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EFFECT OF NUTRITIONAL TREATMENTS ON PHYSIOLOGICAL CHARACTERISTICS AND TUBERIZATION OF POTATO PLANTS UNDER HYDROPONIC SAND CULTURE

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□ *Under hydroponic condition, number and size of potato tubers are usually controlled by nutritional factors such as nitrogen (N), phosphorus (P), and pH. The main objective of the 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 the physiological traits affected by nutrients and pH. A factorial experiment based on completely randomized design with four replications was conducted. 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. Neither N, P, nor pH treatments affected total nitrogen concentration of potato leaf, stem, and tuber. Higher phosphorus concentration increased the level of endogenous abscisic acid (ABA) and indole-3-acetic acid (IAA), induced tuberization and thereby increased net photosynthesis rate of potato plants.*

Keywords: *Solanum tuberosum* L., hydroponic sand culture, tuberization, nitrogen, phosphorus, pH

INTRODUCTION

In commercial production of potato minitubers under greenhouse conditions the aim is to achieve a large number of minitubers per unit area so that multiplication cycle and the associated costs are reduced. Tubers number and size are usually controlled by many environmental factors such as nutrients, particularly nitrogen (N) and phosphorus (P). Therefore,

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under hydroponic production of potato tubers, an appropriate combination of nutrients should be applied to improve crop productivity.

Tuberization in potato plants is a complex process is inhibited by nitrogen nutrition (Li, 1985). Nowadays some of hydroponic commercial methods use nutrient interruption especially nitrogen deficiency for retarding shoot growth and inducing tuber formation. In a water culture experiment, tuberization was completely prevented by continuous supply of nitrogen via the roots (Krauss and Marschner, 1982). Certain intensity of nitrogen deficiency induced tuberization in potato without inflicting a significant injury to the plants (Kang et al., 1996). Bouma (1970) reported that four days after transfer of plants grown with adequate nitrogen to solution without N, leaf area had declined to 84% of the values for the control plants. Discontinuous N supply declined the rate of potato shoot and root growth rapidly (Krauss, 1978). Bot et al. (2001) observed that leaf area and growth rate of vegetable crops reduced by interruption of nitrate in nutrient solution. Chang et al. (2008) 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.

Other nutritional elements including P have been used to increase tuber set (Sanderson et al., 2003) and number (Rosen and Bierman, 2008) in potato plants. Intensive tuber initiation and high photosynthetic capacity during tuber bulking require adequate phosphorus nutrition. Under hydroponic minituber production, root density and thus root ability of potato plants to recover phosphorus is low, and therefore phosphorus deficiency could be a limiting factor (Pursglove and Sanders, 1981). Tukaki and Mahler (1990) reported that P concentrations between 10 and 35 μg mL^{-1} are optimum for tuber production and total tuber weight. They observed that tuber number per plantlet was highest within this range of phosphorus concentration. It has also been reported that increased phosphorus concentration in a nutrient solution increased tuber numbers and reduced the ratio of big tubers (>65 mm) (Jenkins and Ali, 2000). McArthur and Knowles (1993) increased phosphorus concentration in a nutrient solution up to 77 mg L^{-1}, and reported tuber growth was enhanced. They observed that the concentration of N, K, magnesium (Mg), or iron (Fe) in shoots was not affected by increased phosphorus supply. Therefore, adjusting phosphorus concentration of fertilizer is adopted as a new approach to improve potato seed production in commercial hydroponic systems.

Intermittent reduction of solution pH could be a means to stimulate tuber production under hydroponic conditions. Research to date conducted worldwide indicates potato plants adapt well to acidic conditions with optimum growth obtained between pH 5 and 6 (Cao and Tibbitts, 1994). Short term lowering of solution pH at certain growth stages induced tuber initiation in potato plants grown under solution culture (Wan et al., 1994).

These stimulating nutritional and pH treatments may have their effects through changes in photosynthesis, nitrogen distribution, and hormone

concentration. Eckstein et al. (1995) reported that net photosynthesis was higher in an actively growing fruit trees. Frier (1977) observed that potato photosynthesis was stimulated at tuber initiation stage; therefore any nutritional treatment inducing tuber initiation (sink demand) in potato plants may affect leaf gas exchange. Olesinski et al. (1989) found a positive effect of nitrogen nutrition on photosynthesis and stomatal conductance throughout the growing season. In another study conducted by Vos and van der Putten (1998), it was found that increased nitrogen concentration resulted in light saturated photosynthesis (P_{max}) reduction, but the effects were not systematic. They concluded the main effect of nitrogen was on leaf size rather than light saturated photosynthesis (P_{max}) or leaf nitrogen content. Marshall and Vos (1991) measured P_{max} on terminal leaflets of potato plants supplied with 0–36 g N m^{-2} between 100 and 154 days after planting. The results showed that increased leaf nitrogen was not associated with P_{max}. They also reported that stomatal conductance was not influenced by nitrogen supply. Campbell et al. (2010) reported that increasing in soil nitrogen resulted in increased leaf nitrogen content.

It is assumed that nutrients can affect plant growth characteristics (such as tuberization) by changes in hormone levels and activities. Interaction between hormonal and nutritional regulation of tuber formation has been suggested by Xu et al. (1998). The possible role of nitrogen and ABA in potato tuberization has been investigated with a number of experiments. Abdullah and Ahmad (1980) reported that exogenous abscisic acid (ABA) promoted tuberization of potato plants. Krauss and Marschner (1982) observed that ABA content of potato plants increased with an interruption of nitrogen supply, however resumption of N decreased the ABA content. They reported that continues nitrogen supply resulted in delayed tuberization and lower production of tubers. Marschner et al. (1984) supplied nitrogen during tuber formation and observed that ABA content was reduced. They assumed that ABA is a promoting hormone in potato tuberization. In contrast to nitrogen, less attention has been paid to the effect of phosphorus and pH on ABA content.

Little is known about a possible role of indole-3-acetic acid (IAA) in tuber formation. Obata-Sasamoto and Suzuki (1979) reported that the IAA content was high at the stage before tuber initiation and decreased during tuber development. Koda and Okazawa (1983) observed the maximum level of IAA in swelling stolons of potato plants.

The main objective of the present study was to find the best combination of N, P, and pH to produce the maximum number of minitubers. Various morphological characters of the plants and some physiological traits (including rate of photosynthesis, stomatal conductance, transpiration, 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.

TABLE 1 The basic nutrient solution composition (mg/L)

N	P	K	Ca	Mg	S	Fe	Zn	Cu	Mo	Mn	B
160	42	239	152	38	40	1.7	0.6	0.2	0.1	1.2	0.8

MATERIALS AND METHODS

Experimental Setup and Treatments

The experimental set up was an opened sand and perlite (1:1 volume) hydroponic system. This inert, sustain and relatively inexpensive growth 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 $25 \pm 5^{\circ}$C with an approximately 14-h natural photoperiod and 300-600 μmol m^{-2} s^{-1} Photosynthetic Photon Flux Density (PPFD) measured at the top of the canopy. Ten days after emergence (DAE), uniform plants based on leaf number, were selected and thinned to a single main stem per pot.

The basic nutrient solution (Table 1) was contained in a 1000 L reservoir tank inside the greenhouse in a dark chamber to avoid alga growth. Plants were irrigated with basic nutrient solution 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^{-1} respectively (Farran and Mingo-Castel, 2006). The EC of drainage was measured every week and when the drainage EC just passed 3 mS cm^{-1}, the growth media was irrigated with well water (EC 0.5 mS cm^{-1}) to reduce the salinity of substrate (Novella et al., 2008).

A $2 \times 2 \times 2$ factorial experiment based on completely randomized design with four replications was conducted. Each plot included four pots. The experimental treatments included N (N_1: constant consumption of 160 ppm N through the growth period, N_2: constant consumption of 160 ppm N until 65 DAE followed by 0 ppm N for 10 days), P (P_1: constant consumption of 42 ppm P through the growth period, P_2: constant consumption of 42 ppm P until 65 DAE followed by 84 ppm P for 10 days) and pH (pH_1: constant 6 through the growth period, pH_2: intermittent reduction of pH to 3.5: three times for two hours). Use of basic nutrient solution (Table 1) continued until 65 DAE. Between 65 DAE to 75 DAE, the nutrition system was turned off and the 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 sulfuric acid (H_2SO_4) and then applied to related plots on 73 DAE for two hours.

After two hours, the growth media in these pots was washed with normal well water (7.2 pH). After two hours pH treatment was repeated two times.

MEASUREMENTS

Leaf Gas Exchange

Leaf gas exchange (net photosynthesis rate, leaf stomatal conductance, and rate of transpiration) was measured at 75 DAE (at the end of nutritional and pH treatments) using a portable CI-340 Ultra-Light Photosynthesis System (CID Bioscience Inc., Camas, WA, 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 μmol m^{-2} s^{-1}. The external carbon dioxide concentration and the leaf temperature varied between 303–346 μmol mol^{-1} and 25–27°C, respectively.

Plant Growth Characteristics

At 75 DAE, one plant from each plot was harvested and separated into leaves, stems, roots and stolons. Length of stems and stolons was measured. Leaf and stolon numbers were counted. Leaf area was measured by LI-COR-3100C Leaf Area Meter (LI-COR, Lincoln, NE, USA). Plant parts were rinsed with distilled water three times. Dry weight of leaves, stems, roots, and stolons were determined after oven dried at 70°C until constant weight was reached.

Total N Concentration

The second plant from each plot was harvested and separated into leaves, stems, roots and tubers at 75 DAE. Separated plant parts were thoroughly washed by two dippings of five minutes each, in distilled water, then were dried at 105°C and total nitrogen concentration was determined using macro-Kjeldahl method (AOAC, 1984).

ABA and IAA Concentration

In order to analyze ABA and IAA concentration, 75 DAE leaf samples of third plant from each plot were harvested and frozen in liquid nitrogen and stored at -80°C until analysis. Concentrations of ABA and IAA were measured in the leaves of treatments with highest ($N_1P_2pH_1$) and lowest ($N_1P_1pH_1$) tuber number. Leaf samples of these treatments were washed thoroughly with running water, 10 grams of leaf tissue per sample was homogenized with 70% (v/v) methanol and stirred overnight at 4°C. Extracts were passed through a Whatman filter (GE Healthcare, Little Chalfont, UK) and the methanol was removed under reduced pressure at 35åC. The aqueous residue was adjusted to pH 8.5 with 0.2 M potassium and then partitioned two times

against ethyl acetate. After removal of the ethyl acetate phase, the pH of the aqueous residue was adjusted to 2.5 with 0.2 M hydrochloric acid (HCl). The solution was partitioned two times against ethyl acetate. Ethyl acetate was removed under reduced pressure and the newly obtained residue solved in 0.5 mL methanol immediately. The solution was filtered by 45% polytetrafluoroethylene filter and then injected into a high performance liquid chromatography (HPLC) C_{18} column and eluted with a linear gradient of methanol (100%), containing 0.2% acetic acid at a flow rate of 0.7 mL min^{-1}.

Tuber Characteristics

The fourth plant from each plot was harvested 90 DAE. The number of tubers was counted. For dry weight determination, the surface of randomized selected tubers cracked, these tubers oven-dried at 70°C until constant weight was reached.

Statistical Analysis

SAS software (SAS Institute, Cary, NC, USA) 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 the Least Significant Difference test (LSD).

RESULTS

Leaf Gas Exchange

Net photosynthesis of potato plants was neither affected by nitrogen interruption nor pH intermittent reduction (Table 2). However, increased phosphorus concentration resulted in net photosynthesis increment (Figure 1A).

TABLE 2 Summary of variance analysis for leaf gas exchange of potato plants under nutritional and pH treatments

	Mean square		
	Net photosynthesis rate	Rate of transpiration	Stomatal conductance
N	19.14 ^{ns}	0.71 ^{ns}	17415.84*
P	81.63*	0.12 ^{ns}	602.47 ^{ns}
pH	5.21 ^{ns}	0.79 ^{ns}	3229.06 ^{ns}
N × P	23.82 ^{ns}	0.03 ^{ns}	2178.16 ^{ns}
N × pH	21.07 ^{ns}	3.78 *	2982.42 ^{ns}
P × pH	5.14 ^{ns}	0.07 ^{ns}	2172.88 ^{ns}
N × P × pH	5.45 ^{ns}	1.37 ^{ns}	1016.21 ^{ns}

*,** indicates significant difference at $P < 0.05$ and $P < 0.01$, respectively. ns: non-significant. N, P and pH indicate nitrogen, phosphorus and pH respectively.

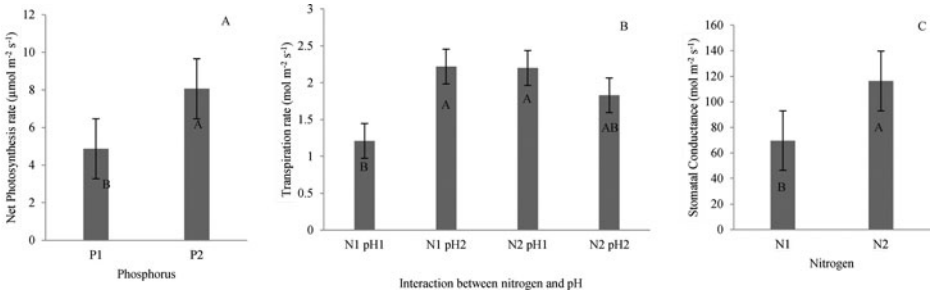

FIGURE 1 Effect of nutritional and pH treatments on leaf gas exchange of potato plants.

There was a significant ($P < 0.05$) interaction between nitrogen and pH for the rate of transpiration (Table 2). Under the normal nitrogen nutrition (N_1), transpiration rate was significantly higher in pH_2 compared to the normal pH_1, whereas under the nitrogen interruption (N_2), transpiration rate was not significantly different between pH levels (Figure 1B).

Stomatal conductance was affected only by nitrogen treatment (Table 2). Nitrogen interruption (N_2) significantly increased stomatal conductance from 69.63 to 116.29 mol m^{-2} s^{-1} (Figure 1C).

Plant Growth Characteristics

Stem and leaf dry weights and leaf numbers were not affected by any of the nutritional and pH treatments (Table 3). However, stem length was reduced by pH reduction from 88.12 cm to 83.62 cm (Figure 2A). Significant interaction between phosphorus and pH with respect to leaf area was observed (Table 3). Under normal phosphorus nutrition (P_1), leaf area of potato plants was increased by pH reduction, while under the increased phos-

TABLE 3 Summary of variance analysis for plant growth characteristics under nutritional and pH treatments

	Mean square				
	Stem dry weight	Leaf dry weight	Stem length	Leaf number	Leaf area
N	0.1069 ^{ns}	0.0190 ^{ns}	60.50 ^{ns}	4.50 ^{ns}	284823.75 ^{ns}
P	0.2610 ^{ns}	0.2346 ^{ns}	28.12 ^{ns}	2.12 ^{ns}	2831.28 ^{ns}
pH	0.1365 ^{ns}	2.3871 ^{ns}	162.00*	1.15 ^{ns}	46436.28 ^{ns}
N × P	0.4875 ^{ns}	0.0338 ^{ns}	10.12 ^{ns}	3.12 ^{ns}	157.53 ^{ns}
N × pH	0.3220 ^{ns}	0.0242 ^{ns}	84.50 ^{ns}	0.50 ^{ns}	136633.78 ^{ns}
P × pH	0.6873 ^{ns}	2.0808 ^{ns}	21.12 ^{ns}	18.00 ^{ns}	1132136.28*
N × P × pH	0.1667 ^{ns}	2.2578 ^{ns}	66.12 ^{ns}	10.12 ^{ns}	251163.28 ^{ns}

*,** indicates significant difference at $P < 0.05$ and $P < 0.01$, respectively. ns: non-significant.
N, P and pH indicate nitrogen, phosphorus and pH respectively.

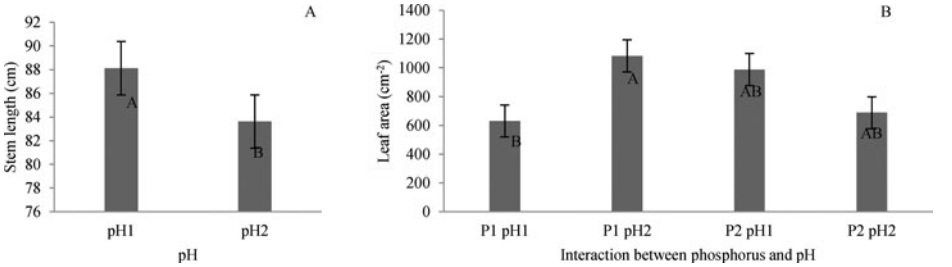

FIGURE 2 Effect of intermittent reduction of pH on A) stem length and B) interaction between phosphorus and pH on leaf area of potato.

phorus concentration (P_2), leaf area was not significantly different between pH levels (Figure 2B).

Plant Underground Characteristics

Intermittent reduction of pH decreased root dry weight from 0.86 g to 0.63 g (Figure 3A). Stolon dry weight was not affected by any of the nutritional and pH treatments (Table 4). However stolon length was influenced only by nitrogen treatment (Table 4). Nitrogen interruption (N_2) resulted in 27.5% reduction in stolon length (Figure 3B). There was not only a significant interaction effect on stolon number between N and pH, but there was also a highly significant effect of nitrogen supply (Table 4). Under the normal nitrogen nutrition (N_1), intermittent reduction of pH (pH_2) significantly lowered stolon numbers, while under the nitrogen interruption (N_2), stolon numbers of potato plants were not affected by pH treatment (Figure 3C).

Tuber numbers and tuber dry weight were the main characteristics of potato plants evaluated in this research. Tuber number per plant was significantly increased with an increase in phosphorus concentration (Figure 3D). However the effect of other nutritional and pH treatments on this trait was not significant (Table 4). Tuber dry weight was not affected by any of the nutritional and pH treatments except for N×P×pH interaction. The highest

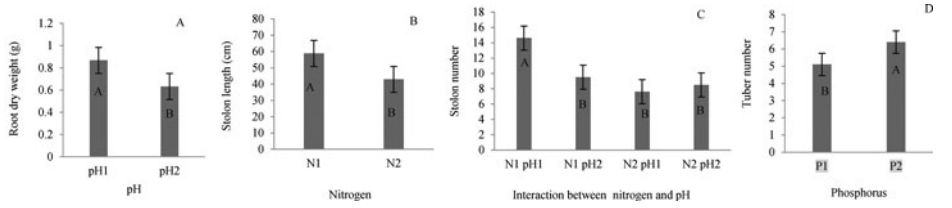

FIGURE 3 Effect of different nutritional and pH treatments on underground growth characteristics of potato plants.

TABLE 4 Summary of variance analysis for underground plant growth characteristics under nutritional and pH treatments

	Mean square					
	Root dry weight	Stolon dry weight	Stolon length	Stolon number	Tuber number	Tuber dry weight
N	0.2000 ns	0.0124 ns	3310.94*	128.00**	3.12 ns	383.57 ns
P	0.0750 ns	0.0108 ns	126.00 ns	4.50 ns	12.50*	204.07 ns
pH	0.4394*	0.0603 ns	1898.82 ns	36.12 ns	6.12 ns	44.58 ns
N × P	0.0427 ns	0.0282 ns	468.94 ns	15.12 ns	2.50 ns	14.21 ns
N × pH	0.0116 ns	0.0457 ns	14.44 ns	72.00*	10.12 ns	2.80 ns
P × pH	0.0247 ns	0.0148 ns	689.13 ns	2.00 ns	2.35 ns	72.99 ns
N × P × pH	0.0132 ns	0.0331 ns	532.19 ns	91.12 ns	2.00 ns	1277.27*

*,** indicates significant difference at $P < 0.05$ and $P < 0.01$, respectively. ns: non-significant. N, P and pH indicate nitrogen, phosphorus and pH respectively.

and lowest dry weights of tubers were observed in $N_1P_2pH_1$ and $N_1P_1pH_1$, respectively.

Total N Concentration

According to the variance analyses (Table 5), none of the nutritional and pH treatments affected total nitrogen concentration of potato leaf, stem, or tuber. However, root nitrogen concentration responded significantly to nitrogen and phosphorus treatments; both nutritional treatments (nitrogen interruption and increased phosphorus concentration) increased total nitrogen concentration of roots (Figure 4A and B). Significant differences ($P < 0.05$) among potato plant parts with respect to the total N concentration were observed. Total N concentration of leaves, tubers, stems, and roots were 4.02%, 2.33%, 1.67%, and 0.90%, respectively (Figure 5).

TABLE 5 Summary of variance analysis for total nitrogen concentration in different plant parts under nutritional and pH treatments

	Mean square			
	Leaf	Stem	Root	Tuber
N	0.080ns	0.020 ns	0.126*	0.002 ns
P	0.005 ns	0.001 ns	0.252**	0.004 ns
pH	0.003 ns	0.015 ns	0.015 ns	0.126 ns
N × P	0.182 ns	0.001 ns	0.038 ns	0.256 ns
N × pH	0.030 ns	0.059 ns	0.018 ns	0.015 ns
P × pH	0.430 ns	0.108 ns	0.018 ns	0.002 ns
N × P × pH	0.020 ns	0.145 ns	0.504**	0.001 ns

*,** indicates significant difference at $P < 0.05$ and $P < 0.01$, respectively. ns: non-significant. N, P and pH indicate nitrogen, phosphorus and pH respectively.

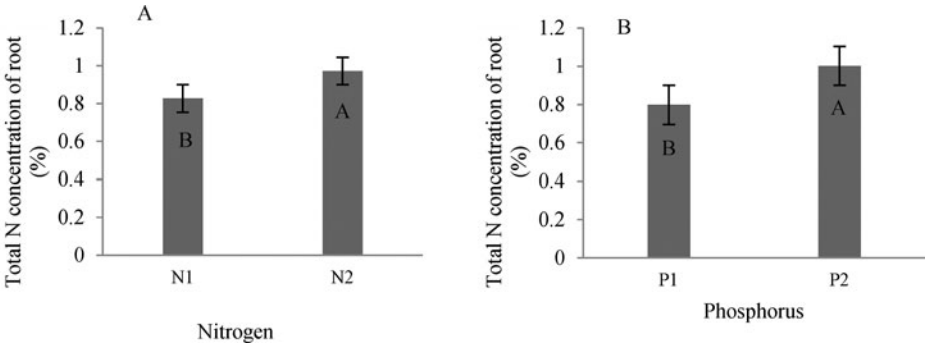

FIGURE 4 Total N concentration of potato root under A) nitrogen and B) phosphorus nutrition.

ABA and IAA Concentrations

The concentration of ABA and IAA was only influenced by phosphorus concentration. In selected plots ($N_1P_1pH_1$ and $N_1P_2pH_1$); higher phosphorus concentration in nutrient solution increased both hormones (ABA and IAA) concentration in potato plant leaves (Figure 6).

DISCUSSION

Leaf Gas Exchange

Photosynthesis is the process of converting light to chemical energy and storing it in the bonds of sugar. Plants need light energy, carbon dioxide (CO_2) and water (H_2O) to make sugar. The deference between photosynthesis and respiration termed net photosynthesis was measured in our research. The observed higher net photosynthesis rate of P_2 as compared to the P_1 revealed that the increasing of phosphorus concentration in nutrient solution positively affects the potato plants net photosynthesis rate. There were likely two reasons for increased net photosynthesis rate by phosphorous.

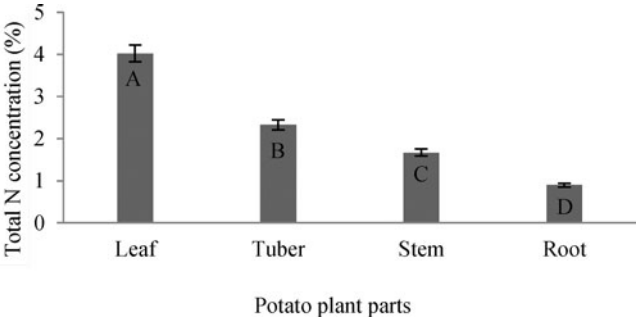

FIGURE 5 Total N concentration of different potato plant parts.

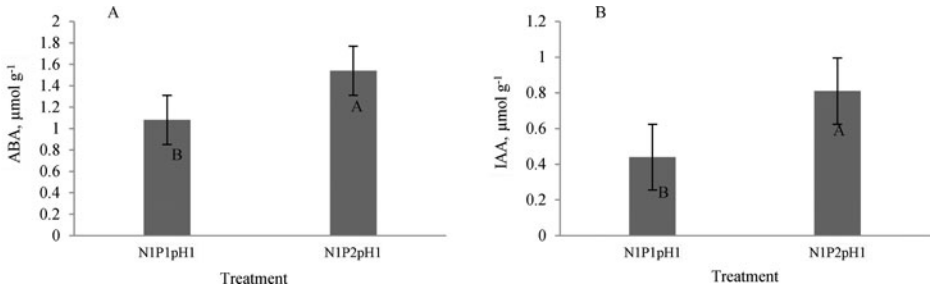

FIGURE 6 Comparison of A) ABA and B) IAA concentrations in the leaves of treatments with highest ($N_1P_2pH_1$) and lowest ($N_1P_1pH_1$) tuber number.

First, this nutrient plays an important role in photosynthesis and intermediary metabolism. Phosphorous in the form of nucleotides such as adenosine triphosphate (ATP) and adenosine diphosphate (ADP), as well as inorganic phosphate (Pi) and phosphorylated sugars also play an integral role in the energy metabolism of cell. Second, Phosphorus, which is especially important in promoting early crop growth (Jenkins and Ali, 1999), can increase tuber set of potato (Freeman et al., 1998; Jenkins and Ali, 2000; Sanderson et al., 2003). Therefore, it is postulated that the promoted early potato crop growth and increased tuber set by phosphorus (Figure 3D) 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, it is probably an increased demand for assimilates in the sinks that causes the rate of net photosynthesis to be increased. Therefore, sink-to-source ratios change during development, which implies that assimilate production must be adjusted to the changing needs of distant tissues (Vreugdenhil et al., 2007).

Net photosynthesis rate of potato plants was not significantly influenced by other treatments (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. However, there was no N effect on leaf area in the current study. Marshall and Vos (1991) suggested that an increasing proportion of leaf nitrogen was not associated with the performance of the photosynthetic system. The present findings are in contrast with previous observations of Chang et al. (2008), who reported that photosynthetic rates of potato plants decreased by 10 days of nitrogen interruption.

As previously mentioned, there was a significant interaction between nitrogen and pH for the rate of transpiration. Since the media was washed three times at intermittent pH reduction (pH_2), it was moistened amply in the pots. Therefore, under normal nitrogen nutrition (N_1), higher transpiration rate of pH_2 compared to the normal pH_1 was probably due to more water availability of potato plants. However, under nitrogen interruption,

higher stomatal conductance has masked the little difference in transpiration rate of pH_1 and pH_2 (Figure 1B). Therefore, there was not a significant difference between transpiration rate of N_2pH_1 and N_2pH_2.

Plant Growth Characteristics

Among the vegetative growth characteristics studied in this research, stem length of potato plants was significantly reduced by intermittent reduction of pH (Figure 2A). Wan et al. (1994) reported that alteration of solution pH did not reduce vegetative growth of potato plants.

Nutritional treatments did not significantly affect the vegetative plant growth characteristics (Table 3). Therefore, our findings showed that nitrogen withdrawal from nutrient solution and increased phosphorus concentration for 10 days did not affect aboveground vegetative growth of potato plants. It is plausible to postulate that initial supply of nitrogen until 65 DAE provided necessary amount of N for vegetative growth. Secondly, at the same time as removing nitrogen from the solution, plants shifted the assimilate partitioning towards tubers. Therefore, nitrogen withdrawal had not any effect on plants vegetative growth. Increased phosphorus concentration (84 ppm) for 10 days had no toxicity effect on potato plants and did not reduce vegetative growth (Rolot and Seutin, 1999).

Plant Underground Characteristics

Root dry weight, stolon length, and stolon number (underground plant growth characteristics) of potato plants was significantly affected by pH, nitrogen interruption, and interaction between N and pH, respectively (Table 4). Root dry weight was not significantly influenced by nitrogen interruption and increased P concentration. Conversely, Bhargava and Banerjee (1994) and Jami Moeini et al. (2010) reported that potato root dry weight increased at lower concentration of nitrogen. While in this study nitrogen nutrition was only interrupted for 10 days, in previous research, lower nitrogen concentration was applied throughout trial time. Stolon length of potato plants was reduced by nitrogen interruption (Figure 2C). One likely reason is the effect of nitrogen on reorientation of the cortical microtubules in stolon tips. Inducing conditions such as short days or nitrogen interruption reduce gibberellic acid (GA) levels and activities in potato plants (Struik et al., 1999). GA inhibits the reorientation of the cortical microtubules, necessary to enable radial cell expansion to occur in stolon tips (Sanz et al., 1996). Therefore, nitrogen withdrawal stimulated stolon swelling (decreased stolon elongation) by possibly decreasing GA content.

Stolon dry weight was not influenced by any of nutritional and pH treatments (Table 4). Wan et al. (1994) reported stolon dry weight did not differ

significantly among the three pH treatments, yet with intermittent pH reduction, stolons appeared to be shorter and thicker than with higher pH.

Potato tuber initiation is usually controlled by some nutrients, particularly of nitrogen and phosphorus. The present findings showe that tuber numbers of potato plants did not significantly influenced by nitrogen interruption (Table 4). 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 numbers found to be significantly increased by phosphorus fertilization (Figure 3). Rolot and Seutin (1999) increased phosphorus concentration to 160 mg L^{-1} in nutrient solution to induce tuberization in potato plants. Results showed 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 (Nielsen et al., 1998). AGPase is exquisitely sensitive to allosteric regulation being activated by 3PGA and inhibited by Pi (Preiss, 1988). Sowokinos and 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.

In this study, tuber numbers of potato plants did not significantly influenced by intermittent pH reduction (Table 4). On the contrary, tuber initiation was induced in the plants subjected to intermittent pH reductions compared to constant pH 5.5 (Wan et al., 1994). This could be due to a difference in growth media was used in two experiments. In the last study, a recirculating solution culture system was used to grow potato plants, but our experiment was conducted in sand culture.

Total N Concentration

Total nitrogen concentration of potato leaf, stem, and tuber was not affected by any of the nutritional and pH treatments. This is in conformity with the findings of Sattelmacher and Marschner (1979), who observed that after nine 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. On the contrary, mineral analysis of potato cultivar leaves indicated that total nitrogen concentration in potato leaves

from nitrogen interruption treatments decreased by 12-15% compared with those of control plants (Chang et al., 2008).

The effect of other nutritional treatment (increased phosphorus concentration) and intermittent pH reduction of solution on total N concentration of potato plants was not significant. Therefore, our finding showed increased phosphorus concentration from 42 to 84 mg L^{-1} did not affect nitrogen absorption by potato plants. Also intermittent pH reduction of growth media had not significant effect on nitrogen accumulation in potato.

ABA and IAA Concentration

Leaf samples which were harvested in treatments with highest ($N_1P_2pH_1$) and lowest ($N_1P_1pH_1$) tuberization, analyzed for ABA and IAA determination. The results showed that increment of phosphorus concentration increased both hormones (ABA and IAA) concentration in potato plant leaves. In favor of these findings, Krauss and Marschner (1982) reported an increase of ABA level under tuber-inducing conditions. 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.

One likely way for tuber number increasing by P is an increment of ABA and IAA, which play important roles in cessation of stolon elongation and initiation of active cell division respectively (Koda and Okazawa, 1983). The promotive effects of ABA on tuberization appear to be due to the antagonistic effects of ABA and GA (Xu et al., 1998). Such antagonism could be at the level of cortical microtubules, where ABA was shown to promote longitudinal arrays of microtubules and was able to reverse the effect of GA3 on microtubule orientation (Shibaoka, 1993).

CONCLUSIONS

Among studied nutritional treatments (N, P, and pH), increased phosphorus concentration significantly enhanced tuber numbers of potato plants in hydroponic sand culture. This nutrient increased net photosynthesis, ABA, and IAA concentrations 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 $N_1P_2pH_1$.

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