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Effect of Soil pH on the Growth, Photosynthesis and Mineral Element Content in Own-Rooted Saplings and Grafted Saplings of Blueberry

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Abstract The adaptation of blueberry to higher soil pH is poor. To explore the effect and probable mechanism of the adaptation of grafted blueberry (*Vaccinium* spp.) saplings to the soil environment with higher pH value. We took three types of saplings, (i) own-rooted Wufanshu (*V. bracteatum*), an economical shrub belonging to the same genus and family with blueberry, also known as oriental blueberry, (ii) own-rooted 'Sharpblue', a southern highbush blueberry (*V. corymbosum*) cultivar, and (iii) grafted-bluebedrry as experimental materials, which were grown in quartz sand medium for 90 d, irrigated with nutrient solution of three levels of pH values, respectively, *i.e.* pH 4.6, pH 5.8 and pH 7.0. Results showed that root development, shoot growth and dry biomass accumulation of grafted blueberry were significantly better than those of own-rooted blueberry grown in higher pH medium; compared with own-rooted blueberry saplings, leaf chlorophyll content, chlorophyll a/b ratio, chlorophyll fluorescence chemical efficiency and photosynthetic efficiency of grafted blueberry saplings grown in higher pH medium were all significantly improved. Results also showed that mineral element content of leaves, especially Mg and Fe elemental content of grafted blueberry was significantly higher than that of own-rooted blueberry saplings grown in higher pH media. Grafting-propagation of blueberry saplings taking Wufanshu as rootstock significantly enhanced the adaptation of blueberry to soil environment with higher pH value.

Key words Blueberry, Wufanshu, Grafting, pH stress, Adaptation

1 Introduction

The fruits of blueberry (*Vaccinium* spp.) are rich in anthocyanin, rhodopsin, niacin and other biologically active components, which can resist cancer and enhance the human immune system. It is one of the five major human health foods listed by FAO[1-2], and it is also one of the species of fruit trees rapidly developed in many countries. However, the blueberry plant root system is fine, the main root is not clearly seen, and the root hairs are rare and very underdeveloped; it is shallowly distributed in the soil, with a small stretch, and the soil physicochemical properties have a much greater impact on its growth and development than on other fruit trees[3-4]. The blueberry has a particular preference for the acidic or strongly acidic soil, the suitable soil pH is 3.8 to 5.0, and many countries in the world use the sulfur powder and other substances to adjust the soil acidity when cultivating the blueberry on the soil with pH of greater than 5.0[5-7]. These eco-physiological characteristics greatly limit the suitable growth range and development areas of blueberry, and also significantly increase the cost of blueberry production. Therefore, to explore a better cultivation method of blueberry saplings to improve the adaptation of blueberry fruit to the soil environment has important significance in blueberry cultivation practice.

Grafting is an ancient worldwide plant technology of infinite vitality, and has irreplaceable value in many aspects of agriculture and forestry[8-10]. China is recognized as the first country in the world to invent grafting technology. Currently, there are few studies on the blueberry grafting breeding and cultivation[11-13], and there is no literature report about the use of grafting to improve the adaptation of plants to soil pH habitat. Wufanshu (*V. bracteatum*), an economic shrub belonging to the same family and genus with blueberry, is uniquely produced throughout southern China. It is a pigment source plant of a famous tourism food "Wumifan" in Jiangsu Wuxi[14], and it has long been used in landscaping in southern China[15], having strong adaptation to the soil physical and chemical habitat.

In this experiment, we took Wufanshu as rootstock for grafting of southern highbush blueberry (Sharpblue), to explore the adaptation of grafted blueberry saplings to high soil pH habitat as well as the possible mechanisms, and reveal the growth and dry matter accumulation, leaf chlorophyll content and composition, chlorophyll fluorescence chemical performance and photosynthetic efficiency, and the difference in the content of major mineral elements in the leaves concerning the blueberry plant on Wufanshu rootstocks, a new plant grafted under the irrigating and cultivation with the nutrient solutions at different pH.

2 Materials and methods

2.1 Materials We took the economic shrub Wufanshu unique-

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ly grown in southern China and belonging to the same family and genus with blueberry as rootstock, took the southern highbush blueberry cultivar Sharpblue as scion, and took the own-rooted Wufanshu saplings, own-rooted blueberry saplings and the grafted blueberry saplings on Wufanshu rootstocks as the test materials.

2.2 Methods

2.2.1 Test methods. In the growing season (early June), from the Wufanshu and Sharpblue blueberry plant cultivated in the cutting orchard, the robust half-lignified shoots were clipped into the 10 cm – 12 cm long stem sections as the shoots for cutting. After 60 min of treatment in the 300 mg/L indole acetic acid solution, the shoots were inserted in the 20 cm high plastic film bag with six holes in the middle and lower part and upper opening diameter of 15 cm. The seedling medium (V/V) was peat: perlite: river sand (1 mm≤grain diameter≤2 mm) = 3:1.5:0.5.

A total of 2 weeks before cutting, 2.5 g of sulfur powder per bag was applied to adjust the pH of medium to (5.0 ± 0.2). The own-rooted saplings were cultivated in the plastic greenhouse, and the greenhouse was covered with a shading net in the first month after cutting. We focused on the temperature and spraying management, and kept the indoor temperature (day/night) 28 (±1.0) ℃/20 (±1.0) ℃ and relative humidity 85 (±2.0)% or above.

After that, it was followed by the regular water and fertilizer management. At the end of October in the same year, the average rooting rate of Wufanshu and blueberry was 88.5% and 82.0%, respectively, and the average height was 21.5 cm and 14.8 cm, respectively. We continued to cultivate one year. In late February of the third year after cutting, two kinds of own-rooted saplings were cut 5 cm to 10 cm from the ground, and some own-rooted Wufanshu saplings with a diameter of more than 6 mm were selected to graft blueberry, and the scions and scion buds were clipped from the full and robust shoots of the southern highbush blueberry cultivar Sharpblue.

The grafting method was single bud scion grafting. The "single bud" for grafting was the bud cut out with some branches, the cutting surface below bud was without xylem, the incision, scion buds and scions were tightly wrapped with the plastic film. We continued to cultivate one year after grafting or cutting, and the method was similar to the conventional breeding method. In late February next year, the well-developed shapely grafted saplings and two kinds of own-rooted saplings were selected for sand culture experiment.

2.2.2 Sand culture experiment. The own-rooted Wufanshu saplings, own-rooted blueberry saplings and grafted blueberry saplings on Wufanshu rootstocks (3-year-old roots, 1-year-old branches) were planted in the PVC plastic pots in mid-February, 1 plant per pot. The lower plots had holes, and the plots were 22 cm tall, with upper opening diameter of 25 cm and lower opening diameter of 18 cm. With quartz sand as medium, the tray was set at the bottom of pots. The cultivation free from rain was conducted in the greenhouse, and it received the natural light.

The 1/2 Hoagland solution (using 0.05 mol/L H_2SO_4 or 0.5 mol/L KOH solution to adjust the pH value to 5.0) was used for irrigating and cultivation until the completely normal growth in mid-May, and the well-developed plants of uniform size were further selected for higher pH habitat treatment. The irrigating liquid was complete Hoagland solution, and three pH treatments including pH 4.6 (simulate strongly acidic soils, CK), pH 5.8 (simulate moderately acidic soils), pH 7.0 (simulate neutral soils) were set, and 6 plants from three kinds of saplings were selected for each treatment, with three replicates.

Depending on weather conditions, the treatment solution was used for irrigating once every 1 d – 2 d, 300 mL of solution was used for watering once per pot, and the quartz sand was rinsed with tap water once every 2 times of irrigating.

2.3 Determination items and methods 90 d after treatment (cultivation) (mid-August, slow growth), the plant height, basal diameter and crown diameter of saplings under the treatments were measured, and the sampling was conducted to analyze the content of N, P, K, Ca, Mg and Fe in the leaf, and the fresh biomass of root, stem and leaf was measured, respectively.

One week before the plant was removed from the pot, the sunny weather was selected to test the leaf chlorophyll content and fluorescence parameters of saplings under each treatment: initial fluorescence (F_o), maximum fluorescence (F_m), PS II primary conversion efficiency of light energy (F_v/F_o). And the gas exchange parameters were also tested: photosynthetic rate (P_n), transpiration rate (T_r) and photosynthetic water use efficiency (*WUE*).

The drying oven was used for drying the root, stem, leaf samples of plant under different treatments, and the constant dry weight of three organs was calculated to get the dry biomass of whole plant. The leaf chlorophyll content was measured according to the method of Arnon[16], and the leaf chlorophyll fluorescence parameters were determined using plant efficiency analyzer. The leaf gas exchange parameters were determined using LI-6400X portable photosynthesis testing system.

As for the determination of the leaf mineral element (including nitrogen), the fourth to sixth mature leaves were selected from the development branches, and five leaves were selected from each seedling under each treatment according to the position, and rinsed with water after washing with 0.1% neutral detergent. After rinsing with deionized water three times, the surface moisture was dried, deactivated for 15 min in the 105 ℃ oven, dried to constant weight at 80 ℃, ground and sieved.

The leaf total nitrogen content was determined by H_2SO_4-H_2O_2 digestion-automatic Kjeldahl method[17]; the total phosphorus content was determined by H_2SO_4-H_2O_2 digestion, Mo-Sb ratio colorimetric method[18]; the K, Ca, Mg, Fe content was determined using the inductively coupled plasma atomic emission spectrometry (ICP-AES)[19].

The *t*-test was used to analyze the difference significance of the test data.

3 Results and analysis

3.1 Effect of cultivation medium pH on the growth of three kinds of grafted blueberry saplings As can be seen from Table 1, after using the nutrient solutions with pH 4.6 (CK), 5.8 and pH 7.0 to irrigate for 90 d, respectively, there were significant differences in the growth response of three kinds of grafted blueberry saplings, and it was in the descending order of own-rooted Wufanshu saplings > grafted blueberry saplings > own-rooted blueberry saplings. With increasing pH value, the plant height, basal diameter and crown diameter of three kinds of saplings were inhibited, especially for the crown diameter.

By the cultivation on the medium with pH 5.8, there were no significant differences in the plant height, basal diameter and crown diameter of own-rooted Wufanshu saplings and grafted blueberry saplings compared with the control, while the differences in these indicators for the own-rooted blueberry saplings were significantly reduced compared with the control. By the cultivation on the medium with pH 7.0, the plant height and basal diameter of own-rooted Wufanshu saplings and grafted blueberry saplings were not significantly inhibited, while the crown diameter was significantly reduced compared with control, but the values of the three indicators for the own-rooted blueberry saplings were significantly lower than in the control.

Table 1 The differences in the plant height, basal diameter and crown diameter among three kinds of saplings in habitat with relatively high pH

Saplings	Treatment	Plant height//cm	Basal diameter//mm	Crown diameter//cm
Own-rooted Wufanshu saplings	pH 4.6	74.7 ±5.0 a	6.7 ±0.5 a	46.0 ±6.0 a
	pH 5.8	73.0 ±7.0 a	6.6 ±0.6 a	43.3 ±5.8 a
	pH 7.0	70.3 ±8.7 a	6.5 ±0.7 a	37.7 ±4.5 b
Own-rooted blueberry saplings	pH 4.6	45.0 ±5.0 a	6.2 ±0.7 a	38.3 ±4.0 a
	pH 5.8	38.3 ±7.6 b	5.4 ±0.5 b	29.0 ±2.6 b
	pH 7.0	26.2 ±7.5 c	5.0 ±0.4 b	20.6 ±2.9 c
Grafted blueberry saplings	pH 4.6	60.2 ±5.5 a	6.9 ±0.5 a	48.0 ±5.3 a
	pH 5.8	58.6 ±7.0 a	6.7 ±0.6 a	44.7 ±5.0 a
	pH 7.0	54.4 ±6.2 a	6.2 ±0.6 a	40.2 ±5.0 b

Note: The different lowercase letters behind the same column of data about the same kind of saplings indicated significant difference ($P<0.05$).

Similar to the response of plant height, basal diameter, crown diameter, by the cultivation on two media with pH 5.8 and pH 7.0, the root, stem, leaf biomass of own-rooted Wufanshu saplings and grafted blueberry saplings was slightly reduced, not significantly different from the control (pH 4.6); the leaf biomass of own-rooted blueberry saplings was significantly lower than in the control, and the difference reached a significant level.

Meanwhile, because of additive effects, the whole plant biomass of three kinds of saplings had more significant response to the cultivation medium pH, the own-rooted Wufanshu saplings and grafted blueberry saplings cultivated on the medium with pH 7.0 were significantly different from the control, while the own-rooted Wufanshu saplings and grafted blueberry saplings cultivated on the medium with pH 5.8 were not significantly different from the control; the own-rooted blueberry saplings cultivated on the media with pH 5.8 and pH 7.0 were significantly different from the control, and there was a significant pairwise difference.

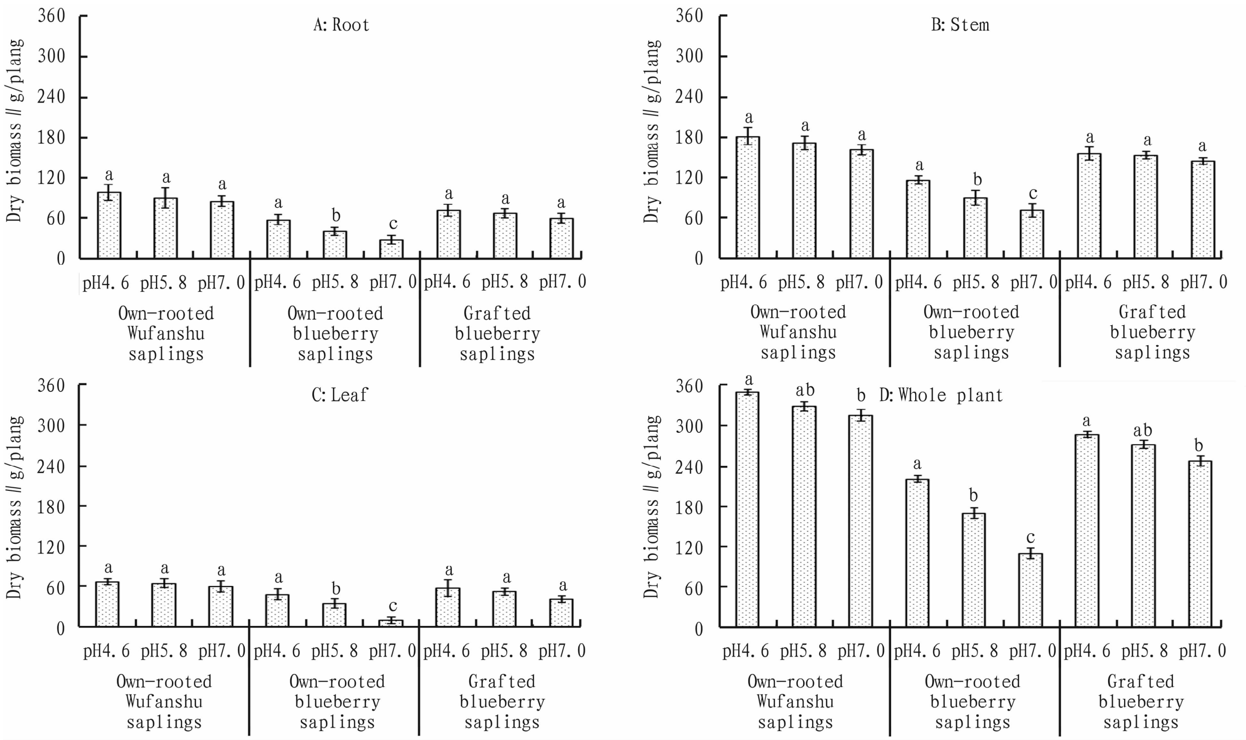
The biomass measurement results further showed that the adaptation of grafted blueberry saplings with Wufanshu as rootstock to high pH habitat was significantly enhanced compared with the traditional own-rooted blueberry saplings (Fig. 1).

3.2 Effect of the cultivation medium pH on the leaf chlorophyll content, chlorophyll fluorescence parameters and gas exchange parameters about the three kinds of saplings From Fig. 2, it was found that with the rise of irrigating solution pH, the leaf chlorophyll a, chlorophyll b, chlorophyll (a + b) content and the chlorophyll a/b ratio concerning the three kinds of saplings were all reduced, the amplitude of reduction was in the order of chlorophyll a > chlorophyll b, the response of chlorophyll a/b ratio was more sensitive than the response of the chlorophyll content, and the own-rooted blueberry saplings had a more sensitive response than the own-rooted Wufanshu saplings and grafted blueberry saplings.

Among the three kinds of saplings, only the the leaf chlorophyll a and chlorophyll b and chlorophyll (a + b) content of own-rooted blueberry saplings cultivated on the medium with pH 7.0, was significantly less than in the control, while there was no significant difference between the other two kinds of saplings and the control.

When the own-rooted blueberry saplings were cultivated on the medium with pH 5.8 and pH 7.0, the leaf chlorophyll a/b ratio was significantly lower than in the control; when the grafted blueberry saplings were cultivated on the medium with pH 7.0, the leaf chlorophyll a/b ratio was significantly reduced compared with the control.

From Table 2 and Table 3, it was found that with the increasing pH of irrigating solution, the initial fluorescence (F_o) of the leaf chlorophyll in three kinds of saplings increased, the maximum fluorescence (F_m), PS II primary photochemical efficiency (F_v/F_o) decreased, photosynthetic rate (P_n) and transpiration rate (T_r) decreased, and the photosynthetic water use efficiency (WUE) increased, but the response of chlorophyll fluorescence parameters was more sensitive than that of the gas exchange parameters, the change of these parameters concerning the leaves of own-rooted Wufanshu saplings and grafted blueberry saplings was significantly smaller than the change of these parameters concerning the leaves of own-rooted blueberry saplings, and there were significant differences among different types of saplings.



Note: The different lowercase letters in the bar graph indicated significant difference ($P<0.05$).

Fig. 1 The differences in the root, stem, leaf and whole plant biomass of three kinds of saplings cultivated on the medium with relatively high pH

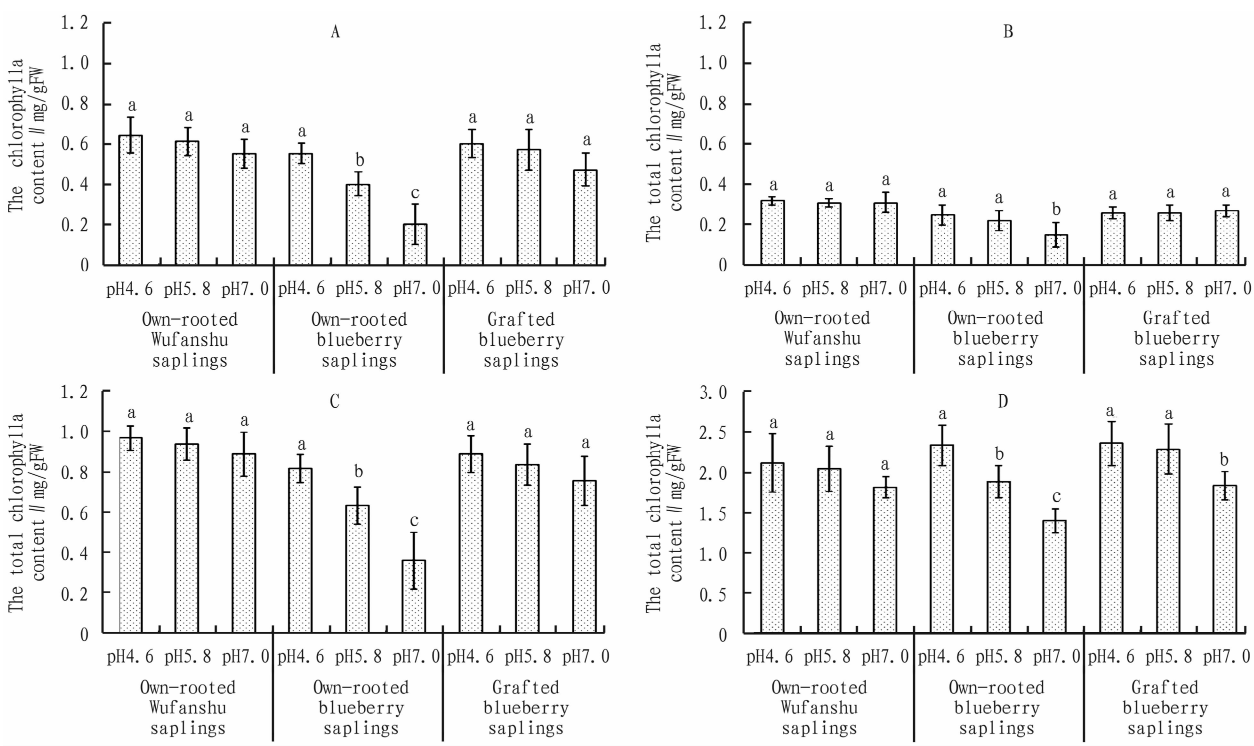


Fig. 2 The differences in the leaf chlorophyll content and composition of three kinds of saplings cultivated on the medium with relatively high pH

By the cultivation on the media with pH 5.8 and pH 7.0, there were not significant differences in the leaf chlorophyll Fo and Fm values between three kinds of saplings and the control, respectively; by the cultivation on the medium with pH 7.0, the Fv/Fo value of own-rooted Wufanshu saplings and grafted blueberry saplings was significantly less than that of the control, by the cultivation on the media with pH 5.8 and pH 7.0, the Fv/Fo value of own-rooted blueberry saplings was significantly less than that of the control, and there were significant pairwise differences.

The response of the leaf Pn, Tr and WUE to the cultivation medium pH was similar to the response of fluorescence parameters, and by the cultivation on the media with relatively high pH, there were not significant differences between the control and own-rooted Wufanshu saplings or grafted blueberry saplings, while the values of own-rooted blueberry saplings when pH was 7.0 were all significantly lower than those of the control.

Table 2 The differences in the leaf chlorophyll fluorescence parameters about three kinds of saplings cultivated on the medium with relatively high pH

Saplings	Treatment	F_o	F_m	F_v/F_o
Own-rooted Wufanshu saplings	pH 4.6	0.25 ±0.03 a	0.90 ±0.12 a	2.58 ±0.25 a
	pH 5.8	0.26 ±0.03 a	0.90 ±0.10 a	2.39 ±0.13 a
	pH 7.0	0.28 ±0.03 a	0.85 ±0.13 a	2.02 ±0.20 b
Own-rooted blueberry saplings	pH 4.6	0.22 ±0.03 a	0.69 ±0.05 a	2.05 ±0.32 a
	pH 5.8	0.24 ±0.09 a	0.61 ±0.05 a	1.55 ±0.97 b
	pH 7.0	0.25 ±0.01 a	0.48 ±0.12 b	1.01 ±0.62 c
Grafted blueberry saplings	pH 4.6	0.22 ±0.02 a	0.82 ±0.15 a	2.45 ±0.23 a
	pH 5.8	0.23 ±0.01 a	0.77 ±0.12 a	2.33 ±0.17 a
	pH 7.0	0.24 ±0.03 a	0.72 ±0.06 a	1.98 ±0.15 b

Table 3 The differences in the leaf gas exchange parameters about three kinds of saplings cultivated on the medium with relatively high pH

Saplings	Treatment	P_n (μmol/m² · s)	T_r (μmol/m² · s)	*WUE*
Own-rooted Wufanshu saplings	pH 4.6	6.32 ±0.36 a	3.01 ±0.21 a	2.10 ±0.22 a
	pH 5.8	6.13 ±0.34 a	2.85 ±0.16 a	2.15 ±0.20 a
	pH 7.0	5.92 ±0.38 a	2.66 ±0.22 a	2.23 ±0.09 a
Own-rooted blueberry saplings	pH 4.6	4.77 ±0.21 a	2.21 ±0.26 a	2.16 ±0.21 b
	pH 5.8	3.46 ±0.41 b	1.57 ±0.33 b	2.20 ±0.18 ab
	pH 7.0	2.15 ±1.06 c	0.89 ±0.19 c	2.42 ±0.50 a
Grafted blueberry saplings	pH 4.6	5.75 ±0.66 a	2.81 ±0.49 a	2.06 ±0.14 a
	pH 5.8	5.33 ±1.19 a	2.53 ±0.47 a	2.10 ±0.08 a
	pH 7.0	5.25 ±1.15 a	2.32 ±0.42 a	2.26 ±0.10 a

3.3 Effect of the cultivation medium pH on the content of six kinds of mineral elements in the leaves of three kinds of saplings From Fig. 3, it was found that the content of 6 kinds of mineral elements in the leaves of three kinds of saplings was in the descending order of Ca > N > K > Mg > P > Fe; the solutions with pH of 4.6, 5.8, 7.0 were used for irrigating for 90 d, with pH increase, the content of six kinds of mineral elements in the leaves of three kinds of saplings decreased, but the content of P, K, Ca decreased slightly.

There were not significant differences between the three kinds of saplings cultivated on the two kinds of media with high pH and the control (pH 4.6), and the significant difference detected was from the response of N, Mg and Fe. By the cultivation on the medium with pH 5.8, the content of N, Mg, Fe in the leaves of own-rooted blueberry saplings significantly decreased compared with the control, while there were not significant differences between the control and the own-rooted Wufanshu saplings or grafted blueberry saplings; by the cultivation on the medium with pH 7.0, the content of N, Mg, Fe in the leaves of own-rooted blueberry saplings further decreased, significantly different from the control and the saplings cultivated on the medium with pH 5.8.

The content of N in the leaves of grafted blueberry saplings did not decrease significantly, and the content of Mg and Fe was significantly lower than in the control, but not significantly different from that in the saplings cultivated on the medium with pH 5.8.

4 Discussions

Growth suppression is the result of a plant response to stress. There are reports about the blueberry growth inhibition by the cultivation on the medium with high pH, reaching a consistent understanding[20-21]. The test results confirmed the previous view.

The test results showed that different from the conventional own-rooted blueberry saplings, the grafted blueberry saplings with economic shrub Wufanshu as rootstock had significantly improved adaptation to the cultivation medium pH habitat, and the growth was evidently good. The study on the physiological properties and regulation of blueberry cultivated with sand culture mode and grafted saplings as test materials has not hitherto been reported.

The good growth of grafted blueberry saplings on the medium with relatively high pH is mainly attributed to the excellent growth characteristics of Wufanshu rootstock, which can be fully reflected from the differences in the plant height, basal diameter, crown diameter and the biomass of roots, stems, leaves and the whole plant of three kinds of saplings, especially the degree of development of the root system. The traits of grafted saplings are dominated by rootstock and also affected by scions, and are greatly influenced by the rootstock in terms of resistance, which has been proved by a large number of production practices and researches[22-23].

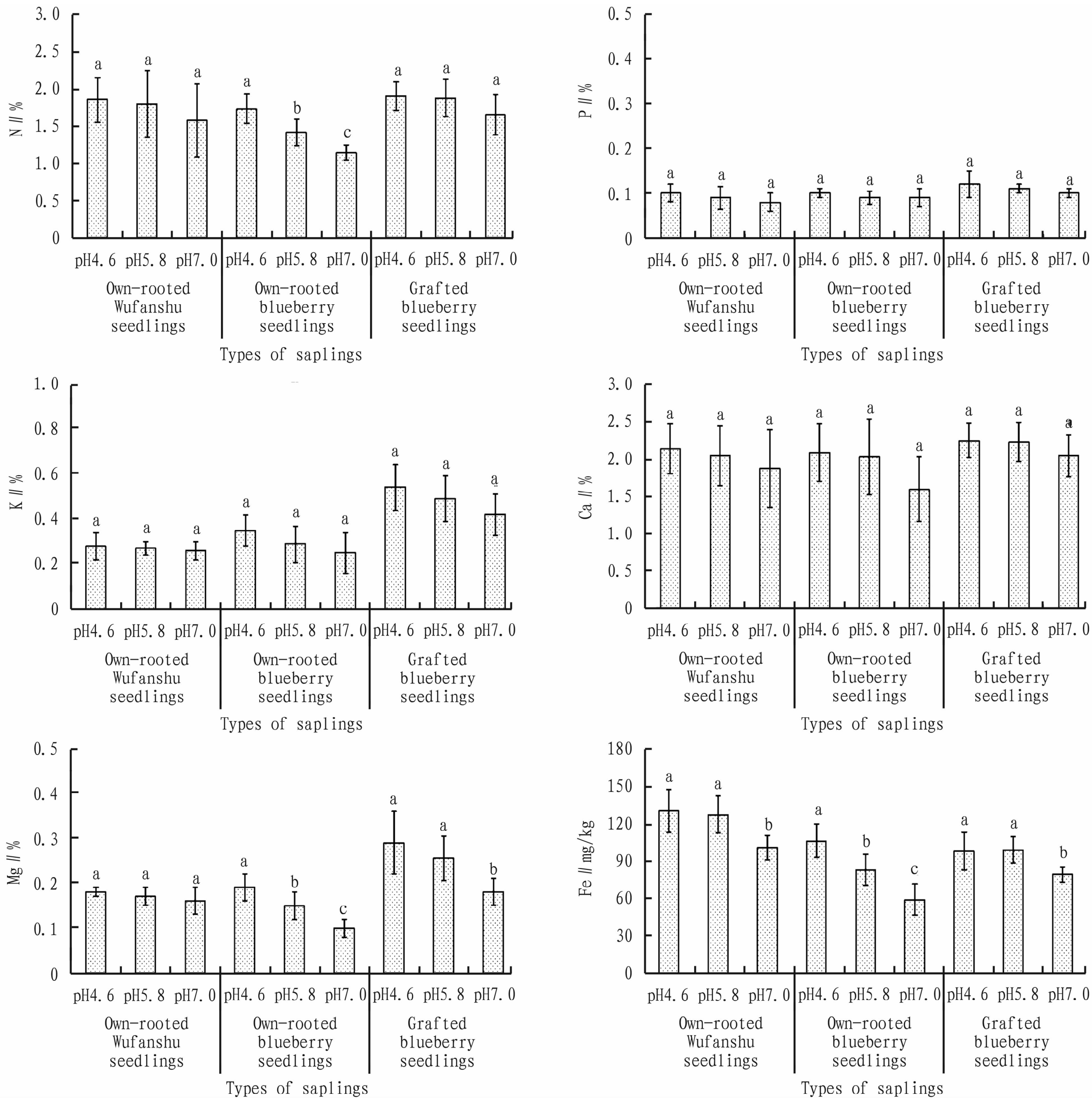


Fig. 3 The differences in the content of 6 mineral elements in the leaves of three kinds of saplings cultivated on the medium with relatively high pH

In this test, the grafted blueberry saplings made full use of the developed root system, strong growth potential and wide adaptation characteristics of Wufanshu rootstock. Meanwhile, the Wufanshu rootstock's expression of genes in blueberry scions also had a significant impact. There is a need to further study the use of other plant species of *Vaccinium* spp. for grafting blueberry, and its response to different pH habitats.

The basis of plant organs and plant growth is cell growth. In plant cells, chloroplast is the organelle most sensitive to stress[24]. Many studies have shown that the leaf chlorophyll content and composition change is an important aspect of plant response and adaptation to adversity. The most principal function of chlorophyll is to capture light and drive the electron to transfer to the optical reaction center, forming very important influence on the plant growth and yield.

Under pH stress, the change in blueberry leaf chlorophyll content, composition and fluorescence and gas exchange parameters has been reported rarely. The results herein showed that by the cultivation on the medium with relatively high pH, the leaf chlorophyll a content, total chlorophyll content and chlorophyll a/b ratio of grafted blueberry saplings were all significantly improved compared with the own-rooted blueberry saplings, especially the stability of chlorophyll a content was significantly enhanced, which was an important physiological basis for maintaining high photosynthetic efficiency under stress.

As one of chemical properties of soil medium, pH has a significant impact on the mineral nutrition of plants. After the blueberry is cultivated on the soil with unsuitable pH, the leaves generally suffer from "yellowing" and even death[25]. The present test results were consistent with the previous results, but the content of P, K, Ca in the leaves of three kinds of saplings was not significantly affected by the medium pH. The content of N only significantly decreased on the medium with relatively high pH, the content of Mg and Fe was greatly affected by the medium pH, and the reduction rate of grafted blueberry saplings was significantly lower than that of own-rooted saplings.

This indicated that the significant reduction of N content, especially Mg and Fe content in the leaves of own-rooted blueberry saplings might be an important reason for the leaf yellowing in the pH 5.8 and pH 7.0 habitat, so it is of great significance to improve the effective state Mg and Fe levels.

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