and perlite (1:1 volume) hydroponic system. This inert, sustain and relatively inexpensive growing media was placed in 6-L pots and certified potato cv. Sante seed tubers (20 -25 mm mean diameter) were planted in these pots at a depth of 5 cm in the spring of 2011 at the research greenhouse of Seed and Plant Certification and Registration Research Institute (SPCRI) in Karaj, Iran. Pots were kept at  $25\pm5$  °C with an approximately 14-h natural photoperiod and 300-600 µmol m<sup>-2</sup> s<sup>-1</sup>Photosynthetic Photon Flux Density (PPFD) measured at the top of the canopy.

Plants were irrigated with basic nutrient solution (Table 1) through a network of tube, with a hole on the top of each pot. The pH and Electrical Conductivity (EC) of the nutrient solution were kept at 5.8-6 and 2 mS cm<sup>-1</sup> respectively (Farran and Mingo-Castel, 2006).

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Table1. Composition of basic nutrient solution $(mgL^{-1})$											
Ν	Р	K	Ca	Mg	S	Fe	Zn	Cu	Mo	Mn	В
160	42	239	152	38	40	1.7	0.6	0.2	0.1	1.2	0.8

A  $2 \times 2 \times 2$  factorial experiment based on completely randomized design with 4 replications was conducted. Experimental factors included N (N1: Constant consumption of 160 ppm N through the growth period, N2: Constant consumption of 160 ppm N until 65 DAE followed by 0 ppm N for 10 days), P (P1: Constant consumption of 42 ppm P through the growth period, P2: Constant consumption of 42 ppm P until 65 DAE followed by 84 ppm P for 10 days) and pH (pH1: Constant 6 through the growth period, pH2: Intermittent reduction of pH to 3.5: 3 times for 2 hours). Use of basic nutrient solution (Table 1) continued until 65 DAE. Between 65 DAE to 75 DAE, nutrition system was turned off and stimulating nutrient solution (the second level of N, P and pH) was applied to the related plots. Afterwards the composition of nutrient solution was returned back to the basic form as before. For intermittent pH treatment, the pH of well water was lowered to 3.5 by adding 1.0 M H2SO4 and then applied to related plots on 73 DAE for two hours. After two hours, the growing media in these pots was washed with normal well water (7.2 pH). After two hours pH treatment was repeated two times.

### Measurements

<u>Net photosynthesis rate:</u> Net photosynthesis rate was measured at 75 DAE (at the end of nutritional and pH treatments) using a portable CI-340 Ultra-Light Photosynthesis System (CID, Inc., USA). Measurements were taken on terminal leaflet of the youngest fully expanded leaf of three plants from each plot. During the measurements, the PPFD at the top of plant canopy was between 300 and 500  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>.

<u>Total N concentration:</u> One plant from each plot was harvested and separated into leaves, stems, roots and tubers at 75 DAE. Separated plant parts were thoroughly washed by 2 dippings, of 5 minutes each, in distilled water, then were dried at 105°C and total nitrogen concentration was determined using macro-Kjeldahl method (AOAC,1984).

<u>ABA and IAA concentration</u>: In order to analyze ABA and IAA concentration, 75 DAE leaf samples of second plant from each plot were harvested and frozen in liquid nitrogen and stored at -80°C until analysis. ABA and IAA concentration was measured in the leaves of treatments with highest (N1P2pH1) and lowest (N1P1pH1) tuber number.

<u>Tuber characteristics</u>: Third plant from each plot was harvested 90 DAE and number of tubers was counted. For dry weight determination, surface of randomized selected tubers cracked, these tubers oven-dried at  $70^{\circ}$ C until constant weight was reached.

<u>Statistical Analysis:</u> SAS software (version 9.0) was used for statistical analysis and means were compared by Duncans Multiple Range Test at a p of 5%. In

addition, concentration of ABA and IAA in specific treatments compared by Least Significant Different (LSD).

# RESULTS AND DISCUSSION Leaf gas exchange

Net photosynthesis rate of potato plants was not affected by either of nitrogen interruption and pH intermittent reduction. However increased phosphorus concentration resulted in net photosynthesis increment(Figure 1). There were likely two reasons for increased net photosynthesis rate by phosphorous. First, this nutrient plays an important role in photosynthesis and intermediary metabolism. Phosphorous in the form of nucleotides such as ATP and ADP as well as inorganic phosphate (Pi) and phosphorylated sugars also plays an integral role in the energy metabolism of cell. Second, it is postulated that the promoted early potato crop growth and increased tuber set by phosphorus (Figure 4) increased the development of new sinks. Creation of strong sinks, the newly formed tubers, could result in increased demand for assimilate. According to the second hypothesis, increased demand for assimilates in the sinks probably caused the rate of net photosynthesis to be increased.

Net photosynthesis rate of potato plants was not significantly influenced by other studied factors (nitrogen interruption or intermittent reduction of pH). In favor of this finding, Vos and van der Putten (1998) reported that the dominant effect of nitrogen supply was on leaf size and not on the rate of photosynthesis. Also Marshall and Vos (1991) suggested that an increasing proportion of leaf nitrogen was not associated with the performance of the photosynthetic system. Our findings are in contrast with previous observations of Chang et al., (2008) reported that photosynthetic rates of potato plants decreased by 10 days nitrogen interruption.

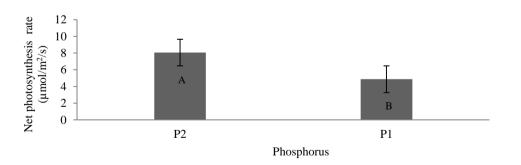


Figure1. Effect of phosphorusconcentration on net photosynthesis rate of potato plants

## Total N concentration

According to the variance analyses, none of the nutritional and pH factors affected total nitrogen concentration of potato leaf, stem and tuber. This is in conformity with the findings of Sattelmacher and Marschner (1979) who observed that after 9 days of nitrogen withdrawal, the concentration of nitrogen in the plants with discontinues nitrogen was particularly the same as in the plants with continues nitrogen. However, both nutritional factors (nitrogen interruption and increased phosphorus concentration) increased total nitrogen concentration of roots (Figure 2).

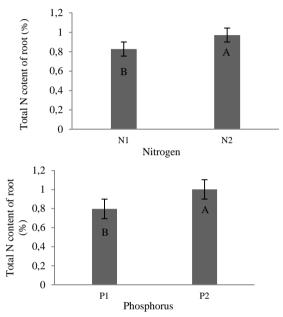


Figure 2. Total N content of potato root under nitrogen (A) and phosphorus (B) nutrition

# ABA and IAA concentration

Concentration of ABA and IAA was only influenced by phosphorus. In selected plots (N1P1pH1 and N1P2pH1) higher phosphorus concentration in nutrient solution increased both hormones (ABA and IAA) in potato plant leaves (Figure 3). In favor of our finding, Chang et al. reported ABA levels increased in cv. Superior as a result of nutrient interruption (Chang et al., 2008). The promoting effect of exogenous ABA on tuberization was demonstrated by the increasing numbers of tubers (Abdullah and Ahmad, 1980). Chang et al. (2008) reported ABA levels increased in cv. Superior as a result of nutrient interruption. It is postulated that higher phosphorus concentration increased the level of endogenous ABA and IAA, induced tuberization and thereby increased net photosynthesis rate.

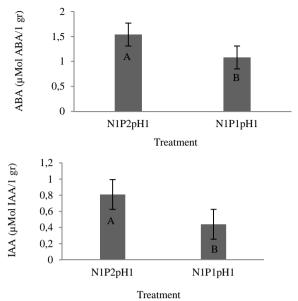


Figure 3. Comparison of ABA (A) and IAA (B) content in the leaves of treatments with highest  $(N_1P_2pH_1)$  and lowest  $(N_1P_1pH_1)$  tuber number

### Tuber characteristics

Tuber number per plant was significantly increased with an increase in phosphorus concentration (Figure 4). Rolot and Seutin (1999) reported that more phosphorus had a positive effect on multiplication rate and increased tuber numbers from 6.4 (in peat culture) to 6.96 per plant. Rosen and Bierman (2008) reported that phosphorus fertilizer application increased total number of tubers per plant. Sucrose synthase (SuSy) and ADP-glucose pyrophosphorylase(AGPase) are two key enzymes involved in sucrose to starch conversion. Expression of AGPase is decreased by phosphate. AGPase is exquisitely sensitive to allosteric regulation being activated by 3PGA and inhibited by Pi (Preiss, 1988). Sowokinosand Preiss (1982) reported that AGPase from potato tubers resembles the leaf enzyme. Therefore during tuber development, expression and activity of AGPase may be inhibited by increased Pi concentration in amyloplasts. Under activity inhibition of AGPase by Pi in developing tuber, produced assimilates can be directed to the new initiated tubers. However the effect of nitrogen and pH on this trait was not significant. Chang et al. (2008) reported nitrogen interruption increased tuber numbers in cv. Superior (medium-early season) and did not influence on tuber numbers of cvs. Atlantic (mid-late) and Jasim (late). On the contrary, in water culture of potato plants nitrogen withdrawal increased tuber numbers (Sattelmacher and Marschner, 1979). Tuber dry weight was not affected by any of studied factors (nutritional and pH factors).

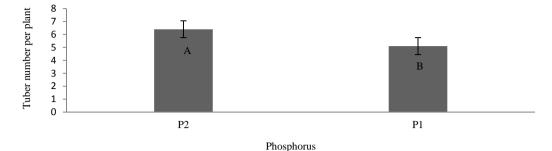


Figure4. Effect of phosphorus on tuber number of potato plants

#### CONCLUSION

Among studied nutritional factors (N, P and pH), increased phosphorus concentration significantly enhanced tuber numbers of potato plants in hydroponic sand culture. This nutrient increased net photosynthesis rate, ABA and IAA concentration of potato plant leaves and did not affect nitrogen absorption by potato. Therefore the best combination of N, P and pH (with respect to tuber number) in nutrient solution under hydroponic sand culture was N1P2pH1.

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