

# Effect of potassium on the agronomic traits and fruit quality of *Lycium barbarum* L

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## Article

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# 2 Effect of potassium on the agronomic traits and fruit quality of 3 *Lycium barbarum* L.

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## 11 ABSTRACT

12 To investigate the effects of potassium (K) application on the agronomic traits and fruit quality of *Lycium barbarum* L. (Goji), three  
13 levels of K fertilizer, namely LK (25 g/plant), CK (50 g/plant), and HK (75 g/plant), were applied to plants in phytotron for observing  
14 and measuring relevant indicators. The investigation involved seven agronomic traits: plant height, plant stem diameter, new branch  
15 increment, yield of fresh fruits per plant, dry fruit quantity within 50 g, ratio of different grade fruits, and ratio of longitudinal  
16 diameter to transverse diameter of Goji fruits. The results showed that K application level had significant effect on ratio of the longi-  
17 tudinal diameter to the transverse diameter of fresh Goji fruits, and that the influence on other agronomic traits was slight. In the  
18 meanwhile, the concentrations of amino acids, betaine, polysaccharides and flavonoids of Goji fruits in different levels of K fertilizer  
19 were tested. The K treatment increased the content of glutamic acid, and decreased that of flavonoids ( $P < 0.05$ ), whereas the content  
20 of other amino acids, polysaccharides and betaine were unaffected. A total of 132 flavonoid metabolites was identified. Among them,  
21 K treatment up-regulated 36 metabolites and down-regulated 30 metabolites ( $P < 0.05$ ). The results provided a basis for balanced K  
22 supply to regulate the agronomic traits and nutrients of Goji fruits.

## 23 KEYWORDS

24 *Lycium barbarum* L. fruit; potassium; flavonoid; quality; agronomic traits

## 25 Introduction

26 *Lycium barbarum* L., also known as Goji<sup>[1]</sup>, belongs to the genus *Lycium* in the family Solanaceae. It is one of the major  
27 crops in Northwest China, being mainly cultivated in Ningxia, Qinghai, and Tianjin<sup>[2]</sup>. Ningqi series belong to *L. barba-*  
28 *rum* are recorded as a medicine<sup>[3-4]</sup> and have high nutritious value<sup>[5-6]</sup>. The active constituents are polysaccharides,  
29 flavonoids, betaine, vitamins and other nutrients<sup>[7]</sup>.

30 Potassium (K) is an essential nutrient for plant growth and development, and consequently of fertilizers. Its content in  
31 the plants is second only to nitrogen (N)<sup>[8]</sup>. In addition, K application can also increase the disease resistance of plants  
32<sup>[9]</sup>. For example, with the increase of K application in tobacco leaves, the incidence of black shank disease and black rot  
33 disease also showed a clear downward trend<sup>[10]</sup>. Similarly, the *Chrysanthemum morifolium* Ramat. in K deficiency will  
34 contribute to a significant decline in the physiological resistance of plants. It is prone to leaf blight disease, owing to  
35 physiological lack of K, resulting in yield reduction<sup>[11]</sup>. This phenomenon also occurs in Goji, causing abscission of  
36 leaves, flowers and fruits, and reduced fruit yield<sup>[12]</sup>. Potassium is essential for plant growth and function<sup>[13]</sup>. A suffi-  
37 cient supply of K improves the contents of amino acids (cystine, methionine, tyrosine, and tryptophan), sugar and acid-  
38 ity in fruits<sup>[14]</sup>, mainly because a number of enzymes requiring K<sup>+</sup> activation, making it essential for normal cell metab-  
39 olism<sup>[15]</sup>. In the northern part of China, even soils with slightly higher K content can suffer from K deficiency in high-  
40 yielding and K-loving crops due to factors such as soil drought. Nevertheless, excessive application of K will not only  
41 pollute the environment, but also destroy the nutrient balance and lead to the deterioration of crop quality<sup>[11, 16-18]</sup>.  
42 Therefore, the rational application of K fertilizer is crucial for the healthy growth and quality improvement of plants.  
43 The effect of K on the quality of many crops is already explored. The study has shown that spraying the foliar fertilizer  
44 potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) on grapes can increase the expression of anthocyanin synthesis genes in  
45 fruits<sup>[19]</sup>. N and K can change the content of soluble sugars and organic acids in grapes by inducing the expression of  
46 the relevant genes in saccharide metabolism, and in turn, regulate anthocyanin synthesis<sup>[20]</sup>. For the root of *Hedysarum*

47 *polybotrys* Hand. -Mazz., spraying of moderate concentrations of K salts is beneficial to plant growth and isoflavonoid  
 48 accumulation [16]. It has demonstrated that with the increase of K application, the content of soluble sugars [10], total  
 49 phenols, flavonoids [10,11,17,18], chlorogenic acid [11], ascorbic acid [17] and lignin [18] in plant leaves and flowers increased  
 50 gradually. The studies indicate that the PAL [11,18], PPO and POD [18] activities are positively associated with K applica-  
 51 tion, but the content of soluble amino acid is negatively correlated with K application [11]. It has shown that flavonoid  
 52 content in both *C. morifolium* and *Labisia Pumila* Benth showed an increasing and then decreasing trend with increasing  
 53 K application. This is because the activities of antioxidant enzymes and nutrients are lower in excessive K content [11, 17].  
 54 Liu [21] held that K deficiency could significantly reduce the flavonoid content in *C. morifolium* by 31.4%. Therefore,  
 55 insufficient or excessive supply of K fertilizer would reduce the activities of metabolic enzymes, the synthesis of phe-  
 56 nolics and lignin, and the yield of crops [11, 17, 18].  
 57 In *Lycium barbarum* L., the content of flavonoids differs among varieties in response to different genetic backgrounds. It  
 58 has a wide range of functions in plant tissue colouration and response to adversity [22]. There are studies on the changes  
 59 of flavonoid content in leaves and fruits before ripening of Goji fruits [23], as well as studies on key genes related to  
 60 flavonoid synthesis [24, 25], and studies on the yields and nutrient compositions of *Lycium ruthenicum* Murray after spray-  
 61 ing with  $\text{KH}_2\text{PO}_4$  [26]. Although there have been some studies on the changing of flavonoid content in Goji fruits during  
 62 growth and development, the effects of K fertilizer on agronomic traits and quality of Goji fruits are not clear. In this  
 63 study, the main cultivar "Ningqi 7" was used as the test crop, and we observed the effects of K treatments on the agro-  
 64 nomic traits and nutrients of the plants during different harvesting periods. The data, thus provide a theoretical basis  
 65 for balanced K application to regulate the quality of Goji fruits.

## 66 Results

### 67 Effects of K fertilization on different agronomic traits

68 The agronomic traits data was analyzed statistically. The results showed that K treatments have no effects ( $p > 0.05$ ) on  
 69 the plant height (Figure 1A), plant stem diameter (Figure 1B), new branch increment (Figure 1C), yield of fresh fruits  
 70 per plant (Figure 1D), dry fruit quantity of 50 g (Figure 1E) and the ratio of different grade fruits (Figure 1F). As Goji  
 71 fruit was picked four times (6 June, 25 June, 4 July, 12 July), the agronomic traits at each time were analyzed. The results  
 72 showed that in three K treatments, the yield of fresh fruits at the second harvest was the highest, and the first harvest  
 73 was the lowest (Figure 1G). Moreover, the dry fruit quantity of 50 g at the fourth harvest was more than that at the third  
 74 harvest, followed by the second harvest at three K levels (Figure 1H). It indicated that the fruits at the fourth harvest  
 75 were the smallest, while those at the second harvest were the largest size and highest yield. Owing to larger fruits  
 76 having higher market prices and greater purchasers, the second-harvest fruits are more popular. The ratio of different-  
 77 grade fruits was analyzed (Figure 1I). It clarified that the proportion of first-grade fruits was the highest in both CK  
 78 (49.17%) and HK (50.75%) treatments.

79 The results also showed that K had a significant effect on the ratio of longitudinal and transverse diameters of fresh Goji  
 80 fruits ( $p < 0.01$ ) (Figure 2). The longitudinal diameter and transverse diameter of Goji fruits differ from K treatments  
 81 (Figure 2A). They were maximum at HK. The ratio of longitudinal diameters and transverse diameters increased with  
 82 the increasing K application (LK: 0.93; CK: 1.00; HK: 1.07). At the first and second harvests, the ratio had a significant  
 83 difference between LK and HK treatments ( $p < 0.05$ ). The ratio value of HK was higher than that of LK. There were no  
 84 differences in both third and fourth harvests ( $p > 0.05$ ). It indicated that the influence of K on the ratio was mainly  
 85 influenced by the first and second harvests. The fruit shape was significantly different under three levels of K, being  
 86 flatter at LK, rounder at CK, and longer at HK.

### 88 Effect of K on nutrient composition of Goji fruits

89 The fruits at the second harvest (25 June) had the highest yield (Figure 1D) and the largest size (Figure 1E), while the  
 90 fruits at the fourth harvest (12 July) had the smallest size. To observe the nutrient differences at these two harvest times,  
 91 the key components (amino acids, betaine, polysaccharides, and total flavonoids) of the fruits were detected.

92 A total of 17 amino acids were detected (Figure 3A). Potassium (K) had a significant effect on glutamate between HK  
 93 and LK ( $p < 0.05$ ). The glutamate content of HK (1.087 g/kg) was higher than that of LK (0.954 g/kg). It also found that  
 94 the content of nine amino acids (proline, alanine, valine, leucine, threonine, aspartic acid, methionine, glycine, and ty-  
 95 rosine) had significant effects between the second and fourth harvests ( $p < 0.05$ ) (Figure 3B). Among them, the contents  
 96 of proline, leucine, valine, glycine, and aspartic acid increased over time, while those of threonine, alanine, methionine,  
 97 and tyrosine decreased. The total content of amino acids on 25 June was lower than that on 12 July in both LK and CK  
 98 treatments ( $p < 0.05$ ) (Figure 3C). However, there was no significant difference in HK ( $p > 0.05$ ).

The content of flavonoids negatively correlated with K fertilization (Figure 4A). It was significantly different from LK and HK ( $p < 0.05$ ). However, K treatments had no effect on the content of betaine (Figure 4C) and polysaccharides ( $p > 0.05$ ) (Figure 4E). The results also showed that the betaine contents on 12 July (0.7265 g/100 g) were significantly higher than those on 25 June (0.5495 g/100 g) ( $p < 0.01$ ) (Figure 4D). However, the content of flavonoids (Figure 4B) and polysaccharides (Figure 4F) between the second and fourth harvests was unaffected. It indicated that the content of amino acids and betaine in smaller fruits of "Ningqi 7" was higher than that in larger fruits. Therefore, there isn't a positive correlation between fruit size and nutritional value.

#### **KEGG pathway analysis of flavonoid metabolites in *Lycium barbarum* L.**

Since K fertilizer had a significant effect on the flavonoid content of Goji (Figure 4A), flavonoid metabolites were detected. Flavonoids are synthesized through the phenylpropanoid pathway<sup>[9]</sup>. Phenylalanine transformed to p-coumaroyl-CoA and then entered the flavonoid biosynthesis pathway (Figure 5). In this study, 132 flavonoid metabolites, including 42 flavones, 27 flavonols, 2 flavonolignans, 24 flavone C-glycosides, 16 flavanones, 6 isoflavones, 13 anthocyanins, and 2 proanthocyanidins, were identified from Goji fruits. According to the pathway of the Kyoto Encyclopedia of Genes and Genomes (KEGG), there were only 6 flavones, 6 anthocyanins, 6 isoflavones, 9 flavanones, 13 flavonols, and 2 flavonolignans of goji fruits that can be annotated in the pathway (Figure 5A). By comparing LK with CK, 27 flavonoid metabolites, with 15 being up-regulated and 12 being down-regulated, had changed. By comparing HK to CK, 12 flavonoid metabolites, including 7 up-regulate and 5 down-regulate, had significant differences. There were also 27 metabolites of flavonoid that had significantly changed when comparing LK to HK, with 14 and 13 of them being up-regulated and down-regulated respectively. The enrichment classification results indicated that the impact of K on each group was mainly related to the biosynthesis of flavonoids (Figure 5B).

#### **PCA analysis of flavonoids metabolites of *L. barbarum* L. under different K treatments**

In the present study, two principal components, PC1 (42.7%) and PC2 (23.6%) were extracted (Figure 6). The PCA results show that LK, HK, and CK were clearly separated, and the repeated samples were compactly gathered.

#### **Analysis of flavonoid content of *L. barbarum* L. fruit under different K treatments**

The trends of flavonoids at different levels of K fertilization were investigated (Figure 7). Clearly, the total flavone contents decreased with increasing K supply (Figure 7A), which was 0.18 (g/100g FW) at LK and 0.105 (g/100g FW) at HK. There was a significant difference between HK and CK, LK ( $p < 0.05$ ), and also no differences from CK and LK ( $p > 0.05$ ). Among the seven types of flavonoid metabolites of Goji, the content of flavone (Figure 7B) and anthocyanins (Figure 7G) showed a decreasing and then increasing tendency with the increase of K supply. Flavone content was affected by K supply ( $p < 0.05$ ), and it reached the top in HK treatment. The content of anthocyanins was the highest in the LK treatment, which was significantly greater ( $p < 0.05$ ) than that in the CK and HK treatments. The flavanone content (Figure 7D) had a positive correlation with K fertilizer supply, and its content in HK level was obviously different from that in LK and CK levels ( $p < 0.05$ ). The isoflavone content (Figure 7F) featured an increasing and then decreasing trend with increasing K supply. It was the highest in CK and was significantly different from that in LK and HK treatments ( $p < 0.05$ ). Just a slight difference appeared in the content of flavone C-glycosides (Figure 7C), flavonol (Figure 7E), and flavonolignan (Figure 7H) in different K levels.

#### **Metabolic profiling of flavonoid in Goji fruits at different K fertilizer levels**

Heat map cluster analysis of flavonoid metabolites in Goji fruits was performed (Figure 8). Heat map cluster analysis The results demonstrated that 132 flavonoid metabolites grouped according to the relationship between their contents and K treatments. The red group showed a positive correlation with the K supply, while the blue group exhibited a negative correlation. The darker the colour the stronger the correlation.

#### **Discussion**

The research about the effects of K on the agronomic traits and fruit quality of Goji was fewer. However, it is necessary to know the relationship between k fertilization and yield qualities, which is important to companies. In this study, the differences in agronomic traits and nutrients of Goji under three K levels were revealed.

It was found that foliar spraying of  $\text{KH}_2\text{PO}_4$  on *Lycium ruthenicum* Murray significantly increased the yield of a single plant and the weight of 100 fresh fruits. However, it had no significant effects on the longitudinal diameters, transverse

151 diameters, and the fruit type index of black fruits. With the increasing  $\text{KH}_2\text{PO}_4$  spraying concentration, the size of black  
152 fruit tended to increase and then decrease [26]. In present study, K levels had a significant effect on the ratio of the longi-  
153 tudinal diameter to the transverse diameter of Goji fruits, and the influence on plant height, plant stem diameter, new  
154 branch increment, yield of fresh fruits per plant, dry fruit quantity within 50 g, ratio of different grade fruits, was slight  
155 (Figure1, Figure2). The shape of red fruit was flatter at LK, rounder at CK, and longer at HK. It was entirely different  
156 between black and red fruit, mainly because of *Lycium ruthenicum* Murr. and *L. barbarum* belong to different varieties  
157 and have different genetic backgrounds.

158 It showed that the application of K fertilizer had an effect on the improvement of Goji fruit quality. The content of sugar  
159 and vitamin C in fruit was higher than that in those fruits without K fertilizer [12]. However, there were no statistical  
160 analyses of the experiment data, and the nutrient content and metabolites were not analyzed. In previous study, K  
161 fertilizer had the greater effect on the content of polysaccharides, total sugar, and carotenoids of 'Ningqi 1' [27]. Never-  
162 theless, the material was different from this study and the experiment was done in the field, where many uncertainty  
163 factors occur. It illustrated that there was a significant correlation between the yield qualities of Goji and the amount of  
164 N and K applied. With the increase of N and K, the content of polysaccharides, flavonoids, and  $\beta$ -carotene increasing,  
165 while the content of betaine and the yield increased and then decreased [28]. However, whether the effects on yield or  
166 quality of Goji were N or K was unclear. It has been indicated that the flavonoids content in leaves and fruits of  
167 *Lycium barbarum* L. presented a decreasing and then increasing trend before harvesting. It was dropped to the lowest  
168 point when the fruits were ripe [23]. It was the same trend with the expression level of key genes related to flavonoid  
169 synthesis [24, 25]. This experiment showed the trend of flavonoids content before harvesting. The fertilizer influence on  
170 nutrients in the total period of harvest, from mid-June to mid-July, was not researched. During the development of  
171 *Lycium chinense* Miller fruits, the trend of flavonoid content was increased with the gradual maturation of the fruit. It  
172 was shown to be higher at the late stage of development [29]. Similarly, the material was different and it did not show  
173 the effect of K on nutrients of Goji. In present study, the contents of amino acids, flavonoids, polysaccharides, and  
174 betaine of Goji fruit were analyzed under three K treatments at different harvest. It was demonstrated that K fertilization  
175 had affected the content of glutamic acid and flavonoids in Goji fruits. The higher the level of K, the higher the content  
176 of glutamate but the lower the content of flavonoids (Figure 3 and Figure 4). Furthermore, the effect of K on flavonoid  
177 metabolites of Goji fruit was analyzed. The KEGG pathway showed that the flavonoids of Goji were synthesized  
178 through the phenylpropanoid pathway (Figure 5). Using PCA analysis, it was proved that there were large differences  
179 among LK, CK and HK (Figure 6). 132 flavonoid metabolites were identified, of which 36 metabolites up-regulated and  
180 30 metabolites down-regulated. The results presented that the higher the K supply, the lower the total flavanone content  
181 but the higher the flavanone content. The content of flavonol and anthocyanins decreased, and then increased with the  
182 increasing K application. On the contrary, isoflavone content increased and then decreased with the increase of K ap-  
183 plication. The content of flavone C-glycosides, flavonol and flavonolignan was not biologically statistically significant  
184 (Figure 7, Figure 8). Finally, correlating the results of agronomic traits with nutrient, we found that the fruit picked at  
185 the second time had the highest yield, the largest size, and the lower content of total amino acids and betaine, while the  
186 fruit picked at the fourth time had the smallest size and the higher content of total amino acids and betaine. It indicated  
187 that the total amino acids and betaine content in small 'Ningqi 7' fruits were significantly higher than those in large  
188 fruits.

## 189 Conclusion

191 The study discovered that the fruit shape of Goji could be significantly changed by K supply. With the increase of K  
192 content, the fruit shape changed from flat to round and then to long. Potassium (K) fertilization affected the contents of  
193 glutamic acid and flavonoids in Goji fruits. The content of glutamic acid was positively correlated with K supply, while  
194 that of flavonoids was negatively related to K supply. The study also presented that even though larger fruits have  
195 higher market prices and purchasers, their total amino acids and betaine contents were not as good as smaller ones. The  
196 results provided evidence of increasing the quality of Goji fruits, which may influence the hobbies of purchasers.

## 198 Materials and Methods

### 199 Plant Materials

200 The variety was called 'Ningqi 7', and seedlings were provided by the National Wolfberry Engineering Research Center,  
201 Ningxia, China.

### 203 Experimental Design and Conditions

The experiment was conducted in the phytotron of the Wolfberry Scientific Research Institute base of Ningxia Academy of Agricultural and Forestry Sciences, Ningxia, China. The temperature ranged from 24 – 27 °C and the humidity ranged from 70 – 80%. The illumination intensity ranged from 110 to 125  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (14 h light, and 10 h dark). There were fifteen plants assigned to each treatment, which planted in pots (diameter: 25 cm, height: 50 cm) containing 5 kg soil, respectively.

The measurements of soil conditions were as follows. The soil PH was detected using a DDSJ-319L electrode pH meter. In addition, total N (TN), total P (TP), total K (TK), available N (AN), available P (AP), available K (AK), total salt (TS), and soil organic matter (OM) were evaluated by using the standard test methods of the Chinese national standard [30]. Therefore, the conditions of the soil were: pH, 7.84; EC, 0.068 dS/m; TS, 0.42 g/kg; OM, 5.12 g/kg; TN, 0.44 g/kg; TP, 0.72 g/kg; TK, 7.50 g/kg; AN, 23.00 mg/kg, AP, 12.00 mg/kg; AK, 15.00 mg/kg.

Five trees were randomly assigned to each treatment, which had three replications. As the study of Hu [31], 500g of fertilizer was needed to produce 500g of dry fruits. Based on the research experiences of the group, a 5-year-old tree of Goji can produce about 500g of dry fruits a year. It was calculated that the total amount of N, P, and K fertilizer was 200g per plant. According to the previous research [32, 33], the control treatment was N, 85 g/plant; P, 65 g/plant; K, 50 g/plant. The three treatments in this experiment were, low K (LK), high K (HK), and control K (CK), as shown in Table 1. The N, P and K were supplied as urea (N 46%), ammonium dihydrogen phosphate (N 12%, P 61%), and potassium sulfate (K 52%), respectively. The total fertilizers needed for Goji were split into 3 times a year, in late March, early June and mid-August. For N, based fertilizer was 60%, then top dressing 20% for twice. For both P and K, based fertilizer was 40%, then top dressing 30% for twice. Each time, mixed fertilizers with water and supplied them to the pot.

#### Measurement of agronomic traits of Goji under different K treatments

Measurements of seven agronomic traits in three groups are shown as follows. In April, the plant height and the plant stem diameter at 5 cm above the ground were measured with the tape measure and vernier caliper respectively. In the middle of May, when the new branches grew to about 5 cm, a branch of each tree was selected and marked. Every 5 or 6 days, its length on the marked new branches was measured with a tape measure for a total of 5 times. From mid-June to mid-July, ripe Goji fruit was picked four times. At each time, the fresh fruits from each tree were picked, packed, and weighed separately. In the meanwhile, 5 to 6 fresh fruits were packed in 10 ml freezing tubes and stored in liquid nitrogen (-196 °C) rapidly, then keep the samples in a refrigerator at -80 °C to detect metabolites. At the same time, 5 fruits of each treatment when harvested were randomly selected, and their length and transverse diameters were measured. After that, the fresh fruits were transported to the drying room immediately at each time (50 °C for 5 h) and then preserved the samples in a dry and ventilated room to be counted, weighed, and graded. In late July, all the dry fruit samples from each time were prepared. The number of fruits within 50 g was counted before being graded. Because it is necessary to know whether K treatments influence fruit size, relating to the market prices of Goji closely. Finally, graded the dry samples using 3 sieve sizes: the first grade was 180 fruit/50g; the second grade was 220 fruit/50g; and the third grade was 280 fruit/50g. The ratio of the different grades was measured.

#### Nutrient composition of Goji fruit

For each grade the composition of polysaccharides, amino acids, total flavonoids and betaine contents were measured. Dry fruit samples were freeze-dried using a vacuum freeze dryer. Then cut the samples and freeze them using liquid nitrogen. Adding PBS after melting and homogenizing samples to pulp. Then centrifuged for 20 min and removed supernatant. To detect polysaccharides, total flavonoids, and betaine contents, the plant polysaccharide test kit, the plant flavonoid test kit, and the plant betaine test kit were applied separately [32, 34].

To detect amino acid contents, crushed samples were prepared. 0.02 mol/L hydrochloric acid was added, then centrifugation for 10 min. The supernatant added 6–8% sulfosalicylic acid. Then centrifuged for 15 min and obtained supernatant. The liquid samples were passed through the film on the machine, which obtained amino acid contents [32].

#### Quantification of flavonoid metabolites

Fresh fruit samples were freeze-dried, then crushed using a mixer mill. Added 70% aqueous methanol and centrifugation for 10 min. The extracts were absorbed and filtrated, then analyzed using an LC-ESI-MS/MS system. The HPLC conditions, including HPLC column, solvent system, gradient program, flow rate, and injection volume, were regulated to the appropriate state [32, 33]. Mass spectrometry was used to elute metabolites and perform multiple reaction monitoring (MRM) experiments [35]. The qualitative analyses of metabolites was based on the self-built database MWDB

(Metware Biotechnology Co., Ltd. Wuhan, China) and the public database of metabolite information. The quantitative analysis of metabolites was performed using MRM analysis of mass spectrometry [36-38].

### Statistical analyses

The data for agronomic traits and nutrient content were analysed using Microsoft Office Excel 2016, SPSS 23.0 (IBM Corporation, Armonk, NY, USA) and Graphpad Prism 8.0 (Graphpad software, Inc., 7825 Fay Avenue, Suite 230, La Jolla, CA 92037 USA). The results for the flavonoid metabolites were analyzed by using R (<http://www.r-project.org/>), which performs orthogonal signal correction and partial least squares-discriminant analysis (OPLS-DA) [39].

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## Author Contributions

R.W., Z.S. and Y.L. wrote the main manuscript text; T.H., Y.C., W.A. and X.Z. prepared figures 1-4; J.Z. and K.Q. prepared figures 5-8; X.W. and L.Y. investigative data. All authors reviewed the manuscript.

## Additional information

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350  
351 **Competing interests**

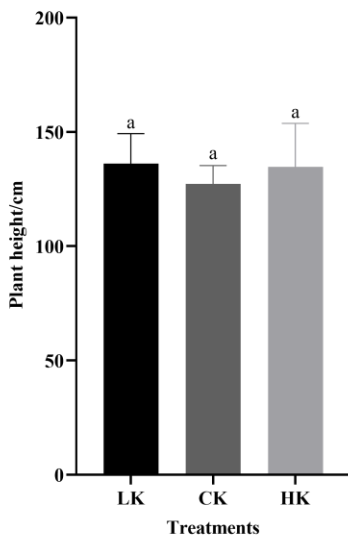
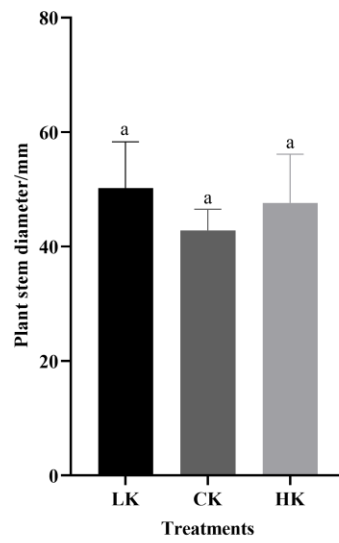
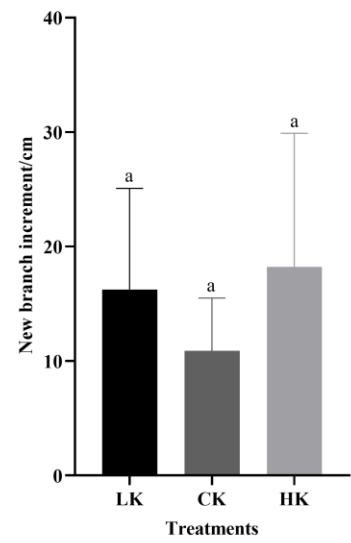
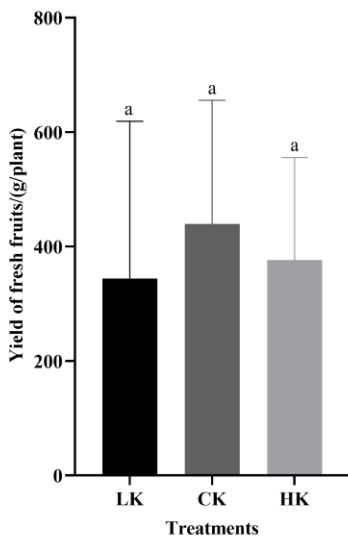
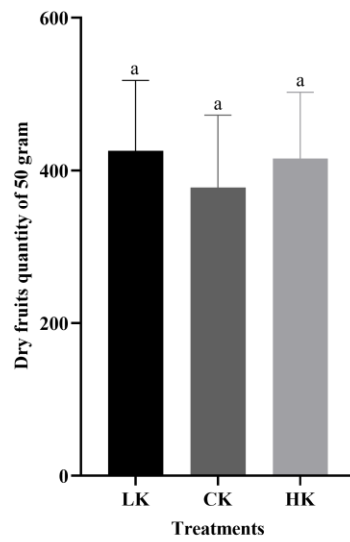
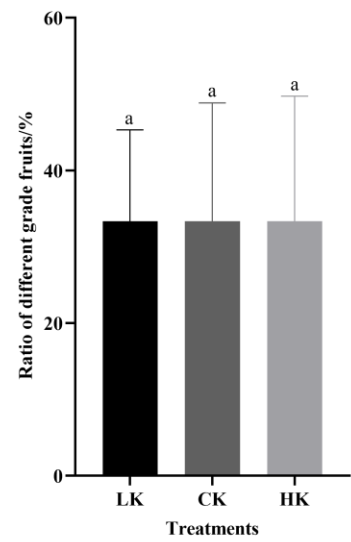
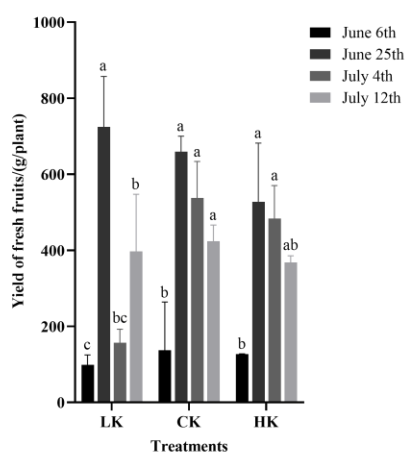
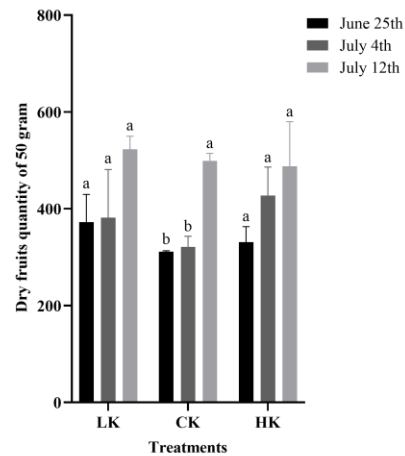
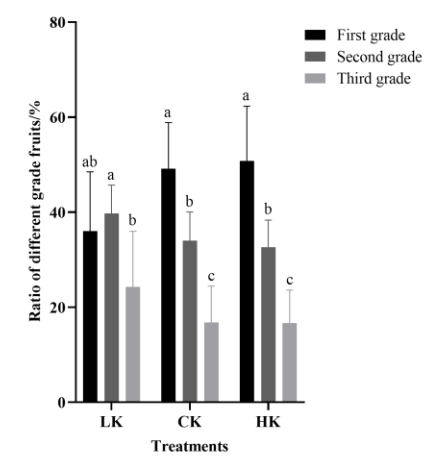
352 The authors declare no conflicts of interest.

353  
354 **Data availability**

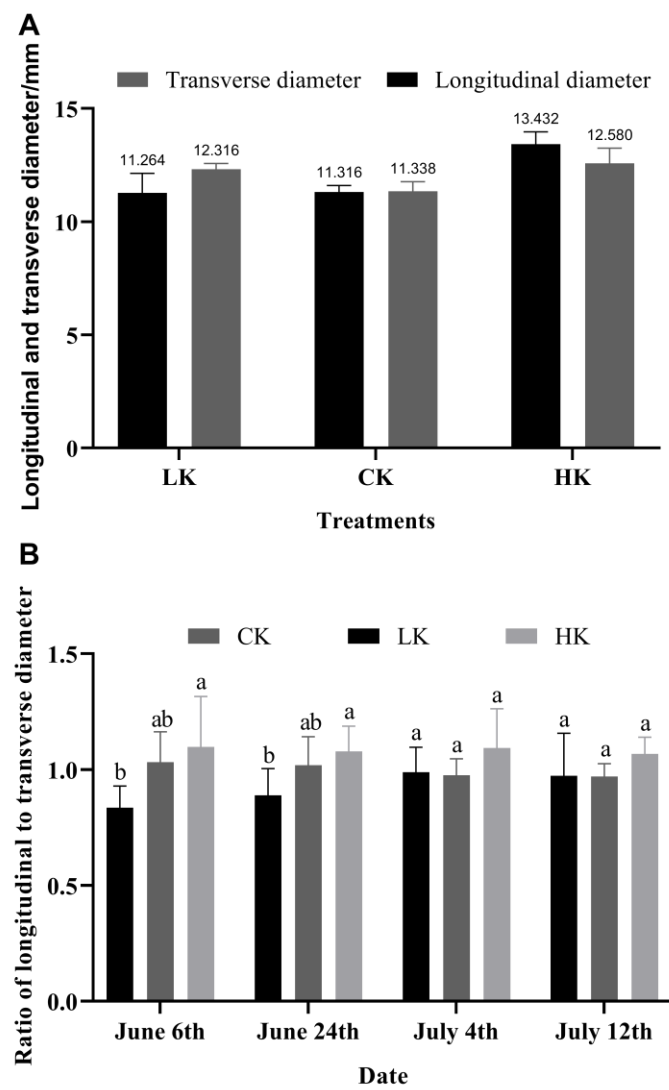
355 The datasets generated and/or analysed during the current study are not publicly available due to all the data are collected by the  
356 author, so it is not convenient to disclose but are available from the corresponding author on reasonable request.

357  
358 **Statement**

359 Experimental research and field studies on cultivated plants, including the collection of plant material, comply with relevant institu-  
360 tional, national, and international guidelines and legislation. All methods were carried out in accordance with relevant guidelines in  
361 the method section. Authors complied with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the  
362 Convention on the Trade in Endangered Species of Wild Fauna and Flora.

**A****B****C****D****E****F****G****H****I**

364 **Figure 1.** Agronomic traits of Goji fruit under different K fertilization. (A) plant height; (B) plant stem diameter; (C) new branch  
 365 increment; (D) yield of fresh fruits per plant; (E) dry fruit quantity of 50 gram; (F) ratio of different grade fruits. Agronomic traits of  
 366 Goji fruit at different harvests: (G) yield of fresh fruits per plant; (H) dry fruit quantity of 50 gram; (I) ratio of different grade fruits.  
 367 Small letters indicate a significant difference ( $p < 0.05$ ), as analyzed by Duncan's multiple tests; values without letters mean no signif-  
 368 icant difference.



369  
 370 **Figure 2.** The relationship between longitudinal diameter and transverse diameter under different K fertilization. (A) Longitudinal  
 371 and transverse diameter of Goji fruits at different levels of K; (B) ratio of longitudinal diameter to transverse diameter of Goji fruits  
 372 at three K treatments; small letters indicate a significant difference ( $p < 0.05$ ), as analyzed by Duncan's multiple tests; values without  
 373 letters mean no significant difference.

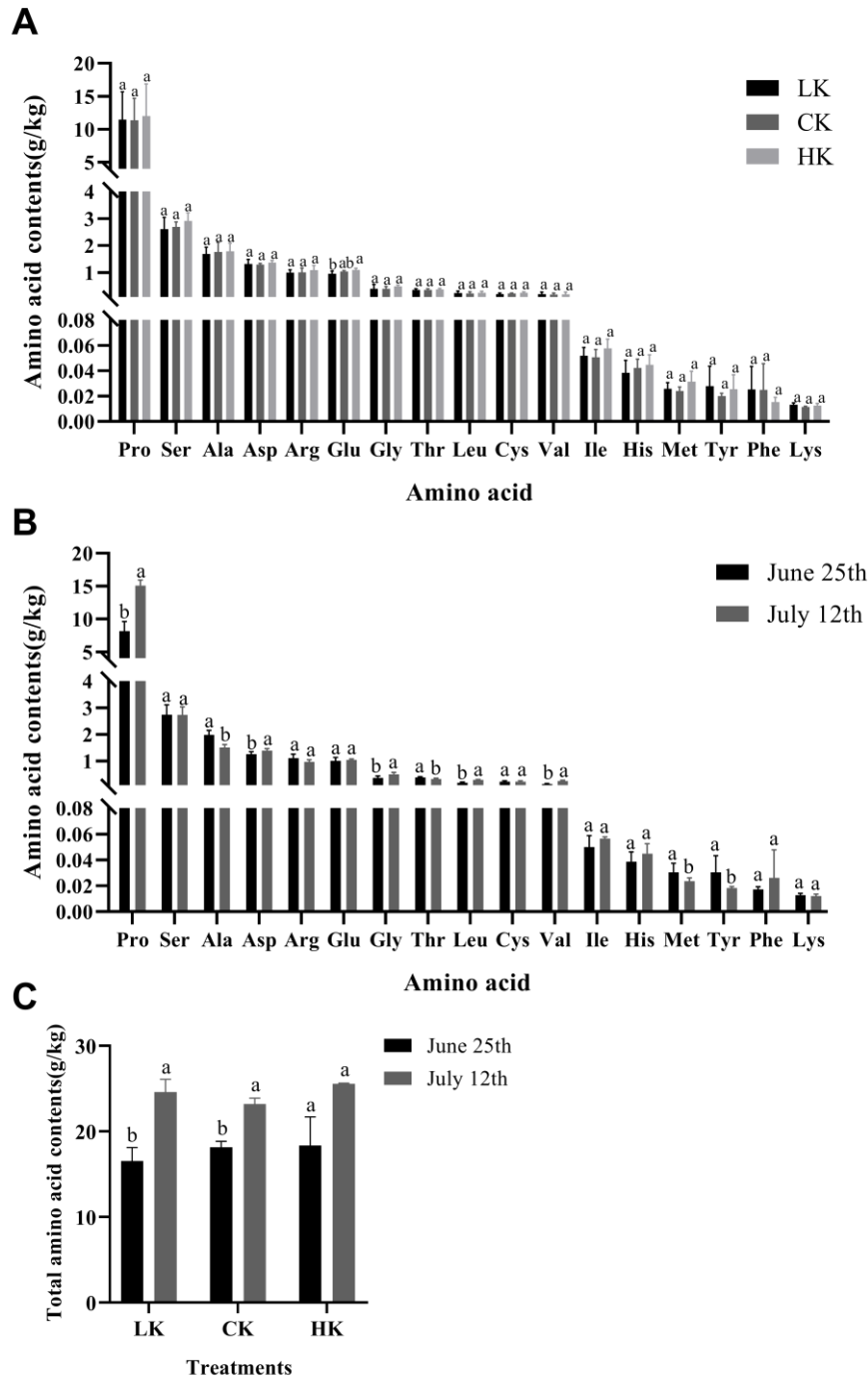


**Figure 3.** Amino acid contents in Goji fruits. (A) Amino acid contents of Goji fruits under different K levels; (B, C) amino acid and total amino acid contents of Goji fruits in different phenology; small letters indicate a significant difference ( $p < 0.05$ ), as analyzed by Duncan's multiple tests; values without letters mean no significant difference.

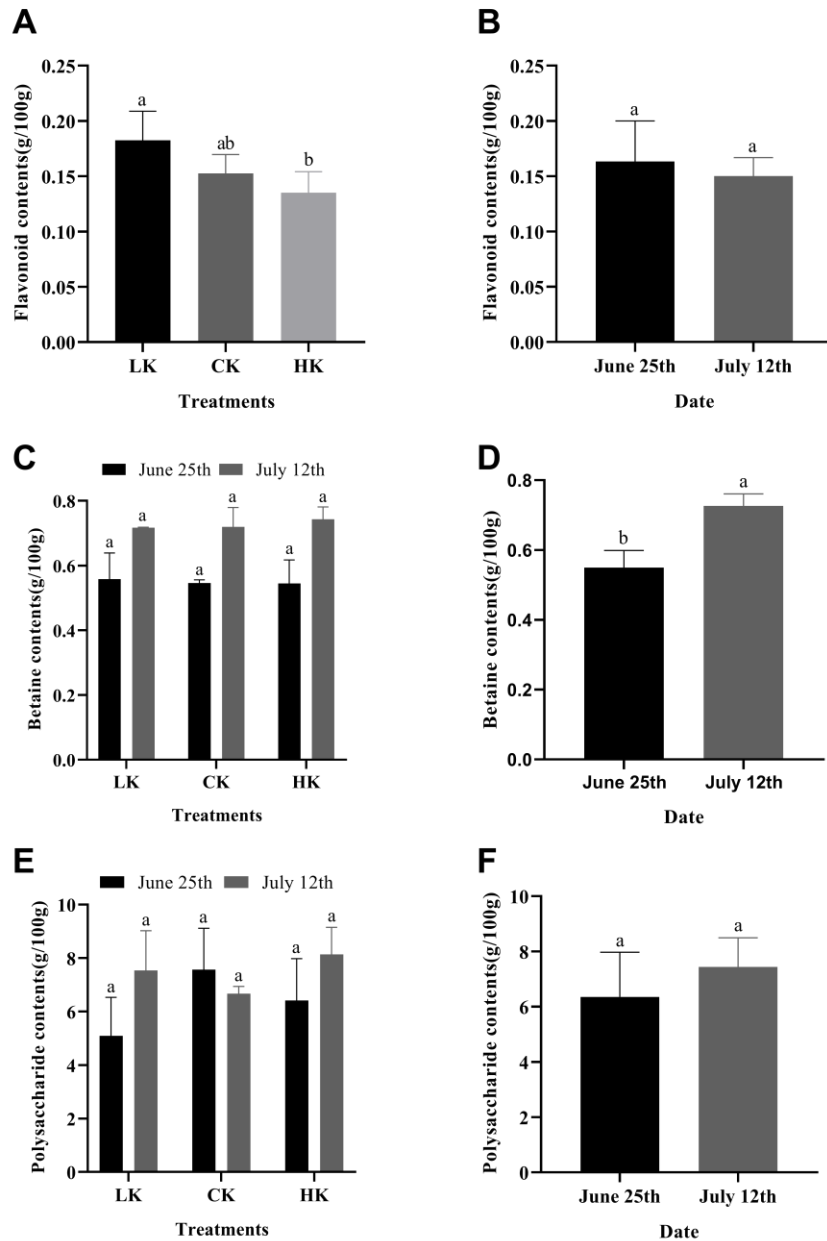


**Figure 4.** Flavonoids, betaine and polysaccharides contents in Goji fruits. (A, C, E) The content of flavonoids, betaine and polysaccharides in Goji fruits under different levels of K; (B, D, F) the content of flavonoids, betaine and polysaccharides in Goji fruits in different phenology; small letters indicate a significant difference ( $p < 0.05$ ), as analyzed by Duncan's multiple tests; values without letters mean no significant difference.

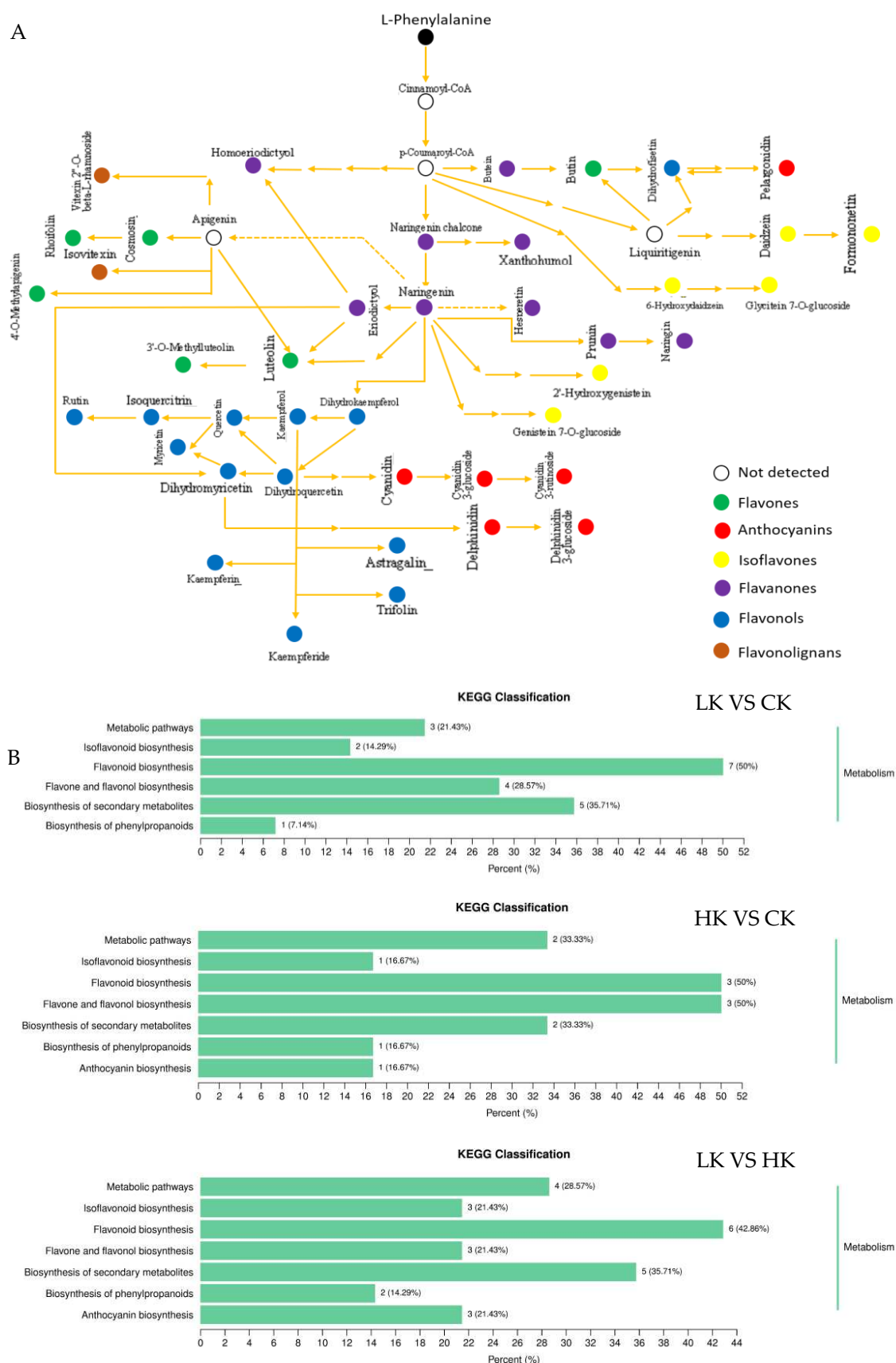


**Figure 5.** Enrichment analysis on Kyoto Encyclopedia of Genes and Genomes (KEGG); the *p*-value represents the degree of enrichment, and the closer the *p*-value is to 0, the more significant the enrichment is. The size of the point indicates the number of differential metabolites.

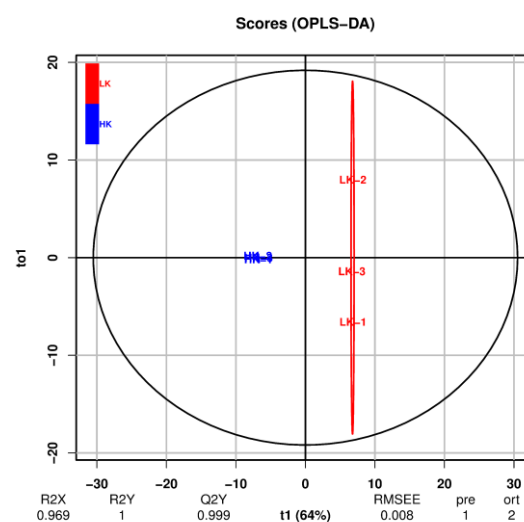
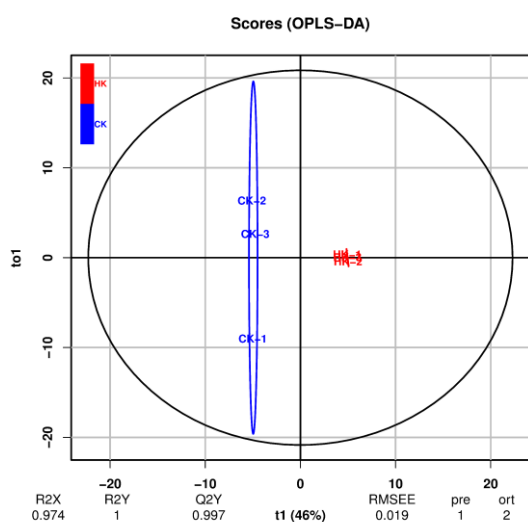
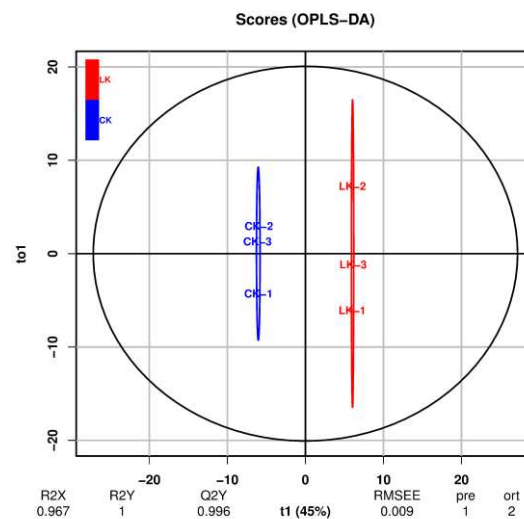
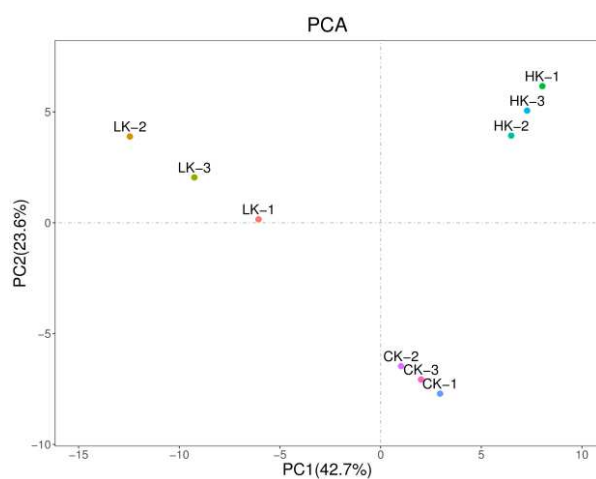
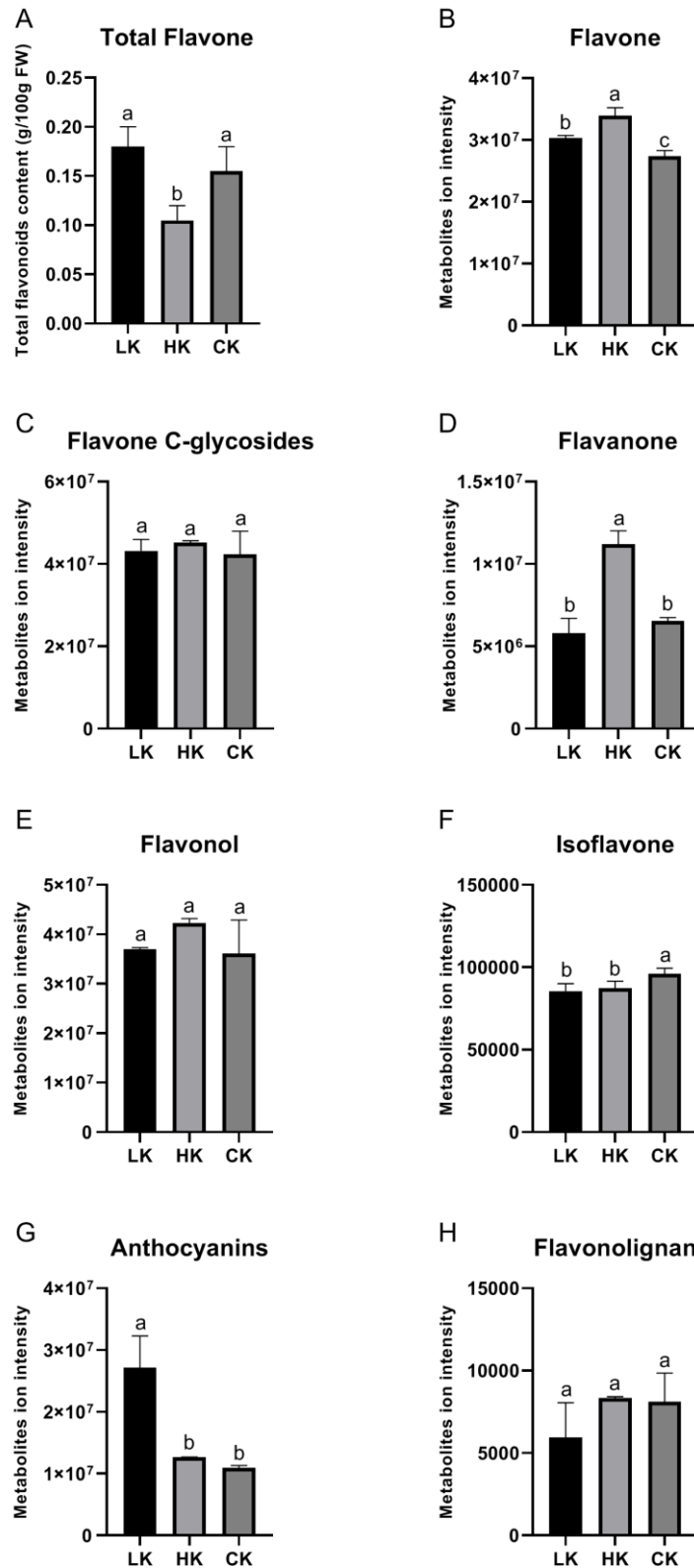


**Figure 6.** Analysis of flavonoid metabolites on the basis of principal component analysis.



389

390 **Figure 7.** Metabolite ion intensity of flavonoids in Goji fruits. (A) Total flavone content of goji fruits; (B, C, D, E, F, G, H) metabolite  
 391 ion intensity of flavone, flavone C-glycosides, flavaone, flavonol, isoflavone, anthocyanins and flavonolignan content in Goji fruit;  
 392 small letters indicate a significant difference among LK, HK, and CK ( $p < 0.05$ ), as analyzed by Duncan's multiple tests.

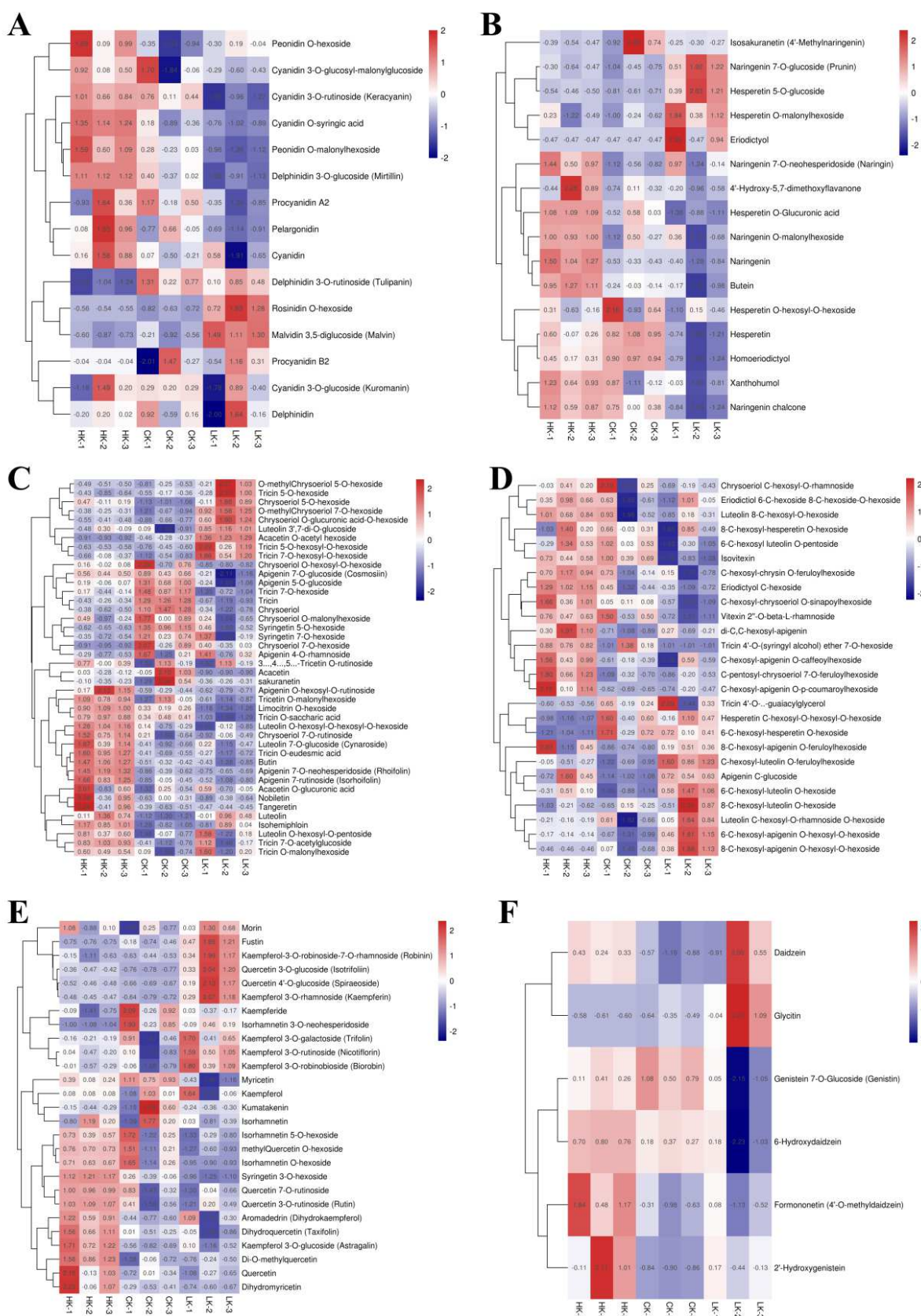


**Figure 8.** Heat map concerning flavonoids of Goji fruit. (A) Anthocyanins and proanthocyanidins of Goji fruit; (B, C) flavanone and flavone of Goji fruit; (D) flavone-C-glycosides and flavonolignan of Goji fruit; (E, F) flavonol and isoflavone of Goji fruit.

| Treatment | Nitrogen (g/plant) | Phosphorus (g/plant) | Potassium (g/plant) |
|-----------|--------------------|----------------------|---------------------|
| LK        | 85                 | 65                   | 25                  |
| HK        | 85                 | 65                   | 75                  |
| CK        | 85                 | 65                   | 50                  |

**Table 1.** Experimental treatments.