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EFFECTS OF NITROGEN, PHOSPHORUS AND POTASSIUM ON PHENOTYPE, PHOTOSYNTHESIS AND BIOMASS ACCUMULATION AT JUVENILE PHASE OF *PRUNUS ARMENIACA* × *SIBIRICA*

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Abstract

The goal of the study was to analyze the effects of three elements (nitrogen, phosphorus and potassium) on the growth and development of *Prunus armeniaca* × *sibirica* cv. Youyi. Hoagland medium was used for treatments with accurate control supply of these elements. The results showed that a lack of nitrogen was associated with changes in color and shape: the color of leaves was changed into yellow-green, veins transferred into white, and their shape was irregularly distorted. When the chlorophyll content in palisades was depleted, the sponge tissue became loose, and cell gaps were increased. The absence of phosphorus was associated with upward curling of leaves and the appearance of white spots on the edge which developed gradually into necrotic spots, while the center part remained dark green in color. Chlorophyll content remained high in the leaves. In the absence of potassium, new leaves were softer and yellower, and the upper part showed a significant distortion reaction. For most of the old leaves, there were focal necrotic spots on the leaf margins. When the supply of exogenous nitrogen, phosphorus and potassium was sufficient, the contents of total nitrogen, phosphorus and potassium in the leaves were 18.81 - 32.13 $mg \cdot g^{-1}$, 8.92 - 9.64 $mg \cdot g^{-1}$ and 34.37 - 38.63 mg^{-1}. While in insufficient scenario, the contents were 8.97 - 12.43 $mg \cdot g^{-1}$, 1.12 - 1.37 $mg \cdot g^{-1}$ and 5.18 - 7.38 $mg \cdot g^{-1}$, respectively. The supply of nitrogen had an effect on the development of the main root, phosphorus on the lateral root, and potassium for the number of fibrous roots. Especially when the elements of nitrogen and potassium were lacking at the same time, the root development was significantly hindered, and the biomass accumulation was significantly decreased. We thus suggest that the current practice of applying fertilization without phosphorus and potassium in early growth goes against scientific evidence.

Key words: *Prunus armeniaca* × *sibirica*; Kernel-apricot; Nitrogen; Phosphorus; Potassium.

Introduction

Kernel-apricot is a general term for apricot plants that are commercially used to produce apricot kernels, which mainly include *Prunus sibirica*, *P. armeniaca* × *sibirica* and *P. mandshurica*. China is the only country in the world that produces and exports apricot kernels, with an annual output of 250,000 to 300,000 tons (National forestry administration, 2019). The apricot kernels produced by *P. armeniaca* × *sibirica* which possess a rich aroma, sweet kernel (the content of amygdalin is only 0.023% - 0.189%), and abundant nutrients, have broad market prospects (Xu *et al*., 2016; Li *et al*., 2019). In addition, *P. armeniaca* × *sibirica* is highly resistant to dry and cold condition, and its cultivation is relatively simple to manage. The plant is widely distributed in arid and semi-arid regions of North China, with an annual output of 110,000-120,000 tons (National forestry administration, 2017; Xu *et al*., 2016), which is about 40.0% of the total annual production of China.Moreover, the plant, is becaming or already became an important ecological and economic tree species in arid and semi-arid regions of 33 N - 43 N.

Nitrogen, phosphorus and potassium are all essential nutrients for plant growth and development (Amini *et al*., 2019; Raza *et al*., 2021). The lack of these elements will thus directly affect the root activity and nutrient absorption of plants, which is expected to result in a significant decrease in biomass accumulation and inhibition of growth. When the elements are replenished in time, the growth of the plants will improve in a short period of time (Gopikumar *et al*., 2004; Ferreira *et al*., 2007; Afrousheh *et al*., 2010; Chen *et al*., 2015; Gama *et al*., 2015; Deus *et al*., 2018; Amini *et al*., 2019). The lack of nitrogen, phosphorus and potassium nutrients is first manifested in plant leaves. Nitrogen deficiency can lead to change of leaf colors to yellow (Fang *et al*., 2018) while phosphorus deficiency is associated with dark-green leaves (Pan 2018). Potassium deficiency can lead to plant leaf loss and necrosis at the edge of the leaves (Linh, 2012). In terms of physiological metabolism, the lack of these elements leads to changes in plant photosynthetic processes and a significant decrease in photosynthetic product accumulation (Lu *et al*., 2001; Molassiotis *et al*., 2006; Pan, 2018; Amini *et al*., 2019).

In the study of apricot plants, scholars mainly studied the effects of different nutrient elements on fruit development and fruit yield (Bussi *et al*., 2003; Li *et al*., 2019). These experimental conditions did not provide data with regards to changes of apricot leaf, root phenotype and photosynthetic physiology. Especially the interaction of nitrogen, phosphorus and potassium on the growth and development of apricot have not been fully in vestigated, and it remains difficult to judge the nutrient status and developmental stage of plants based on solely element.

In this study, we precisely controlled the deficiency of nitrogen (N), phosphorus (P) and potassium (K) individually or in combination (i.e. complex state) and investigated their effect on phenotype and biomass of *P. armeniaca* × *sibirica* in different scenarios, providing a scientific basis for future cultivation regimes.

Materials and Methods

Plant material: *P. armeniaca* × *sibirica*cv. Youyiis the largest cultivation area in China. With features of high yield (1050 kg kernel per ha), cold resistance (-35℃~-40℃) and drought resistance (3.0%~4.0% soil volume water lasting30~35d).The one-year-old seedlings of *P. armeniaca* × *sibirica*cv. Youyi with consistent growth were selected as the research object. In early November 2014, they were transplanted to a depth of 0.5 m and a diameter of 0.6 m plastic flowerpot filled with sterilized clean quartz sand (1 to 2 mm in diameter), and the experimental treatment was conducted from early March to late August 2015.

Test site: The experimental site was located in glass green houses with an effective control system of the Yuanyang experimental base of Non-timber Forestry Research and Development Center, Chinese Academy of Forestry.The geographic coordinates is 113 36′-114 15′ E，34 55′-35 11′ N, which belongs to the northern Henan plain, overlooking the Yellow River to the south and Yu River channels to the north. The main climate type in that region is continental monsoon climate, which makes it distinct in four seasons wide in temperature difference. The average annual rainfall is 571.7 mm and the rainfall is extremely uneven, about 70% of the total rainfall is concentrated in July, August and September and the non-flood season witnesses much less rainfall. The multi-year average evaporation capacity is 1599.0 mm.

Test design: Referring to Ning *et al*., (2013), experiments on macronutrients of *P. armeniaca* × *sibirica* were conducted based on Hoagland medium. A total of nine experimental treatments including +NPK, -N, -NP, -NPK, -NK, -P, -PK, -K and CK (ddH$_2$O, as control check) were set as illustrated in (Table 1). Every treatment was set with six individual plants, and three biological duplications were conducted. A total of 300 mL nutrient solution was given every 10 days, and the normal supply of water was ensured during the whole process, and an appropriate amount of ddH$_2$O was added every 2 days. The illumination time was controlled at 8 - 10 hours per day. In cloudy days, the lighting should be supplemented, and the strong light should be shaded appropriately. The indoor temperature was controlled at 25℃ - 28℃ using activated intelligent control system.Other managements (including pruning, pests and diseases control etc.) were same as of the field.

Determination of chlorophyll content: According to previous experimental results, *P. armeniaca* × *sibirica* need to be cultivated for 60 days, then the function leaves of four position on central branches was selected and sheared with 0.2 - 0.3 mm wide strips, then ethanol (95%) and acetone was added with the ratio of 2: 1 (v/v), subsequently the chlorophyll content was extracted in the absence of light, until the strips were completely white, using Cary-300 ultraviolet spectrophotometer (Agilent Technologies, Inc., Santa Clara, California, USA) in the wavelength of 663 nm and 645 nm was used according to the formula under the conditions of determination of the absorbance value to calculate the chlorophyll content. For more details on the methodology Zhu (2008) is referred.

Table 1. The experiment scheme on deficiency of different elements.

Nutrient element		Treatment								
		+NPK	-N	-P	-K	-NP	-NK	-PK	-NPK	CK
Macro-element (mg·L^{-1})	$Ca(NO_3)_2$	945	-	945	945	-	-	945	-	-
	KNO_3	607	-	607	-	-	-	-	-	-
	$NH_4H_2PO_4$	115	-	-	115	-	-	-	-	-
	$MgSO_4$	493	493	493	493	493	493	493	493	-
	$CaCl_2$	-	443.1	-	-	443.1	443.1	-	443.1	-
	KCl	-	447.3	-	-	447.3	-	-	-	-
	$NaH_2PO_4·H_2O$	-	138	-	-	-	138	-	-	-
	$(NH_4)_2SO_4$	-	-	132.1	-	-	-	132.1	-	-
	$NaNO_3$	-	-	-	510	-	-	510	-	-
Iron arene (g·L^{-1}) pH=5.5	$FeSO_4·H_2O$	5.56	5.56	5.56	5.56	5.56	5.56	5.56	5.56	-
	Na_2EDTA	7.46	7.46	7.46	7.46	7.46	7.46	7.46	7.46	-
Micro-element (mg·L^{-1}) pH=6.0	KI	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	-
	$MnSO_4$	22.3	22.3	22.3	22.3	22.3	22.3	22.3	22.3	-
	Na_2MoO_4	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	-
	$CuSO_4$	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	-
	$CoCl_2$	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	-
	H_3BO_3	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.2	-
	$ZnSO_4$	8.6	8.6	8.6	8.6	8.6	8.6	8.6	8.6	-

Determination of photosynthetic physiological indexes: From May 20 to July 20, 2015, the net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), intercellular CO_2 concentration (C_i) and stomatal limitation (L_s) were measured (using Li-6400 instrument, LI-COR Company, Lincoln, Nebraska, USA) under photosynthetic active radiation (*PAR*) of 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with reference to Xu *et al*., (2016) at 1-month intervals. Then, the instantaneous water use efficiency (*WUE*) changes of the photosynthetic physiological indexes were measured. The chlorophyll fluorescence parameters were measured with a PAM-2500 portable Chlorophyll Fluoroanalyzer (Walz, Effeltrich, Germany), including the *F0*, maximum fluorescence (*Fm*), *Fv/Fm* = (*Fm* - *F0*)/*Fm*, photo photosynthetic electron transport rate (*ETR*), photochemical quenching coefficient (*qP*) and coefficient of non-photochemical quenching (*qN*). For more details on the methodology, Xu *et al.*, (2016) is referred.

Determination of nitrogen, phosphorus and potassium in leaves: Leaf contents of total N, P, and K were determined according to Chinese agricultural industry standards, in which the total amount of N and P in plants were determined using a Seal-AA3 Flow Analyzer (SEAL Analytical, Ltd., Norderstedt, Germany), and the total amount of K was determined using an atomic absorption spectrometer AA800 (PerkinElmer chemagen Technologie GmbH, Waltham, Massachusetts, USA).

Preparation of leaf freehand slices: The fresh leaves were washed and laid flat on a small board, with two-sides quickly cut along main veins by single razor, and slices were put in water, the thin film was used as a temporary slice and investigated with an electron microscope (BX51, Olympus Corp., Shinjuku, Japan) to study the distribution of chlorophyll, as well as the appearance of palisade and spongy tissue.

Determination of root morphological indexes: Three similar plants were included in every process in order to evaluate the root morphology for index determination. Plant roots were sheared and rinsed with distilled water before they were placed in a scanner (WinRHIZO, type of LC4200-Ⅱ, Regent Instruments Inc., Quebec, Canada) to digitize the images. Then the images were submitted to software analyses to obtain the number of primary lateral roots, root length, surface area, volume, root number, and the branch number morphological indexes of samples.

Growth index and biomass accumulation determination: Our samples of *P. armeniaca* × *sibirica* entered hibernation in early November 2015. Following the first use of Vernier caliper, the plant height, ground diameter and crown were measured and the plants were carefully removed from the nursery stock. Harvest roots, stems, leaves and branches were put in the oven at 105℃ for 8 min, then at 75℃ to dry to constant weight. The dried weight was measured and the biomass of surface and underground parts were recorded.

Data processing: The recording and preliminary processing of experimental data were carried out in Microsoft Excel 2010. The single-factor ANOVA and an orthogonal ANOVA were conducted using DPS (Data Processing System) 7.05 software (Tang & Zhang, 2013). The complex range method of Ducan was used to conduct multiple comparison corrections based on the number of treatments. The significance level was set to 0.05 ($p \leq 0.05$), and data were plotted with Microsoft Excel 2007.

Results

Effects of nitrogen, phosphorus and potassium on leaf phenotypes of *P. armeniaca* × *sibirica* at seedling stage: Under the +NPK treatment for 30 days of cultivation, the leaves of *P. armeniaca* × *sibirica* were pale green, but not fully developed, indicating that *P. armeniaca* × *sibirica* was still in the young leaf stage and its physiological function was not fully developed yet. After 60 days of cultivation, the leaves were fully unfolded, with dark green leaves and clear veins, indicating that plants had reached their full photosynthetic capacity. After 90 days, the leaves became lighter in color, with white veins and change in the shape of the leaves, showing an aging trend (Fig. 1).

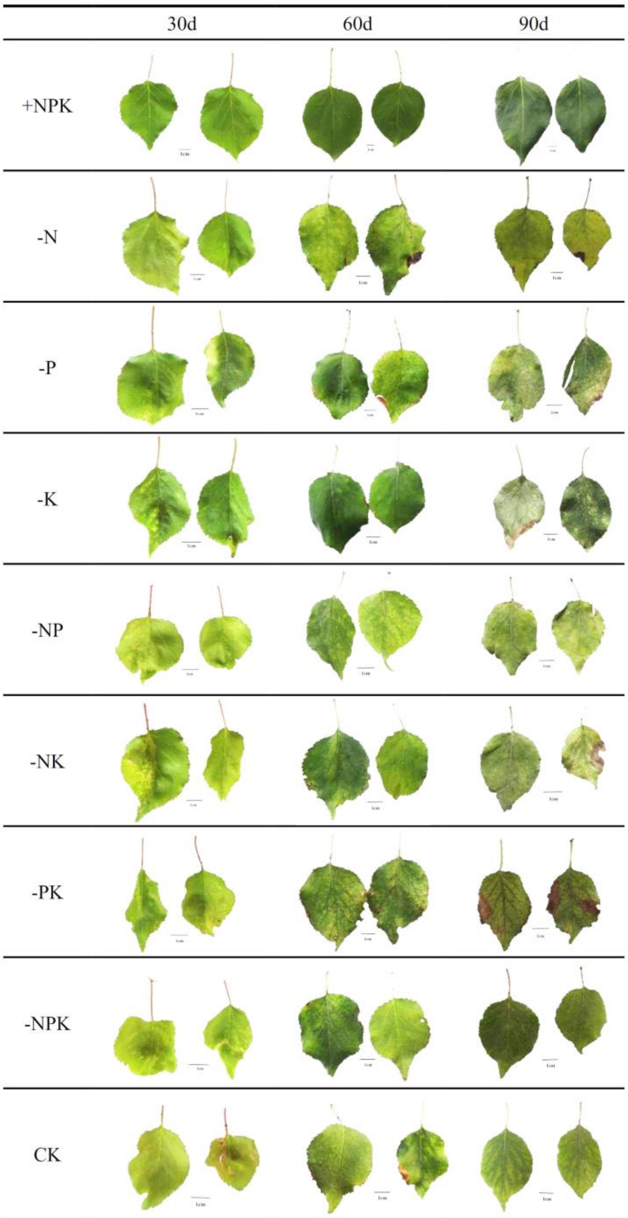

Fig. 1. The changes with cultivation time of leaves phonotype of *Prunus armeniaca* × *sibirica* at different treatments.

At the early stage of plant leaves, deficiency of elements N, P or K contributed to significant shape change, and the phenotypic differences caused by deficiency were more significant with the extension of cultivation time. At the early stage of -N, the leaves were yellow with thin spots and white veins (Fig. 1). On the 60th day of cultivation, there was an increase of the yellow areas on the leaves, and the necrotic spots appeared on the edge of the leaves. On the 90^{th} day of cultivation, the color of the leaves was predominantly yellow. At the early stage of P-deficiency (-P treatment), leaves were dark green with obvious curling and yellow areas showed at the edges of leaves (Fig. 1). With progressing cultivation time, yellow spots on leaves increased, and white spots appeared, accounting for more than 60% of the leaves' surface area at day 90 (Fig. 1). In the scenario with deficiency of K (treatment -K), at the beginning of treatment, the new leaves were soft and yellow, and the leaves curled downward along the mid vein (Fig. 1). After 60 days of cultivation, the tip of the leaves curled down significantly despite the dark color, and there were significant necrosis symptoms after 90 days of cultivation (Fig. 1).

Complex deficiency occurred earlier than single deficiency, yellowing and metamorphosis became more severe over time (Fig. 1). When -NP was applied, leaf yellowing was the most obvious and the tip curled downward, but change in the vein was not obvious (Fig. 1). With the cultivation progressing over time, yellowing was slightly alleviated, but the abundance of yellow spots on leaves was still very high, and the size of leaves was significantly smaller than that of young leaves when compared day 30 to day 90 (Fig. 1). Initial stage of -NK were associated with yellow leaves and curly middle veins with green leaves. After 90 days of cultivation, the leaf color was darkened, but yellow streaks appeared, and leaf edges were absent. At 90 days of cultivation, the number of yellow spots increased, and significant necrotic spots appeared at leaf edges (Fig. 1). In the early stage of -PK, the leaf development of *P. armeniaca* × *sibirica* was significantly hindered, the shape change of leaves occurred along the middle vein, and the leaf edge was scorched (Fig. 1). After 60 days of cultivation, the aging of leaves was serious but the veins were the exception, while the other parts were significantly yellowing (Fig. 1). After 90 days of cultivation, a large area of necrotic spots appeared in the middle of the leaf margin (Fig. 1). The leaf color was yellow and gray, and the vein was green. In the early stage of -NPK, the new leaves (cultured for 30 days) lost their green and yellow color, and the edges of the old leaves (cultured for 90 days) were scorched, and the leaves were always small and thin. After 60 days of cultivation, the leaf color was deepened, but the spots and yellowing were always obvious. Under the condition of using ddH_2O (with CK treatment), the leaf nutrient completely came from the main body of the tree, in the early stage of the leaf development, leaf etiolation was the most significant in all processing, development of leaf shape was irregular, showing significant incision and protruding, which was still apparent after 60 days of cultivation (Fig. 1). At day 90, blade properties were mostly stable, the leaves' color was green and yellow, and the veins were white (Fig. 1).

Effects of nitrogen, phosphorus and potassium on chlorophyll content in seedling stage: Under the condition of total macronutrients +NPK, the palisade tissues of leaves were arranged orderly and tightly, with high chlorophyll content and dark color (Table 2), and the spongy tissues were arranged loosely without gaps (Fig. 2). Under the condition of -N, the palisade and sponge tissues of leaves were arranged loosely, with large intercellular spaces and light color (Fig. 2 and Table 2). Under the condition of -P, the accumulated chlorophyll content in palisade tissue and sponge tissue was less and the gap was increased, but the distribution was more uniform (Fig. 2). Under the condition of -K, the content of chlorophyll in palisade tissue was rich, but there were large cavities observed in the sponge tissue (Fig. 2). Compared with single-element deficiencies, the symptoms of double element deficiencies included lower chlorophyll abundance and more intercellular cavities (Fig. 2). Under the condition of -NP, palisade and sponge tissues had low green content and increased intercellular space (Fig. 2). Under the condition of -NK, both the chlorophyll content of leaves and the cavity ratio were much higher than that of -NP (Fig. 2 and Table 2). Under the condition of -PK, the chlorophyll content in palisade and sponge tissues were from light green to light yellow, and the chlorophyll color was the lightest among the two elements (Fig. 2 and Table 2). Under the condition of -NPK, the color of palisade tissue and sponge tissue was black, indicating that chlorophyll synthesis was blocked. Furthermore, the sponge tissue featured evenly distributed hollow cavities (Fig. 2). Under the CK condition with ddH_2O cultivation, significant black areas were observed, despite residual chlorophyll in the palisade and sponge tissue. This finding may indicate that the chlorophyll could be synthesized by the tree's body nutrition in early development, but that the chlorophyll distribution was compromised.

Table 2. The difference analyse of chlorophyll content at different treatments.

Treatments	Chlorophyll a	Chlorophyll b	Total chlorophyll
+NPK	3.2234 ± 0.03a	1.1817 ± 0.01a	4.4051 ± 0.11a
-N	1.0885 ± 0.01e	0.3996 ± 0.02ef	1.4881 ± 0.07ef
-P	1.2645 ± 0.02c	0.4552 ± 0.03cd	1.7197 ± 0.05c
-K	1.4573 ± 0.01b	0.5496 ± 0.02b	2.007 ± 0.03b
-NP	1.1791 ± 0.02d	0.2796 ± 0.00g	1.4588 ± 0.04de
-NK	1.0401 ± 0.02e	0.3682 ± 0.02ef	1.5083 ± 0.04cde
-PK	1.2024 ± 0.02d	0.4273 ± 0.00de	1.6298 ± 0.02cd
-NPK	0.9173 ± 0.03f	0.3695 ± 0.01f	1.1869 ± 0.03f
CK	0.6366 ± 0.03g	0.4932 ± 0.01bc	1.1298 ± 0.04f

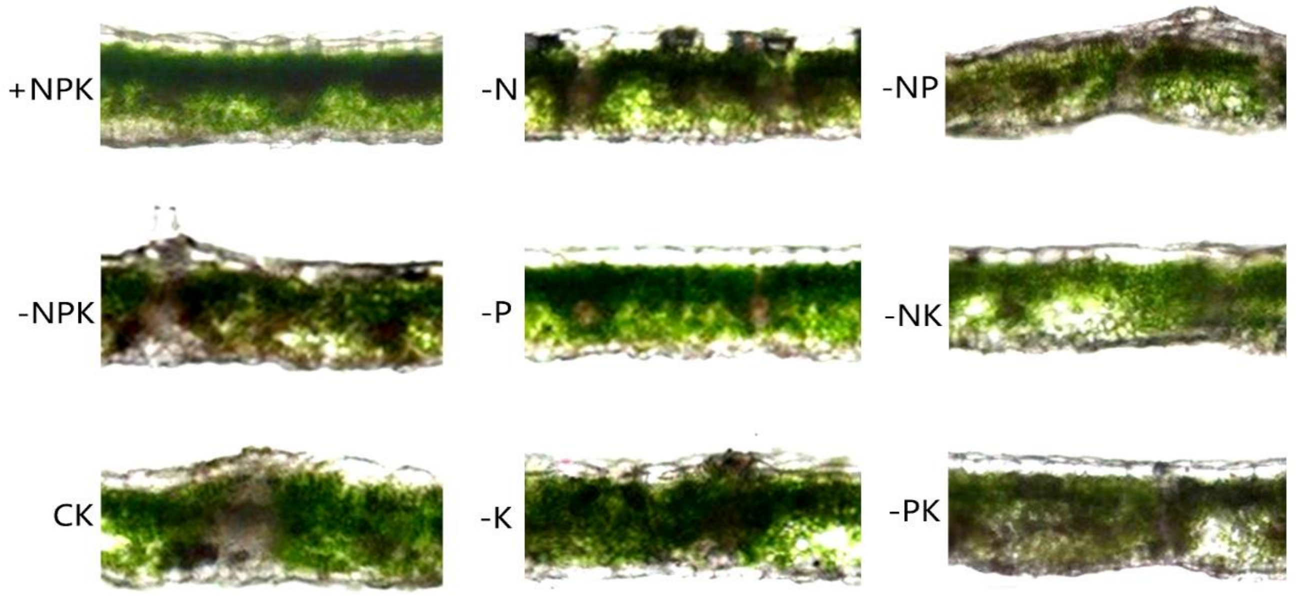

Fig. 2. The distribution of chlorophyll between palisade and spongy tissue of *Prunus armeniaca × sibirica* at different treatment.

Effects of nitrogen, phosphorus and potassium on photosynthetic physiology at seedling stage

Effects of nitrogen, phosphorus and potassium on photosynthetic indexes of *P. armeniaca × sibirica* at seedling stage: Different deficiency treatments had divergent effects on the photosynthetic activity of *P. armeniaca × sibirica* (Fig. 3). In terms of the effect on P_n, in addition to +NPK treatment, other treatments showed a gradually decreasing trend as a function of deficiency time (Fig. 3A). For different cultivation periods and treatments, P_n was significantly ($p \leq 0.05$) higher following treatment with +NPK and -K, compared to other treatments (Fig. 3A). In terms of the effect on G_s, +NPK and -K treatments also showed significantly ($p \leq 0.05$) larger effects than other treatments. However, these effects were related to the time of cultivation. Specifically, the effect of -NK and -PK treatments on G_s was significantly ($p \leq 0.05$) higher compared to -NP after June 20 (Fig. 3B). In terms of the influence on C_i, the effects of +NPK treatment were lower compared to other treatments after June 20, a pattern that lasted even until July 20 (Fig. 3C). In terms of the effects on T_r, treatment with +NPK had the largest effect and was significantly ($p \leq 0.05$) higher than any other treatments irrespective of the cultivation period. In terms of the lack of single element, the influence of P and K were smaller compared to the lack of N (Fig. 3D). Of interest, the effect of +NPK treatment on L_s was significantly ($p \leq 0.05$) lower compared to all other treatments. In terms of single factor treatments, the effect of K fertilizer was the smallest, reaching only a significant effect after July 20.

In the absence of double-elements, -PK treatment before June 20 had the least effect on L_s but the difference in effects caused by other treatments was not found significant. Further, -PK treatment has the greatest effect after July 20, while the difference compared to other treatments was still not significant. In the absence of three or more elements, CK had the largest impact on L_s, followed by -NPK, but the difference between the two treatments was not found significant, either (Fig. 3E).

In terms of the influence on *WUE*, there was no obvious trend on the influence of each treatment. In May 20, besides the -P treatment, the -NPK treatment had the largest impact, but this effect was not significantly different compared to other treatments. On June 20, -P treatment had the largest effect on *WUE*, but this superiority was not found to be significant compared to effects for treatment with +NPK, -K or -NPK. On July 20, -K had the largest effect on *WUE*, but again the difference comparted to +NPK, -N, -P, -NP, -PK and CK remained descriptive (Fig. 3F).

In summary, +NPK treatment had a significant ($p \leq 0.05$) promoting effect on improving P_n and G_s of *P. armeniaca × sibirica* compared to other treatments, and it significantly ($p \leq 0.05$) reduced C_i, T_r and L_s. However, it had no significant effect on improving *WUE*. In terms of the lack of complex elements, treatment with element K has a relatively small effect on the improvement of P_n and G_s, while treatment with element N had the largest effect, followed by treatment with element P.

Effects of nitrogen, phosphorus and potassium on photosynthetic fluorescence parameters of *P. armeniaca × sibirica* at seedling stage: The ratio of *Fv* and *Fm* is large and tends to change only marginally under suitable external conditions, but it decreases substantially under inappropriate external conditions. At the early stage of deficiency (May 20), there was no significant difference between the parameters of each deficiency treatment and the control group (Fig. 4). As of June 20, the parameters of all treatments were significantly lower than that of +NPK, and there was no significant difference between treatments with single element deficiency and combined deficiency in general (Fig. 4). With the extension of cultivation time, the differentiation between different treatments appeared significantly ($p \leq 0.05$). As of July 20, the parameters of all the treatments were still significantly lower than that of +NPK. The difference was that the decline in *Fv/Fm* was significantly ($p \leq 0.05$) higher when only element N was insufficient (Fig. 4A).

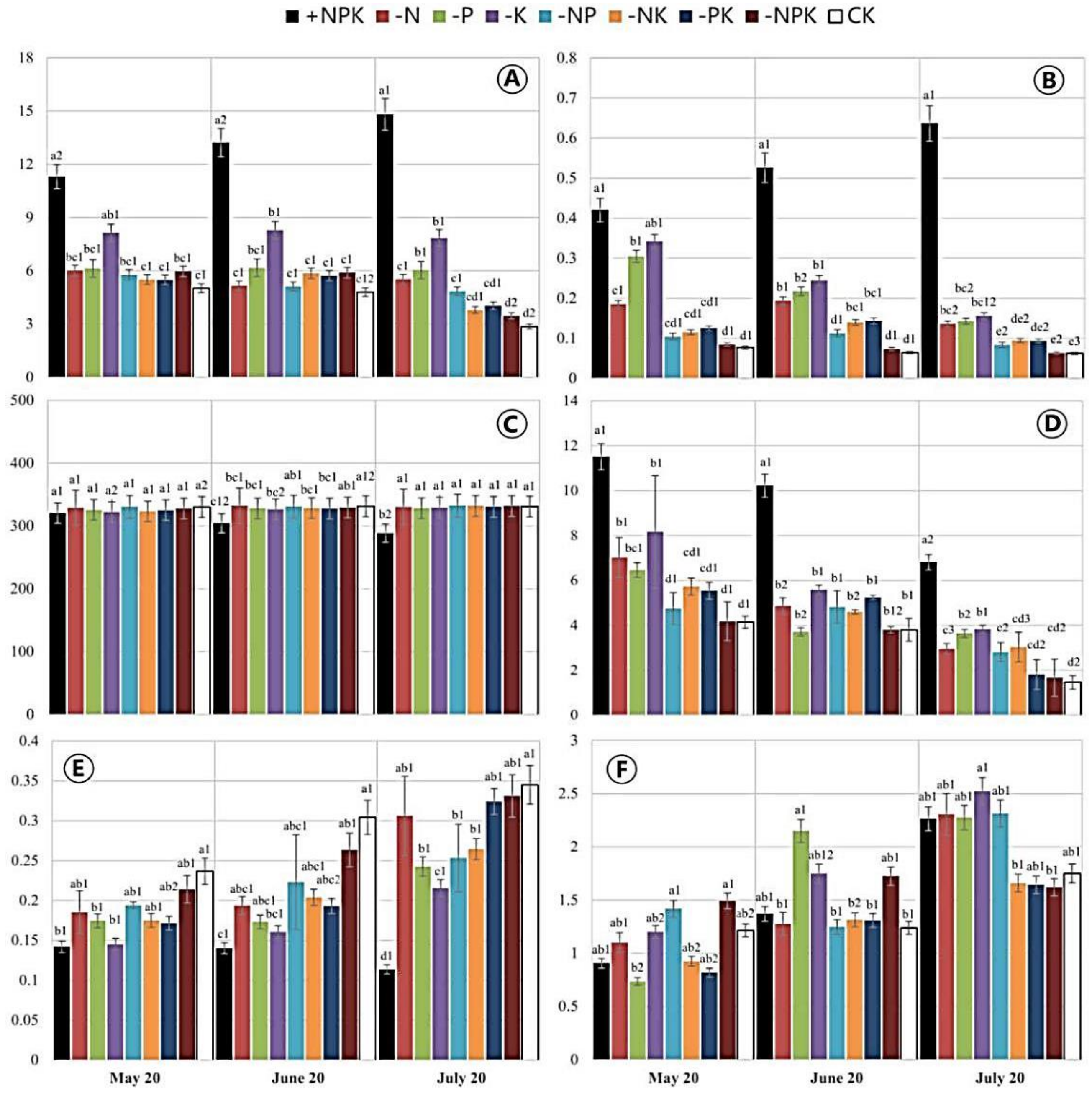

Fig. 3. Effect of Pn (A), Gs (B), Ci (C), Tr (D), Ls (E) and WUE (F) on the seedlings of *Prunus armeniaca* × *sibirica* at different treatments.

In terms of the influence on *Fm*, there was no significant difference in this parameter in the early stage of the deficiency (May 20), but with progressing cultivation time, different effects appeared depending on the treatment (Fig. 4B). By June 20, the deficiency of any element was significantly ($p \leq 0.05$) lower than that of +NPK, among which the deficiency of three or more elements (with treatments of -NPK and CK) was significantly ($p \leq 0.05$) lower than that of double-element, and the influence of double-element on *Fm* was not significantly different among other treatments. By July 20, measurements were consistent with those of June 20, except for *Fm*, which was lower compared to June 20, although this difference was not significant (Fig. 4B).

In terms of the influence on *F0*, there was no significant difference between different treatments, indicating that the effect of element deficiency treatment on *F0* was relatively small (Fig. 4C). In terms of the influence on *qN*, only +NPK and CK showed significant ($p \leq 0.05$) differences in the early stages of cultivation, but no significant ($p \leq 0.05$) differences among other treatments were observed (Fig. 4D). By June 20, there was no significant difference between the deficiency of single element and two elements. However, *qN* was the lowest when three elements were removed (treatments of -NPK and CK), a difference that was found significant (Fig. 4D). By July 20, the difference between the treatment with element deficiency and +NPK reached a peak, and the *qN* value of double-element deficiency was lower than that of the single element deficiency, although this difference did not reach a significant level. CK had the lowest value, showing no significant difference between treatments with defiency of three elements and two elements, but showing significant difference between treatments with deficiency of single element. In terms of the effect on *qP*, the value of +NPK in the early stage of cultivation (May 20) was always the lowest (Fig. 4D). In the early stage of cultivation, the value of *qP* was higher for all treatments (except for CK) compared to +NPK treatment, although these differences remained descriptive (Fig. 4E). By June 20, the *qP* value of the treatment with deficient elements was still higher than that of the treatment with +NPK, and the difference reached a significant ($p \leq 0.05$) level (Fig. 4E). With progressing cultivation time, the difference between deficiency treatments and +NPK treatment showed a trend over time until July 20.

In terms of its influence on *ETR*, the *qP* value of CK was significantly ($p \leq 0.05$) lower than that of other treatments and the difference reached a peak ($p \leq 0.05$) on May 20, while the difference among other treatments was not significant (Fig. 4F). On June 20, the *ETR* of +NPK treatment reached highest value and appeared significant ($p \leq 0.05$) difference between each treatment with other level, in terms of a single element lacks the -N treatment was significantly ($p \leq 0.05$) lower than the other, double elements in a lack of respect - PK was significantly ($p \leq 0.05$) higher than the other two processing, level of N elements significantly ($p \leq 0.05$) influence the *ETR*, three elements and above were lacking *ETR* lowest values and significantly ($p \leq 0.05$) lower than other individual processing (Fig. 4F). On July 20, the characteristics of various treatments were similar to those of the previous period, but the difference was not significant among treatments with deficiency of double elements.

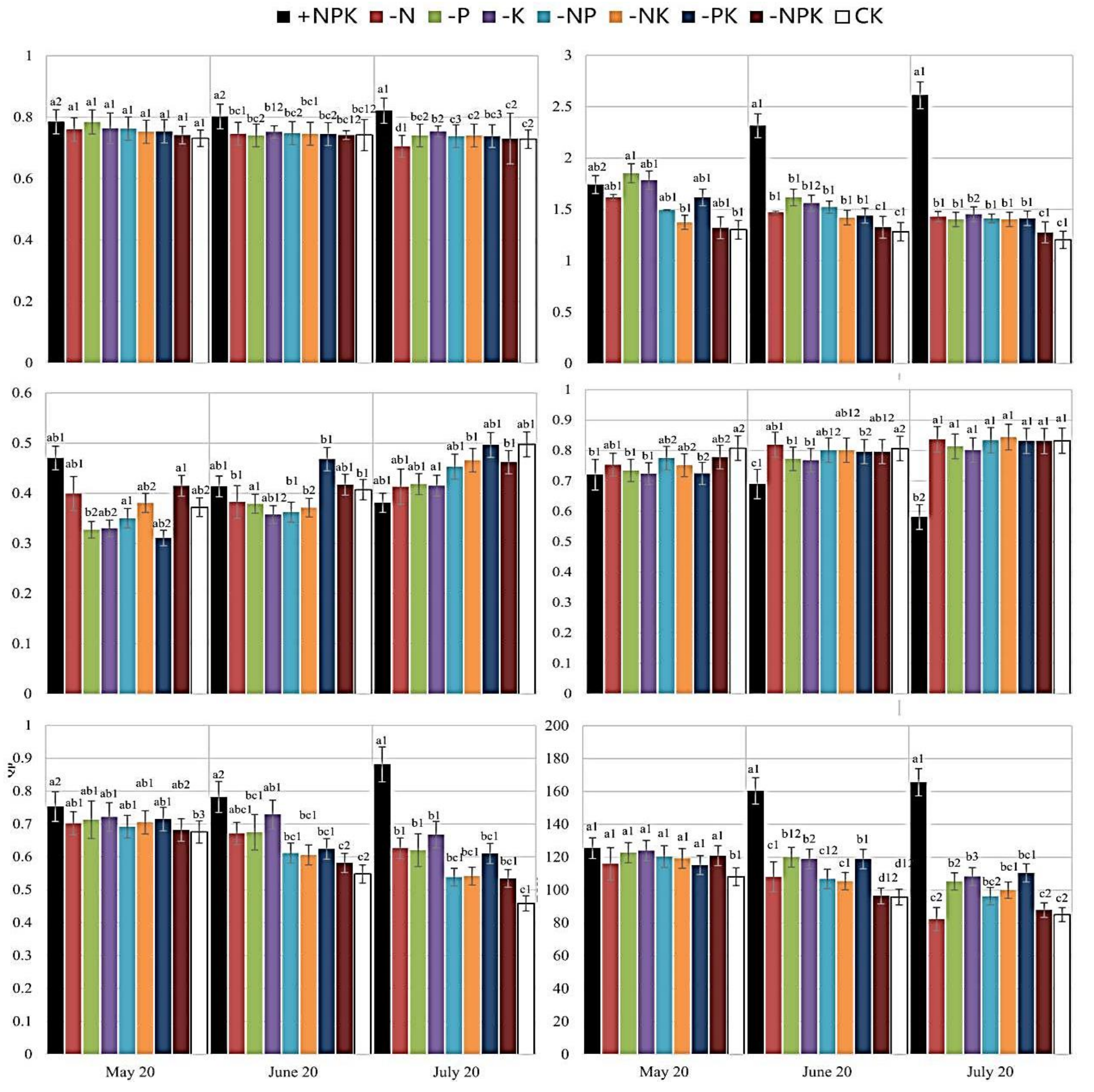

Fig. 4. Change in PSII maximum quantum yield, Fv/Fm (A), maximum fluorescence, Fm (B), minimal fluorescence, F0(C), nonphotochemical quenching coefficient, qN (D), photochemical quenching coefficient, qP (E) and apparent photosynthetic electron transport rate, ETR (F) on the seedlings of *Prunus armeniaca* × *sibirica* at different treatments.

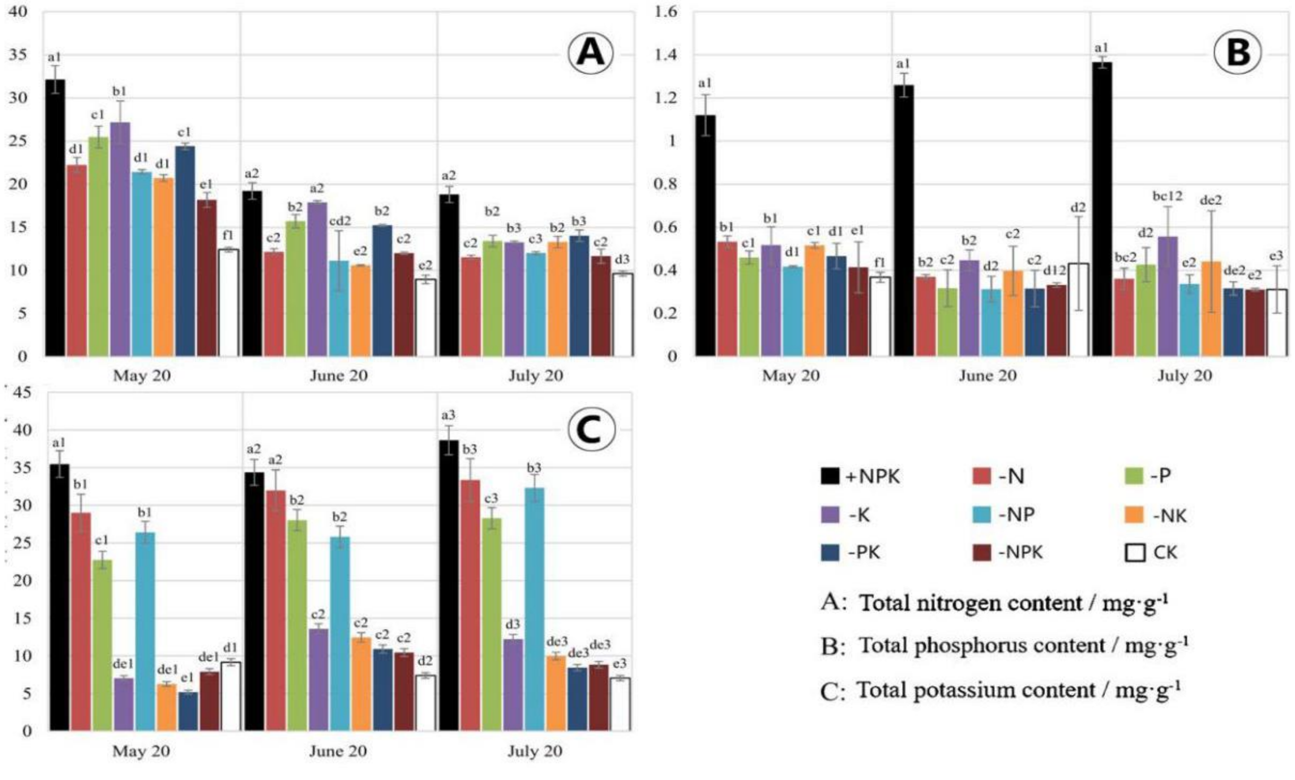

Fig. 5. Effect of different nutrients deficiency treatments on Nitrogen (A), Phosphorus (B) and Potassium (C) content on the seedlings of *Prunus armeniaca* × *sibirica* at different treatments.

Effects of nitrogen, phosphorus and potassium on the accumulation of nitrogen, phosphorus and potassium in the leaves of *P. armeniaca* × *sibirica*: There were significant differences in the accumulation of macronutrients with N, P and K in the leaves of *Prunus armeniaca* × *sibirica* under different deficiency treatments (Fig. 5). In terms of the effect on the total nitrogen content in leaves, the +NPK treatment was always at a high level (18.81 - 32.13 mg·g^{-1}) (Fig. 5A). The abundance and deficiency of exogenous N element was positively correlated with the accumulation of total N content in leaves, and the combined deficiency was significantly ($p\leq0.05$) greater than the lack of a single element. Especially the additive effect of the lack in element of N and P on the total nitrogen content in leaves was remarkable (Fig. 5A). With progressing cultivation time, the total nitrogen content in leaves decreased gradually. Of interest, the total nitrogen content in leaves treated with CK was stable at a low level of 8.97 - 12.43 mg·g^{-1}.

In terms of the effects of different treatments on total phosphorus content in leaves, +NPK treatment was stable at a high level (8.92 - 9.64 mg·g^{-1}) (Fig. 5B), while the lack of exogenous P significantly ($p\leq0.05$) affected the accumulation of total phosphorus content in leaves, especially the addition of N and P reflected a change of total phosphorus content in leaves. Under the condition of element deficiency, the total phosphorus content in leaves remained at a low level and fluctuated slightly with progressing cultivation time. The total phosphorus content in leaves treated with CK was stable at a low level of 1.12 - 1.37 mg·g^{-1}.

Total potassium content in different treatment in leaves was at a high level when processing + NPK (34.37 - 38.63 mg·g^{-1}) (Fig. 5C). However, when supplied with exogenous K, capacity of phosphorus was significantly ($p\leq0.05$) affected, which impacted the overall phosphorus accumulation level in the leaves of *Prunus armeniaca* × *sibirica*. It was noteworthy that the combined deficiency of elements had significantly larger negative effect than the lack of a single element. Especially the lack of elements P and K reduced the total potassium content in leaves, suggesting an additive effect (Fig. 5C). With progressing cultivation time, the total potassium content in leaves was stable for each treatment. Among all treatments, the total phosphorus content in leaves was the lowest (5.18 mg·g^{-1}) at day 30 of cultivation (20 May) with -PK treatment, and was maintained at a low level of 7.04 - 7.38 mg·g^{-1} after 60 days of cultivation under CK treatment.

Altogether, exogenous nitrogen, phosphorus and potassium were positively correlated with the accumulation of total nitrogen, phosphorus and potassium in leaves. Further, the total nitrogen content in each treatment gradually decreased with progressing cultivation time, indicating that N may be increasingly transferred when plants age. Noteworthy, the contents of P and K fluctuated slightly with progressing cultivation time but were relatively stable in leaves, indicating that the mobility of P and K was significantly weaker than that of element N.

Effects of nitrogen, phosphorus and potassium on root growth of *P. armeniaca* × *sibirica* at seedling stage: There were differences in the effects of different deficiency treatments on the root phenotype of *P. armeniaca* × *sibirica* (Fig. 6). Under the treatment of +NPK, the taproots, lateral roots and fibrous roots were more balanced, the taproots were stout, more lateral roots were evently distributed on the taproots, and the fibrous roots were abundant (Fig. 6, +NPK). Under the -N treatment, the primary roots were not apparent, the lateral roots were significantly thickened, and the fibrous roots were significantly increased, but amount was significantly less compared to the treatment with +NPK (Fig. 6, -N). Under the -P treatment, the taproot was significantly thickened, but the lateral roots were significantly thinner and more numerous, and the fibrous roots were more developed (Fig. 6, -P). Under the treatment of -K, taproot, lateral root and fibrous root were all stunted, especially the number of lateral roots and fibrous roots were significantly reduced (Fig. 6, -K). Under the treatment of -NP, the taproot was shorter and less developed, and the number of lateral and fibrous roots were decreased, while becoming significantly longer (Fig. 6, -NP). Under -NK treatment, the taproot was less developed and significantly longer, the lateral root was more developed but less abundant, and the fibrous root was significantly longer but less abundant (Fig. 6, -NK). Under the -PK treatment, both the primary and lateral roots were underdeveloped, the fibrous roots were sparse, and all the primary, lateral and fibrous roots were significantly longer (Fig. 6, -PK).

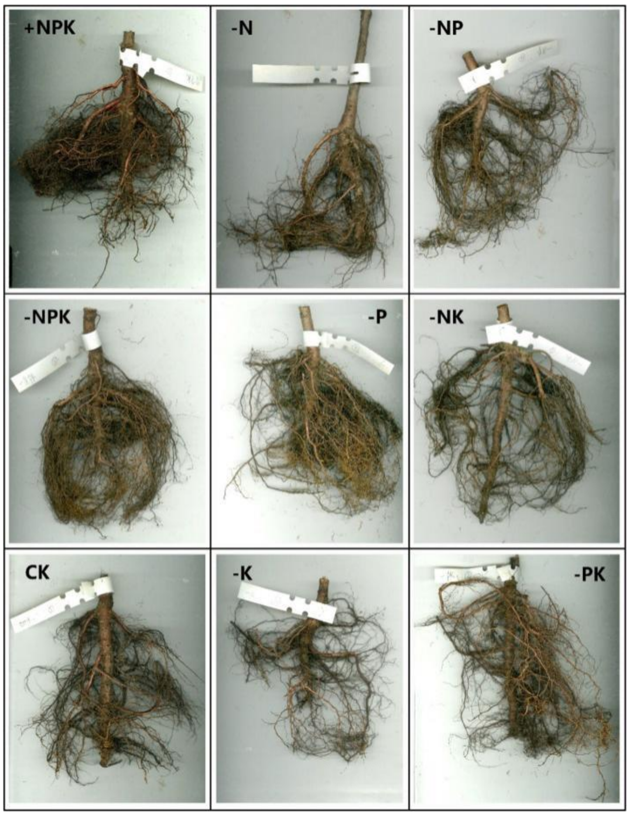

Fig. 6. Effect of root phenotype of *Prunus armeniaca* × *sibirica* at different treatments.

Under the -NPK treatment, both the primary and lateral roots were underdeveloped, and the number of lateral roots was obviously small, while the number of fibrous roots was large (Fig. 6, -NPK). Under CK condition, the principal roots were normally developed, but the lateral roots and fibrous roots were both underdeveloped and sparsely distributed (Fig. 6, CK).

Under different cultivation conditions, different treatments have divergent effects on the root development of *P. armeniaca* × *sibirica* (Table 3). The number of lateral roots under +NPK treatments was the largest, while the number was the smallest for CK treatment when compared to other treatments (Table 3). For single element deficiency treatment, the number of lateral roots under -K treatment was the smallest, and it was significantly less than that of -N and -P. Under double element deficiency treatment, the number of lateral roots for -NK was the smallest, but it was not significantly different comparted to -NP (Table 3). This finding indicated that the lack of element K alone already reduced the formation of lateral roots, and that there was an additive effect when element N was also depleted. Regarding total root length, its increase was the largest for +NPK treatment, while CK treatment resulted in the shortest roots, followed by -NPK treatment. The differences among these three treatments were significantly different ($p \leq 0.05$), also comparted to other treatments (Table 3). The combined deficiency significantly reduced the total root length of *P. armeniaca* × *sibirica* and the extent to which the length was reduced was larger than that of single element deficiency treatment. In terms of the influence on the projection area of root system, each treatment showed a consistent relation with regards to the total root length: for total surface area, when element N was insufficient, the root total surface area was the smallest for -N, -NP and -NK treatments and to a similar extent, indicating that N played a positive role in promoting the total surface area of the root system. In terms of the influence on the total volume of roots, when element K was lacking, the total volume of roots treated with -K and -NK was the smallest, indicating that element K played a positive role in promoting the total volume of roots. In terms of the influence on the number of root tips, elements of N and K had the largest influence with evidence that the number of root tips were the lowest in treatments -N, -K and -NK, an effect that was significant ($p \leq 0.05$) compared to other treatments (Table 3).

Effects of nitrogen, phosphorus and potassium on biomass accumulation of *P. armeniaca* × *sibirica* at seedling stage: Different treatments had divergent effects on biomass accumulation of *P. armeniaca* × *sibirica* (Table 4). Treatment with +NPK resulted in a significantly ($p \leq 0.05$) higher growth rate than other treatments, and the growth rate following combined deficiency was significantly ($p \leq 0.05$) greater compared to the single element deficiency treatment, with the lowest growth rate under CK treatment (Table 4). For single element deficiency treatment, the effect of deficiency in N and P elements on plant height was the lowest. Regarding ground diameter, the difference between -NPK and CK treatment was significant, so was the comparison between each treatment and these two treatments (Table 4). However, the difference between treatment with single and double element deficiency was not found significant. Regarding effects on crown amplitude, treatment with +NPK resulted in the highest crowns, while treatment with -NPK and CK resulted in the smallest. For treatment with single element deficiency, effects of -N and -P treatments were the lowest (Table 4). In terms of the effect on the dry weight of the aboveground parts, the weight was significantly higher for +NPK treatment compared to other treatments, while dry weight was found to be the lowest under CK treatment (Table 4). For treatment with single element deficiency and treatment with combined element deficiency, the latter had significantly ($p\leq0.05$) higher impact on dry weight (Table 4). Further, elements N and K resulted in greater impact on dry weight for aboveground parts when compared to element P. In terms of the influence on the dry weight of the underground part, data was comparable. However, this effect was found for each treatment with single element deficiency as well as the treatment with combined deficiency, with no significant differences among those treatments. However, the biomass accumulation was the lowest under -NP treatment. Lastly, in terms of the effect of different treatments on the root/shoot ratio, CK treatment had the largest effect on root/shoot ratio and +NPK treatment had the smallest effect on the root/shoot ratio (Table 4). These differences were significant ($p\leq0.05$) compared to other treatments. Further, treatment with combined deficiency increased the root-crown ratio significantly compared to treatment with single element deficiency.

Table 3. Effect on root development of *Prunus armeniaca* × *sibirica* at different treatments.

Treatments	The number of primary lateral roots	Total roots length	The square of roots projection	Total superficial area of roots	Total volume of roots	The number of root tips
+NPK	27 ± 3.0a	7415.02 ± 126.09a	289.00 ± 4.00a	213.20 ± 7.72a	50.49 ± 4.29a	43233 ± 80.0a
-N	24 ± 0.2b	3958.71 ± 62.86c	161.01 ± 7.19cd	105.50 ± 4.14cd	27.69 ± 5.88b	37568 ± 990.5b
-P	26.5 ± 2.5ab	4676.44 ± 255.34b	187.45 ± 12.44b	145.12 ± 18.83b	26.77 ± 4.56b	42902 ± 620.5a
-K	20 ± 2.5cd	4032.20 ± 362.14c	165.43 ± 7.89c	120.45 ± 5.98bc	19.36 ± 2.97c	28175 ± 45.0c
-NP	19 ± 4.5cd	3545.17 ± 207.07c	146.84 ± 9.68cd	92.32 ± 4.85de	14.59 ± 0.06c	23547 ± 311.5e
-NK	18 ± 1.5de	3462.09 ± 91.07c	140.10 ± 1.93d	98.67 ± 1.34cde	14.44 ± 0.81c	20330 ± 157.0f
-PK	23 ± 1.0bc	3538.96 ± 142.31c	141.42 ± 2.89d	110.51 ± 8.50cd	22.68 ± 1.98bc	28316 ± 789.5c
-NPK	19 ± 2.5d	2745.02 ± 60.44d	107.28 ± 1.68e	76.74 ± 0.47e	17.19 ± 1.58bc	24853 ± 748.5d
CK	15 ± 1.0e	2674.87 ± 54.52d	104.74 ± 0.86e	76.53 ± 0.99e	13.20 ± 2.22c	18670 ± 715.5g

Table 4. Effect on biomass accumulation of *Prunus armeniaca* × *sibirica* at different treatments.

Treatment	Plant height/ m	Ground diameter / mm	Crown diameter/ m	Dry weight of aboveground / g	Dry weight of underground / g	Root/shoot ratio
+NPK	1.30 ± 0.02a	14.49 ± 1.50a	1.60 ± 0.07a	131.80 ± 11.30a	83.70 ± 6.20a	0.64 ± 0.07g
-N	0.91 ± 0.02bc	12.59 ± 0.95ab	0.94 ± 0.13cd	75.62 ± 3.63bc	50.65 ± 0.55bc	0.67 ± 0.02ef
-P	0.95 ± 0.03b	13.06 ± 0.96ab	1.04 ± 0.16bc	88.38 ± 5.16b	61.30 ± 1.30b	0.69 ± 0.04de
-K	0.85 ± 0.00cd	12.03 ± 0.80ab	1.08 ± 0.14bc	83.08 ± 4.94b	53.95 ± 2.15bc	0.65 ± 0.03fg
-NP	0.85 ± 0.01cd	12.11 ± 0.34ab	0.90 ± 0.18cd	65.10 ± 3.63c	46.60 ± 4.00c	0.71 ± 0.04cd
-PK	0.96 ± 0.02b	12.64 ± 0.61ab	1.02 ± 0.12bc	63.90 ± 3.48c	49.10 ± 6.50bc	0.79 ± 0.19b
-NK	0.96 ± 0.03b	12.12 ± 0.76ab	1.00 ± 0.18b	61.02 ± 2.46c	48.65 ± 6.05bc	0.80 ± 0.11b
-NPK	0.80 ± 0.01de	11.60 ± 0.37b	0.79 ± 0.08d	63.95 ± 2.03bc	46.80 ± 3.50bc	0.74 ± 0.09c
CK	0.76 ± 0.02e	11.44 ± 0.38b	0.72 ± 0.06d	50.22 ± 3.13d	45.60 ± 0.80c	0.91 ± 0.08a

Discussion

Nitrogen, phosphorus and potassium are essential elements for plant growth and development. Results of this study showed that when nitrogen, phosphorus and potassium were deficient, the leaf phenotypic reactions and shape change were apparent in early growth stages. The deficiency of these elements increased with progressing cultivation time. Nitrogen is an important component of chlorophyll synthesis. In this study, leaves were yellow-green in color with white veins and irregular convoluted leaves (Fig. 1) when N was deficient (i.e. for treatment with -N, -NP and -NK), which may be related to the phenomenon of low chlorophyll content in palisade tissues, loose arrangement of spongy tissues and more intercellular spaces in leaves (Fig. 2). The nitrogen deficiency symptoms of *P. armeniaca* × *sibirica* were similar to those reported for teak sandalwood (Barrett *et al.*, 1997) and *Mastic berbera* (Trubat *et al.*, 2006). Phosphorus is involved in the synthesis of phospholipids, nucleic acids and other phosphorous compounds in plants. When element P was deficient, the leaves of *P. armeniaca* × *sibirica* curled upward, white spots appeared at the edge of the leaves, which gradually developed into necrotic spots. However, the color of the middle part of the leaves was dark green (Fig. 1), indicating that chlorophyll content in the palisade tissue was still high (Fig. 2). Potassium is an activator of plant enzymes, which is involved in osmotic regulation, photosynthetic physiology and other important processes to maintain life. Existing studies have found that potassium deficiency can lead to accelerated growth rate in the middle of leaves (Trubat *et al*., 2006) and destruction of chlorophyll (Jia *et al.*, 2014; Amini *et al*., 2019), leading to tip curling and leaf necrosis. In this study, the new leaves of *P. armeniaca* × *sibirica* were soft and yellow when K was absent, and the upper part of the leaves showed a significant distortion reaction. Most of the old leaves were normal in color, but there were spots of scorch and necrosis at the leaf margin. Data showed that the chlorophyll content in palisade tissue was still high, but there were large gaps in the sponge tissue. The symptoms of leaf and root phenotype with of *P. armeniaca* × *sibirica* combined element deficiency appeared earlier than that with single deficiency, and its symptoms in leaves were pronounced. Specifically, leaves aged faster, and there were more and larger gaps in sponge tissues.

With regard to root growth and development, element N mainly affected the development of taproot, where P affected the development of lateral root, and K the number of fibrous roots. The negative effect of combined element deficiency on root growth and development was greater compared to single element deficiency. In particular, when N and K elements were deficient, the root development was significantly hindered and the biomass accumulation was significantly reduced, indicating that elements of N and K were indispensable nutrients for the root development of *P. armeniaca* × *sibirica*. This finding was consistent with the results of Cao *et al*., (2013). Compared to taproot and lateral roots, fibrous roots have stronger absorption capacity and are the main nutrient absorption organs of plants (Sullivan *et al*., 2000). Scientific evidence based on these findings went against the heavy use of applying N fertilizer and only light use of P and K fertilizer in early development of *P. armeniaca* × *sibirica*. In contrast, the supply of exogenous K should be increased to promote the growth and development of the plant's root system.

In this study, mobility of P and K elements in *P. armeniaca* × *sibirica* leaf was significantly weaker compared to that of N, which may explain its absence in *P. armeniaca* × *sibirica* tree body resulted in quickly deficiency phenotype of leaves, chlorophyll content and root development (Figs. 1, 2 and 3). Therefore, in order to avoid errors in the determination of nutrients in leaves caused by N, P and K element transfer, it was recommended to take samples at day 60 after leaf development. When the phenotype of N deficiency was found in the leaves of *P. armeniaca* × *sibirica*, it should be timely supplemented to prevent a disruption of photosynthesis and hence the decreased economic yield.

Conclusion

The levels of exogenous N, P and K directly affected the accumulation of total N, P and K in leaves and body of *P. armeniaca* × *sibirica*. When the supply of exogenous nutrients was sufficient, the total N, P and K in leaves were 18.81 - 32.13 mg·g^{-1}, 8.92 - 9.64 mg·g^{-1} and 34.37 - 38.63 mg·g^{-1}, respectively. When the supply was insufficient, the total N, P and K in leaves were 8.97 - 12.43 mg·g^{-1}, 1.12 - 1.37 mg·g^{-1} and 5.18 - 7.38 mg·g^{-1}, respectively. These measures can be used as the standard to judge the nutrient real-time status in trees and soil.

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