RESEARCH ARTICLE https://doi.org/10.7235/HORT.20210064

Nitrogen and Phosphorus Rates Influence Growth, Flowering, and Nutrient Uptake in *Iris germanica* 'Immortality'

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Received: April 19, 2021
Revised: June 21, 2021
Accepted: July 13, 2021

OPEN ACCESS

HORTICULTURAL SCIENCE and TECHNOLOGY
39(6):726-737, 2021
URL: http://www.hst-j.org

pISSN : 1226-8763
eISSN : 2465-8588

Contribution of the Mississippi Agricultural and Forestry Experiment Station Journal article no. 12755. This work was supported by the Mississippi Agriculture and Forestry Experiment Station; the USDA National Institute of Food and Agriculture Hatch projects MIS-249120, MIS-212050.

Compliance with Ethical Standards (Conflict of Interest)
No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication.

Abstract

In this study, the influence of nitrogen (N) and phosphorus (P) rates on plant growth, flowering, and uptake of essential nutrients was evaluated in container-grown tall bearded (TB) iris (*Iris germanica*) 'Immortality'. Factorial combinations of three N (5, 10, or 15 mM) rates and three P (5, 10, or 15 mM) rates were applied to plants via fertigation twice per week from March to September 2013. Plant height, leaf SPAD reading, and flowering data were collected during the growing season. Plants were harvested in December 2013 to measure dry weight (DW) and analyze concentrations of essential mineral elements. Greater N rates had positive effects on plant height, leaf SPAD reading, flowering performance, and uptake of several essential elements, such as potassium (K), calcium (Ca), and iron (Fe). Phosphorous rate did not significantly affect most of the growth parameters. Greater P rates increased P concentration in leaves and roots and decreased boron (B) concentration in leaves but did not significantly influence net uptake of other nutrients except copper (Cu). The average N:P ratio ranged from 4.7 to 7.5, 2.4 to 4.0, and 6.0 to 8.7 in leaves, roots, and rhizomes, respectively.

Additional key words: different tissues, mineral elements, N:P ratio, potassium, SPAD reading

Introduction

Iris germanica is one of the most popular ornamental perennials of the iris genus due to its showy display with multiple colors. A subclass of *I. germanica* cultivars, called re-blooming iris, blooms more than once per growing season, providing a prolonged flowering period and greater ornamental value. However, there is limited information about the management of nitrogen (N) and phosphorus (P) for optimizing the growth of re-blooming iris.

Plant growth is often limited by N and P availability (Vitousek et al., 2002; Iversen et al., 2010). N is the most commonly used mineral nutrient in plants; about 1 − 5% of total plant dry matter consists of N. N plays a pivotal role as a constituent of proteins, nucleic acids, chlorophyll, and other compounds (Marschner, 2012). Greater N fertilization rates can significantly promote shoot growth

(Wang, 1996; Dong et al., 2004; Bi et al., 2007; Scagel et al., 2011).

P is another essential macronutrient that works as a structural element in nucleic acids and phospholipids and plays an important role in energy transfer. Usually, P is associated with root growth during plant development (Zhang et al., 2002; Graciano et al., 2006; Ristvey et al., 2007).

Plant growth and productivity can be affected by the balance between nutrients (Ingestad, 1991; Dighton et al., 1993; Graciano et al., 2006; Hasanuzzaman et al., 2012). Usually, N fertilization can increase extracellular phosphatase activity and hence stimulate P uptake (Fujita et al., 2010). On the other hand, N uptake efficiency was increased by increasing P availability (Iversen et al., 2010). This indicates that the interaction of P and N availability plays an important role in growth-related processes (Cornelissen et al., 1997).

When N availability limits plant growth, uptake of other nutrients is expected to decline accordingly (Marschner, 1995). For example, insufficient N supply caused growth limitation and led to decreased uptake of P, potassium (K), sulfur (S), calcium (Ca), and magnesium (Mg) in azalea (*Rhododendron* L.) (Scagel et al., 2008a). By contrast, increasing N application rate improved uptake of K, Ca, S, and Mg in rhododendron (*Rhododendron* L.) (Scagel et al., 2011). Thus, increased N rate should accompany modified concentrations of other nutrients in a fertilizer formulation to optimize growth (Scagel et al., 2008a).

In *I. germanica*, high N rates had positive effects on the number of inflorescences and inflorescence stem length (Hanley et al., 2008; Zhao et al., 2016), which indicates that high N level may improve the ornamental value of *I. germanica* by producing more flowers. However, excessive N fertilizer will run-off to the soil and pollute the water. Thus, optimal N rates should be determined to protect the environment while optimizing ornamental performance.

N utilization was affected by application level of P in work by Ali (2002), in which a higher P rate increased the N utilization. Thus, P level could influence plant growth or uptake of other nutrients by affecting N utilization. As P availability influences N uptake efficiency, the effects of N on plant growth and development can be accurately examined with sufficient P supply. In this work, factorial combinations of three N rates and three P rates were applied to study the effects of different N and P rates and their interaction on growth, flowering, and uptake of other mineral nutrients in *I. germanica*.

Materials and Methods

Plant Material and Growth Conditions

This study was conducted outdoors in full sun at Starkville, MS (lat. 33°46' N, long. 88°82' W). In August 2012, rhizomes (average caliper = 4.7 cm and length = 5.8 cm) of 'Immortality' Tall bearded iris (Schreiner's Iris Gardens, Salem, OR) were potted into 3.78-L (23-cm diameter; 16-cm height) round plastic pots with one rhizome per pot. The pots were filled with commercial substrate with no starter fertilizer (Fafard 2; Sun Gro Horticulture, Agawam, MA). Two weeks after transplanting, each plant was fertigated with 400 mL of nutrient solution with N-free fertilizer (1.06 $mg·mL^{-1}$; Cornell No N Formula 0-6-27; Greencare Fertilizers, Kankakee, IL) plus 10 mM N from NH_4NO_3 (Sigma Aldrich, St. Louis, MO) twice per week from August through September in 2012 to provide basic nutrients for fall growth.

Experiment Treatments

In April 2013, five plants were harvested for baseline biomass and nutrient composition before spring fertigation treatment started. From April to September 2013, nine N and P rate combinations using a 3 by 3 factorial treatment design were applied twice per week to plants with 20 replicates under each treatment in a completely randomized experimental design. The three rates of N and P were 5, 10, or 15 mM, and each treatment was designed to allow for only N or P rate to change while all other nutrients were held constant, except for chlorine (Cl^-) (different concentrations of KCl were used to maintain a constant concentration of K) Table 1. Other micronutrients [Fe (0.1 mM); Mn (0.01 mM); zinc (Zn, 10^{-3} mM); Cu (10^{-3} mM); B (0.05 mM)] were also added to nutrient solutions. Analytical grade chemicals were used to make fertilizer solutions.

Experiment Methods

During the growing season, flowering data (number of inflorescences and inflorescence stem length), plant height (average height of top three cluster of leaves), and relative leaf chlorophyll content measured as SPAD readings were collected. The leaf SPAD readings were taken using a chlorophyll meter (SPAD-502; Minolta Camera Co., Japan), with one of the first two fully expended leaves selected to measure SPAD.

In December 2013, four plants from each treatment were randomly selected and destructively harvested (senesced leaves were removed). During harvesting, numbers of clustering leaves (cluster of leaves) and floral meristems (FMs) visible to the naked eye were counted. Each plant was divided into leaves, roots, and rhizomes. All samples were dried in a 60°C oven until they reach a constant weight and then samples were ground to pass a 40-mesh sieve in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) for nutrient analysis.

Tissue N was determined by the Kjedahl method (Schuman et al., 1973), and concentrations of other macronutrients and micronutrients in plant tissues were obtained using inductively coupled plasma optical emission spectroscopy (ICP-OES) at the Soil Testing Lab of Mississippi State University. Nutrient content of each tissue was calculated by multiplying dry weight by concentration. Total plant content of each nutrient was calculated by the sum of the content in leaves, roots, and rhizomes. Nutrient uptake was estimated by subtracting the average total nutrients in April 2013 from the average total nutrients in December 2013.

In the following spring (2014), the remaining four plants from each treatment combination were grown outdoors without any fertilizer. In spring 2014, flowering (number of inflorescences and inflorescence stem length), plant height (average height of top three clustering leaves), and leaf SPAD reading data were collected.

Table 1. Chemicals used (in mM) to prepare fertilizer solutions with various nitrogen (N):phosphorous (P) ratios

Chemicals composition (mM)	N:P ratios in fertilizer								
	5:5	5:10	5:15	10:5	10:10	10:15	15:5	15:10	15:15
NH_4NO_3	2.5	2.5	2.5	5	5	5	7.5	7.5	7.5
KH_2PO_4	5	10	15	5	10	15	5	10	15
KCl	10	5	0	10	5	0	10	5	0

Statistical Analysis

The experiment was conducted in a completely randomized design with factorial arrangement of treatments. N rate (three levels) and P rate (three levels) served as the two main factors. Continuous response data were analyzed using linear models with the GLM procedure of SAS (version 9.3; SAS Institute, Cary, NC), and count data were analyzed using generalized linear mixed models with the GLIMMIX procedure of SAS. If the interaction term was not significant, main effects are reported and discussed; if the interaction was significant, simple effects (the effect of a variable at each level of the other variable) are reported and discussed. Mean comparisons were made using Tukey's honestly significant difference (HSD) at $p < 0.05$.

Results and Discussions

Effects of N and P on Plant Growth

In this study, an increasing N rate had positive effects on plant height in June 2013 (approximately 3 months after treatment); SPAD readings were higher in plants with 10 and 15 mM N treatment compared to those with 5 mM N in June 2013 (Table 2). Plant height was measured with a ruler, and SPAD value is an indicator of the greenness or relative

Table 2. Plant height and SPAD reading of container-grown *Iris germanica* 'Immortality' affected by nitrogen (N) (5, 10, or 15 mM) and phosphorus (P) (5, 10, or 15 mM) rates from March to September 2013

Treatments (mM)	Plant height (cm)		SPAD reading	
	June 2013	March 2014	June 2013	March 2014
5N:5P	56	21	45.2	73.0 bc
5N:10P	49	25	50.0	74.6 abc
5N:15P	53	26	46.4	73.9 abc
10N:5P	55	26	50.4	73.1 bc
10N:10P	57	28	51.0	71.8 c
10N:15P	57	25	54.9	77.4 a
15N:5P	61	27	53.2	77.2 a
15N:10P	59	28	52.8	75.6 ab
15N:15P	61	26	52.8	73.0 bc
Main effects of N rates (mM)				
5	53 c[z]	24 b	47.2 b	
10	57 b	27 a	52.1 a	
15	60 a	27 a	52.9 a	
Significant differences				
N	***[y]	*	***	NS
P	NS	NS	NS	NS
N × P	NS	NS	NS	*

[z]Means within a column followed by different lowercase letters denote significant differences affected by the N rate main effect or by the interaction between N and P rate indicated by Tukey's honestly significant difference, $p < 0.05$.
[y]NS, ***, **, * Nonsignificant or significant at $p < 0.001$, 0.01, or 0.05, respectively.

chlorophyll content of leaves. In this study, the results showed that N rates had positive effects on plant height in June 2013 (with treatments of about 3 months); SPAD readings were higher in plants with 10 and 15 mM N treatment compared to 5 mM N treatments in June 2013 (Table 2).

In March 2014, the plant height and SPAD value were measured to reveal the effects of storage nutrients from last year on the plant growth in early spring. In March 2014, plant height was increased by N rates of 10 and 15 mM compared to 5 mM treatment, whereas SPAD readings were affected by the interactions between N and P rate (Table 2).

Effects of N and P on Plant Flowering Performance

In December 2013, the numbers of clustering leaves and FMs were affected by N rate but not by P rate (Table 3). The numbers of clustering leaves and FMs under N rates of 10 and 15 mM were greater than that with 5 mM.

Compared to the 5 mM N treatment rate, 10 and 15 mM treatments also increased the diameter and length of the largest axillary rhizome (Table 3). This is supported by research with Siam tulip (*Curcuma alismatifolia*), in which increasing N rates increased rhizome size (Ruamrungsri and Apavatjrut, 2003). Craver and Harkess (2012) indicated that floral initiation was related to rhizome size, and larger rhizomes were more likely to initiate flowering. Considering that the rhizome size was increased by the 10 and 15 mM rates, the greater number of FMs and rhizome sizes might be positively

Table 3. Flowering data including number of clustering leaves (cluster of leaves) and floral meristems (FMs), and size of rhizome (RZ) of container-grown *Iris germanica* 'Immortality' in 2013 and 2014. Plants were treated with combinations of nitrogen (N) (5, 10, or 15 mM) and phosphorus (P) (5, 10, or 15 mM) from March to September 2013. Number of clustering leaves and FMs, and size of RZ were counted or measured in December 2013 after harvest

Treatments (mM)	Flowering in 2013[z]		Flowering in 2014		Clustering leaves/plant (no.)	FM/plant (no.)	Diameter of RZ(cm)	Length of RZ (cm)
	Stems/plant (no.)	Stem length (cm)	Stems/plant (no.)	Stem length (cm)				
5N:5P	0.7	54	0.4	35	16	3.0	2.8	3.4
5N:10P	1.3	60	0.8	42	14	2.5	2.4	3.0
5N:15P	1.0	60	0.2	40	19	2.8	2.5	3.9
10N:5P	1.2	55	1.4	48	22	3.3	3.0	4.5
10N:10P	1.0	58	0.4	46	18	5.3	3.2	3.7
10N:15P	0.9	57	0.6	43	21	4.3	3.1	4.1
15N:5P	1.1	60	0.6	37	24	5.3	3.1	4.0
15N:10P	1.4	61	0.6	42	20	4.3	3.1	4.3
15N:15P	1.0	62	0.4	40	25	4.0	3.0	4.4
Main effects of N rates (mM)								
5				39 b[z]	16 b	2.8 b	2.5 b	3.4 b
10				46 a	20 a	4.3 a	3.1 a	4.1 a
15				40 b	23 a	4.5 a	3.1 a	4.2 a
Significant differences								
N	NS[y]	NS	NS	*	***	*	**	*
P	NS	NS	NS	NS	NS	NS	NS	NS
N × P	NS	NS	NS	NS	NS	NS	NS	NS

[z]Means within a column followed by different lowercase letters denote significant differences affected by the N rate main effect or by the interaction between N and P rate indicated by Tukey's honestly significant difference, $p < 0.05$.

[y]NS, ***, **, * Nonsignificant or significant at $p < 0.001$, 0.01, or 0.05, respectively.

related in this study.

In spring 2013, neither N nor P rate affected the number of inflorescences (presented as stems/plant) and inflorescence stem length (Table 3). Since the flowers bloomed in late April, which is less than 1 month from when the fertilization treatments started, none of the effects of N or P treatments on florescence were due to a short treatment period.

In spring 2014, inflorescence stems were longer with 10 mM N than with 5 or 15 mM N. Neither N nor P rate affected the number of inflorescences (Table 3). The numbers of FMs were 2.8, 4.3, and 4.5 per plant with 5, 10, and 15 mM N, respectively, in December 2013, which had great potential to flower in the spring. The numbers of flower stems of each plant in spring 2014 were much lower than the number of FMs in December 2013. It is possible that storage nutrients from 2013 were not sufficient to support all the FMs to blooming in spring 2014, even though those FMs initiated in fall 2013.

Effect of N and P on concentration of macro-nutrients in December 2013

Concentrations of N, P, and Mg in leaves (Table 4); concentrations of P, K, and Mg in roots (Table 6); and concentrations of N, P, Mg, and Ca in rhizomes were affected by treatments in December 2013 (Table 8).

Table 4. Macro-nutrient concentrations on a dry weight basis in leaves of container-grown *Iris germanica* 'Immortality'. Plants were treated with combinations of nitrogen (N) (5, 10, or 15 mM) and phosphorus (P) (5, 10, or 15 mM) from March to September 2013

Treatments (mM)	N[z] (%)	P (%)	K (%)	Ca (%)	Mg (%)	N:P
5N:5P	1.85	0.34[y]	5.05	0.94	0.58	5.8
5N:10P	1.78	0.24	4.41	1.04	0.56	7.5
5N:15P	2.23	0.41	3.72	1.18	0.66	5.6
10N:5P	2.11	0.44	4.47	1.25	0.68	4.8
10N:10P	2.38	0.41	4.52	1.06	0.58	5.9
10N:15P	2.25	0.45	4.65	0.98	0.59	5.1
15N:5P	2.42	0.36	4.28	0.85	0.50	7.2
15N:10P	2.26	0.35	4.13	1.08	0.55	6.5
15N:15P	2.08	0.45	3.88	1.02	0.50	4.7
Main effects of N rate (mM)						
5	1.95 b[y]	0.33 b			0.60 a	6.33 a
10	2.24 a	0.43 a			0.62 a	5.28 b
15	2.25 a	0.39 ab			0.52 b	6.12 ab
Main effects of P rate (mM)						
5		0.38 ab				5.98 ab
10		0.34 b				6.61 a
15		0.43 a				5.13 b
Significant differences						
N	**[x]	*	NS	NS	**	*
P	NS	*	NS	NS	NS	*
N × P	NS	NS	NS	NS	NS	NS

[z]Nitrogen (N); phosphorous (P); potassium (K); calcium (Ca); magnesium (Mg); N:P concentration (N:P).

[y]Means within a column followed by different lowercase letters denote significant differences (Tukey's honestly significant difference, $p < 0.05$).

[x]NS, ***, **, * Nonsignificant or significant at $p < 0.001$, 0.01, or 0.05, respectively.

N concentrations in rhizomes and leaves of plants treated with 10 and 15 mM N were higher than those with 5 mM N. N concentration was not affected by either N or P rate in roots. The average N concentration in leaves and rhizomes was greater than 2% compared with less than 1% N concentration in roots.

Phosphorus concentration in roots and rhizomes was affected by the main effect of N rate: 10 and 15 mM N increased P concentration compared with 5 mM N. In leaves, P concentration was influenced by the main effects of N and P rate separately without interaction.

In roots, K concentration was highest in treatment with 5 mM N and 5 mM P, which means higher N and P rates decreased root K concentration (Table 6). In leaves and rhizomes, concentrations of Mg were influenced by the main effects of N rate. Interestingly, general trends of Mg concentration decreased with higher N rates in leaves and increased in rhizomes.

In this study, tissue N:P ratio varied from 4.7 to 7.5, 2.4 to 4, and 6 to 8.7 in leaves, roots, and rhizomes, respectively, under different N and P treatments (Tables 4, 6, and 8). In leaves and roots, higher P rate decreased tissue N:P ratio, but N rate had no influence on N:P ratio. In rhizomes, neither N nor P rate affected tissue N:P ratio.

Table 5. Micro-nutrient concentrations on a dry weight basis in leaves of container-grown *Iris germanica* 'Immortality' in December 2013. Plants were treated with combinations of nitrogen (N) (5, 10, or 15 mM) and phosphorus (P) (5, 10, or 15 mM from March to September 2013

Treatments (mM)	Fe[z] (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)
5N:5P	40.5	12.5	29.3 ab[y]	3.5	36.8
5N:10P	36.5	8.8	20.5 c	2.3	47.5
5N:15P	39.5	12.8	30.3 a	6.0	33.3
10N:5P	37.5	11.3	30.0 a	5.5	31.3
10N:10P	47.8	13.3	33.3 a	5.3	45.8
10N:15P	47.0	14.8	32.8 a	5.0	34.8
15N:5P	49.7	15.5	32.5 a	7.5	39.3
15N:10P	39.8	15.5	26.8 abc	7.5	38.0
15N:15P	48.3	12.5	21.8 bc	9.0	19.8
Main effects of N rates (mM)					
5				3.9 b	
10				5.3 b	
15				8.0 a	
Main effects of P rates (mM)					
5					36.8 ab
10					44.8 a
15					29.3 b
Significant differences					
N	NS[x]	NS	**	**	NS
P	NS	NS	NS	NS	**
N × P	NS	NS	*	NS	NS

[z]Iron (Fe); manganese (Mn); zinc (Zn); copper (Cu); boron (B).

[y]Means within a column followed by different lowercase letters denote significant differences (Tukey's honestly significant difference, $p <$ 0.05).

[x]NS, ***, **, * Nonsignificant or significant at $p < 0.001$, 0.01, or 0.05, respectively.

Effect of N and P on Concentration of Micro-nutrients in December 2013

The N and P treatments had effects on concentrations of only a few micro-nutrients. Concentrations of zinc (Zn), copper (Cu), and boron (B) in leaves (Table 5), concentrations of Zn and Cu in roots (Table 7), and concentrations of iron (Fe) and Cu in rhizomes (Table 9) were affected by treatments.

Iron concentrations in leaves and roots were affected by neither N nor P treatment. In rhizomes, Fe concentration was the highest in plants receiving 10 mM N (Table 9). In leaves and roots, concentrations of Zn were influenced by the interaction of N and P treatments (Tables 4 and 8). Concentration of Cu was only affected by the main effects of N rate in leaves and interaction of N and P treatments in roots and rhizomes (Tables 5, 7, and 9).

Effect of N and P on Net Nutrient Uptake of iris 'Immortality' in December 2013

In this study, net uptakes of N, P, Mg, Mn, Zn, and B were only affected by the main effects of N rates. Uptake of K, Ca, and Fe was affected by neither N nor P rate. Phosphorus rate had no influence on the uptake of most nutrients, except for Cu, which was affected by the interaction between N and P rate (Table 10).

Table 6. Macro-nutrient concentrations in roots of container-grown *Iris germanica* 'Immortality' in December 2013. Plants were treated with combinations of nitrogen (N) (5, 10, or 15 mM) and phosphorus (P) (5, 10, or 15 mM) from March to September 2013

Treatments (mM)	N^z (%)	P (%)	K (%)	Ca (%)	Mg (%)	N:P
5N:5P	0.75	0.21	4.09	0.29	0.19 b	3.6
5N:10P	0.76	0.24	3.73	0.31	0.20 b	3.3
5N:15P	0.73	0.30	3.41	0.32	0.20 ab	2.4
10N:5P	0.75	0.19	3.25	0.29	0.18 bc	4.0
10N:10P	0.79	0.32	3.79	0.34	0.22 a	2.6
10N:15P	0.77	0.31	2.61	0.32	0.20 ab	2.5
15N:5P	0.79	0.20	3.54	0.31	0.19 b	3.9
15N:10P	0.92	0.27	3.23	0.32	0.18 bc	3.4
15N:15P	0.82	0.29	3.12	0.28	0.16 c	2.8
Main effects of N rates (mM)						
5			3.7 a^y			
10			3.3 b			
15			3.2 b			
Main effects of P rates (mM)						
5		0.20 b	3.63 a			3.9 a
10		0.27 a	3.58 a			3.1 b
15		0.30 a	3.05 b			2.6 c
Significant differences						
N	NS^x	NS	*	NS	**	NS
P	NS	***	**	NS	NS	***
N × P	NS	NS	NS	NS	**	NS

^{z}Nitrogen (N); phosphorous (P); potassium (K); calcium (Ca); magnesium (Mg); N:P concentration (N:P).

^{y}Means within a column followed by different lowercase letters denote significant differences (Tukey's honestly significant difference, $p <$ 0.05).

^{x}NS, ***, **, * Nonsignificant or significant at $p < 0.001$, 0.01, or 0.05, respectively.

Table 7. Micro-nutrient concentrations in roots of container-grown *Iris germanica* 'Immortality' in December 2013. Plants were treated with combinations of nitrogen (N) (5, 10, or 15 mM) and phosphorus (P) (5, 10, or 15 mM) from March to September 2013

Treatments (mM)	Fe[z] (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)
5N:5P	49.3	15.5	18.8 ab[y]	3.0 d	15.0
5N:10P	62.3	15.5	14.0 bc	2.8 d	14.0
5N:15P	58.0	14.3	14.5 bc	4.3 cd	13.3
10N:5P	63.3	14.8	13.5 c	4.0 cd	14.0
10N:10P	70.5	16.3	19.5 a	7.3 ab	15.3
10N:15P	65.3	16.3	19.8 a	6.3 bc	13.3
15N:5P	65.7	21.3	20.5 a	9.8 a	14.3
15N:10P	72.0	18.5	18.3 abc	8.5 ab	13.3
15N:15P	48.0	13.0	20.3 a	6.0 bc	16.5
Significant differences					
N	NS[x]	NS	*	***	NS
P	NS	NS	NS	NS	NS
N × P	NS	NS	*	*	NS

[z]Iron (Fe); manganese (Mn); zinc (Zn); copper (Cu); boron (B).

[y]Means within a column followed by different lowercase letters denote significant differences (Tukey's honestly significant difference, $p < 0.05$).

[x]NS, ***, **, * Nonsignificant or significant at $p < 0.001$, 0.01, or 0.05, respectively.

Table 8. Macro-nutrient concentrations in rhizomes of container-grown *Iris germanica* 'Immortality' in December 2013. Plants were treated with combinations of nitrogen (N) (5, 10, or 15 mM) and phosphorus (P) (5, 10, or 15 mM) from March to September 2013

Treatments (mM)	N[z] (%)	P (%)	K (%)	Ca (%)	Mg (%)	N:P
5N:5P	1.59	0.20	1.71	0.46	52.00	8.7
5N:10P	1.52	0.20	1.78	0.46	62.59	7.7
5N:15P	1.61	0.24	1.53	0.47	44.25	7.3
10N:5P	2.15	0.32	1.72	0.48	51.25	6.7
10N:10P	2.20	0.37	1.65	0.57	68.00	6.0
10N:15P	2.18	0.36	1.93	0.47	64.00	6.3
15N:5P	2.51	0.33	1.54	0.45	55.00	8.0
15N:10P	2.30	0.32	1.48	0.39	41.75	7.3
15N:15P	2.46	0.36	1.42	0.39	39.00	7.0
Main effects of N rates (mM)						
5	1.57 b[y]	0.21 b		0.46 ab	0.22 b	
10	2.18 a	0.35 a		0.50 a	0.28 a	
15	2.42 a	0.34 a		0.41 b	0.25 a	
Significant differences						
N	***[x]	***	NS	*	**	NS
P	NS	NS	NS	NS	NS	NS
N × P	NS	NS	NS	NS	NS	NS

[z]Nitrogen (N); phosphorous (P); potassium (K); calcium (Ca); magnesium (Mg); N:P concentration (N:P).

[y]Means within a column followed by different lowercase letters denote significant differences (Tukey's honestly significant difference, $p < 0.05$).

[x]NS, ***, **, * Nonsignificant or significant at $p < 0.001$, 0.01, or 0.05, respectively.

Table 9. Micro-nutrient concentrations in rhizomes of container-grown *Iris germanica* 'Immortality' in December 2013. Plants were treated with combinations of nitrogen (N) (5, 10, or 15 mM) and phosphorus (P) (5, 10, or 15 mM) from March to September 2013

Treatments (mM)	Fe^{z} (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)
5N:5P	52.0	9.0	21.0	7.0 bc^{y}	10.0
5N:10P	62.6	9.8	18.8	5.5 c	9.8
5N:15P	44.3	7.3	17.8	6.0 bc	9.5
10N:5P	51.3	7.8	16.0	5.0 c	9.8
10N:10P	68.0	9.0	23.8	11.3 a	11.5
10N:15P	64.0	13.0	22.5	8.0 abc	11.5
15N:5P	55.0	12.0	21.8	10.0 ab	10.5
15N:10P	41.8	8.0	17.8	8.0 abc	9.5
15N:15P	39.0	11.3	18.5	4.3 c	15.0
Main effects of N rates (mM)					
5	54.0 ab				
10	61.1 a				
15	45.3 b				
Significant differences					
N	*^{x}	NS	NS	NS	NS
P	NS	NS	NS	NS	NS
N × P	NS	NS	NS	*	NS

^{z}Iron (Fe); manganese (Mn); zinc (Zn); copper (Cu); boron (B).

^{y}Means within a column followed by different lowercase letters denote significant differences (Tukey's honestly significant difference, $p < 0.05$).

^{x}NS, ***, **, * Nonsignificant or significant at $p < 0.001$, 0.01, or 0.05, respectively.

Table 10. Net nutrient uptake of container-grown Iris germanica 'Immortality' in December 2013. Plants were treated with combinations of nitrogen (N) (5, 10, or 15 mM) and phosphorus (P) (5, 10, or 15 mM) from March to September 2013

Treatments (mM)	N^{z} (g)	P (g)	K (g)	Ca (g)	Mg (g)	Fe (mg)	Mn (mg)	Zn (mg)	Cu (mg)	B (mg)
5N:5P	0.49	0.06	0.35	0.05	0.03	1.60	0.41	0.90	0.27 cd	0.47
5N:10P	0.44	0.05	0.29	0.05	0.03	1.99	0.39	0.68	0.19 d	0.54
5N:15P	0.49	0.09	0.18	0.05	0.03	1.33	0.33	0.69	0.24 cd	0.41
10N:5P	1.01	0.16	0.63	0.13	0.10	2.47	0.49	0.86	0.26 cd	0.62
10N:10P	0.60	0.12	0.30	0.06	0.05	2.10	0.40	0.84	0.39 bc	0.56
10N:15P	0.93	0.17	0.58	0.09	0.08	3.04	0.78	1.15	0.41 bc	0.68
15N:5P	1.25	0.16	0.49	0.10	0.09	2.70	0.84	1.30	0.63 a	0.71
15N:10P	1.20	0.18	0.54	0.10	0.09	2.09	0.57	1.06	0.51 ab	0.68
15N:15P	1.38	0.22	0.53	0.10	0.09	1.94	0.72	1.14	0.30 cd	0.91
Main effects of N rate (mM)										
5	0.47 c^{y}	0.06 b			0.03 b		0.03 b	0.75 b		0.47 c
10	0.84 b	0.15 a			0.07 a		0.07 a	0.95 b		0.62 b
15	1.28 a	0.18 a			0.09 a		0.09 a	1.17 a		0.77 a
Significant differences										
N	***^{x}	***	NS	NS	**	NS	*	**	***	***
P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
N × P	NS	NS	NS	NS	NS	NS	NS	NS	*	NS

^{z}Nitrogen (N); phosphorous (P); potassium (K); calcium (Ca); magnesium (Mg); iron (Fe); manganese (Mn); zinc (Zn); copper (Cu); boron (B).

^{y}Means within a column followed by different lowercase letters denote significant differences (Tukey's honestly significant difference, $p < 0.05$).

^{x}NS, ***, **, * Nonsignificant or significant at $p < 0.001$, 0.01, or 0.05, respectively.

Discussion

In addition to flowers, the sword-shaped linear leaves of irises give them a high ornamental value. SPAD reading is an indicator of relative leaf chlorophyll content. A higher SPAD reading means healthier and greener leaves. In this study, the effects of N treatment on plant height and SPAD reading indicated that greater N rates promote vegetative growth in iris, which is consistent with previous studies of rhododendron (Bi et al., 2007) and *Curcuma alismatifolia* (Ruamrungsri and Apavatjrut, 2003).

In this study, the P rate did not affect plant height in June 2013 and March 2014. Plant height was affected by the rates of both N and P in maize (Khan et al., 2014). In the bulbous plant gloriosa lily (*Gloriosa rothschildiana*), N did not affect plant height, whereas low P rate reduced plant height (Ruamrungsri et al., 2011). The different responses of plants to changes in P rate indicated that the requirement for P fertilization may vary among plant species.

The P rates used in our study of 155 mg/L (5 mM), 310 mg/L (10 mM), and 465 mg/L (15 mM) were relatively high compared to the 50 and 100 mg/L P rates in Ruamrungsri et al. (2011), suggesting that a 5 mM P rate is likely high enough to satisfy the P requirement for the growth of TB iris 'Immortality'. In many crops, the need for high P rates may have been overemphasized, which causes overapplication of P fertilizer (Wang and Konow, 2002; Ristvey et al., 2007).

N fertilizer usually promotes vegetative growth rather than reproductive growth (Bi et al., 2007; Scagel et al., 2011). In this study, N rates of 10 and 15 mM increased both number of clustering leaves and FMs compared with 5 mM, which means greater N rates also have positive effects on reproductive growth. This result is supported by the research in hybrid *Phalaenopsis* orchids, in which high N rate is necessary for increasing number of inflorescences (Wang, 2000).

N:P ratio has often been used in agriculture and forestry to analyze nutrient limitations (Fenn et al., 1998; Tessier and Raynal, 2003). For instance, in 1-year-old rhododendron (*Rhododendron* L.), N:P ratio was greater than 14:1 without N limitation, and N:P ratio was less than 9:1 when plants were N-deficient (Scagel et al., 2008b). In this study, N:P ratio was lower than 9:1, suggesting N may be the limiting factor to growth, which may explain why most growth-related data were only affected by N rate.

In this study, the uptake of most nutrients was only affected by N rates. This indicates that N altered the uptake ability and demands of the plant for other mineral nutrients. To optimize growth, increasing N rate should accompany modified doses of other nutrients in a fertilizer formulation (Scagel et al., 2008a).

P rate had no influence on nutrient uptake, including plant P uptake, although 15 mM P rate increased P concentration in leaves and roots. One explanation could be the amount of P uptake was more related to DW, which was affected by N rate. In addition, the N:P ratio in our study was low, suggesting plants in this study had a relatively high tissue concentration of P and 5 mM P rate satisfied plant demand.

Conclusions

In summary, plant height, leaf SPAD reading, and flowering performance, including number of FMs and flower stems, were mainly affected by N rate. Relatively high N fertilizer rates are recommended for growing *I. germanica* to obtain high ornamental value. In general, increasing N rates from 5 to 15 mM increased uptake of N, P, Mg, Mn, Zn, and B in TB iris. Thus, fertilization doses of other nutrients should be modified with changes of N rate to optimize plant growth. P rate affected concentrations of P and B in leaf, concentration of P in root, and concentration of Cu in rhizome.

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