Nutritional quality of different potassium efficiency types of vegetable soybean as affected by potassium nutrition

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Author Contributions

Changkai Liu and Qiuying Zhang designed the experiments, supervised the study and Xiaobing Liu revised the manuscript. Changkai Liu, Xue Wang and Bingjie Tu performed research and wrote the manuscript. Houyu Xia, Heng Chen and Yansheng Li helped in planting and seed composition analysis.
Abstract

Pot experiments were conducted in 2017, 2019, and 2020 to examine the effects of potassium nutrition on the nutritional components of vegetable soybeans with different K efficiency at immature and mature stages. Two vegetable soybean varieties with higher K efficiency and two varieties with lower K efficiency were studied in the low available K soil under the condition of no K and normal K fertilization. The results indicated that almost all nutritional components in vegetable soybean were affected by K, genotypes, inter-annual differences, and their interactions. In general, no K fertilization increased protein and amino acid concentrations but decreased oil, soluble sugar, sucrose, K, Mg, and Fe concentrations in immature and mature vegetable soybean. The sensitivity of nutritional components to K nutrition differed among varieties. For instance, K high-efficiency varieties generally exhibited higher protein and amino acid concentrations without K application. K high-efficiency vegetable soybeans are low-K tolerance varieties to isoflavones. The results of this study provide insights for high yield and quality vegetable soybean breeding against soil K deficiency.

Key words: vegetable soybean, amino acids, isoflavones, sucrose, potassium, nutritional quality
Introduction

Vegetable soybean (edamame) is a food-grade soybean (Glycine max (L.) Merrill). The higher protein and phytochemicals but lower saturated fats, makes it a good health food (Makino et al., 2020). As a kind of specialty soybean species, vegetable soybean can be harvested at the R6 stage, when the seeds are still green and the pod is 80-90% filled, or harvested at maturity (R8 stage) (Saldivar et al., 2011). In vegetable soybean, protein accounting for about 40%, oil accounting for about 20%, and carbohydrate accounting for about 20-30% (Saito et al., 2021). It is also rich in isoflavones, phenols, trace elements, and other health components (Chen et al., 2020; Yu et al., 2021). Immature fresh edible vegetable soybean is rich in free amino acids and trace elements with better texture, while mature vegetable soybean has more nutritional values for deep processing (Liu et al., 2019b).

Potassium is one of the principal plant nutrients underpinning crop yield production and quality determination, and is involved in many physiological processes (Pettigrew, 2008). Yield and quality establishment of crops is highly dependent on soil K content (Wang et al., 2017; Loka et al., 2019). Vegetable soybean is a crop with strong responsiveness to K nutrition (Liu et al., 2017; Liu et al., 2019a). K deficiency not only restricted the yield formation of vegetable soybean but also reduced some nutritional compositions, such as sugars (Liu et al., 2017; Tu et al., 2017).

Previous studies found that K efficiency differences existed among vegetable soybean varieties (Liu et al., 2019a). Based on these findings, K high-efficiency vegetable soybean breeding strategies have been carried out. For instance, less yield loss and higher harvest index were found in K high-efficiency vegetable soybean genotypes (Liu et al., 2019a). However, these investigations mostly focused on the effect of yield or dry matter accumulation of vegetable soybean, but less attention has been paid to the effect of K deficiency on its nutritional value. With the upgrading of food consumption, people’s requirements for agricultural products are not only the requirements of output but also the improvement of quality (Asensio et al., 2019; Wang et al., 2021). Since the high nutritional value is an important characteristic of vegetable soybean (Song et al., 2003), it is of great significance to comprehensively explore the effect of different content of K in the soil on soybean quality components.
Therefore, there is an urgent need to clarify the effect of potassium on the nutritional quality of immature and mature vegetable soybean and to examine the difference in genotype response to low K conditions.

To comprehensively evaluate the effect of no K fertilization on vegetable soybean nutritional quality, this investigation studied the changes of protein, crude oil, amino acid components, isoflavones, mineral elements, soluble sugar, sucrose, and mineral elements concentrations of four vegetable soybean varieties belonging to two different K efficiency types at immature and mature stages. The results of this study will provide new insights for further K high-efficiency vegetable soybean breeding and efficient K fertilization management.

Materials and methods

Experimental design

Pot experiments were conducted in 2017, 2019 and 2020 at the Agronomy Farm of Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin (45° 73’ N, 126° 61’ E, and altitude 128 m). Monthly average temperature from April to October covering the whole growth stages of vegetable soybean at Harbin in three years is showed in Fig. 1. Based on the previous selection (Liu et al., 2019a), two K high-efficiency vegetable soybean genotypes Line 19 and Line 20, and two K low-efficiency genotypes Line 7, and Line 36 were used to examine the nutritional quality of vegetable soybean at fresh pod immature stage (R6) and mature stage (R8) (Fehr and Caviness, 1977). The pot size was 32 cm diameter by 27 cm tall. The ratio of sand to soil was 3:1. The available K content of the pot soil is 85, 92, and 86 mg kg\(^{-1}\) (extracted by 1 mol L\(^{-1}\) NH\(_4\)OAC and determined by flame photometry) in 2017, 2019 and, 2020, respectively, which is a potassium-deficient soil (less than 100 mg kg\(^{-1}\)). A uniform fertilizer application of 70 kg ha\(^{-1}\) diammonium phosphate (1.12 g P\(_2\)O\(_5\) pot\(^{-1}\)) and 98 kg ha\(^{-1}\) urea (0.7 g N pot\(^{-1}\)) at seeding were applied. Two K treatments were set, (1) 0 kg K\(_2\)SO\(_4\) ha\(^{-1}\) (K0), (2) 120 kg K\(_2\)SO\(_4\) ha\(^{-1}\) (1.8 g K\(_2\)SO\(_4\) pot\(^{-1}\)) (K120). Three seeds per pot were sown to simulate the field plant density. Plants in pots were artificially watered according to a consistent ration. Ten pots were planted for each K treatment. Samples were collected at R6 and R8 stages. Harvested samples were dried at 105 °C in a forced-air oven for 0.5 h immediately and then dried to a constant weight at 65 °C.
Measurements

Seed crude protein concentration was determined by Elementar-Vario (Elementar Analysensysteme GmbH E-III, Germany), using combustion nitrogen analysis method. Crude protein concentration = 6.25 × total nitrogen concentration (Saldivar et al., 2011). Crude oil concentration was determined using the Soxhlet extractor method (Li et al., 2012).

The concentration of sucrose and soluble sugar was analyzed by resorcinol hydrochloric acid method and anthrone sulfuric acid method, respectively (Li et al., 2012).

Mineral elements were determined using the method described by Madejon et al. (2003). Around 0.5 g seed sample was taken into a porcelain crucible. After carbonization, it was placed in a muffle furnace at 510 °C for 2 h. After cooling, 1 mL hydrochloric acid and 1 mL deionized water were added to dissolve the ash. Volumed up to 25 mL after filtering. The analysis of the trace element was performed by AAS (TAS-990, Beijing).

Isoflavone concentrations were determined using the HPLC analyses by Hoeck et al. (2000). Preparation of soybean seeds for HPLC analysis was described by Kim and Chung (2007).

Amino acid (AA) profiling was determined by the hydrolysis method. To hydrolyze the sample protein into its constituent AAs, the samples were acid digested with constant-boiling with 6 N hydrochloric acid at 110 °C for 24 h (Gonzalez et al., 2012; Li et al., 2020). Analysis of AAs were performed by amino acid analyzer (BIOCHROM 30+, Cambridge). Total essential amino acids (TEAA) were considered the sum of arginine, phenylalanine, histidine, isoleucine, leucine, lysine, methionine, threonine, and valine. The content of tryptophan in the samples was not determined.

Statistical Analysis

Excel 2016 and SPPS 25.0 were used for the analysis of statistical data. All data were subjected to an analysis of variance (ANOVA) using the General Linear Model (GLM) in SPSS 25.0 to identify significant treatment effects and interactions. Differences in protein, oil, sugars, and elements among treatments were examined by analysis of variance (ANOVA), and the means were separated by LSD test at the 5 % level. The figures were created using Sigmaplot 12.0.
Results

Effect of K nutrition on vegetable soybean yield

As shown in Figure 2A, no K application treatment (K0) significantly reduced the yield of vegetable soybean, especially in K low-efficiency varieties. In K high-efficiency vegetable soybean varieties, yield decreased by 3.7~6.5 % in 2017, 22.9~24.7 % in 2019, and 17.2~24.0 % in 2020. While, the yield of K low-efficiency genotypes decreased by 7.3~15.8 %, 34.6~37.9 %, and 34.5~39.5 %, respectively. In particular, the yield of K low-efficiency variety Line 36 decreased by 15.8~34.6 % over the three years. The highest yield was also found in variety Line 36 in K120 treatment, with 25.0 g plant⁻¹, 20.9 g plant⁻¹ and 14.6 g plant⁻¹ in 2017, 2019 and 2020. K high-efficiency variety Line 19 was least affected under no K fertilization, decreased by 3.7 %, 22.9 % and 24.0 % in 2017, 2019 and 2020. ANOVA showed that year, genotype, K fertilization and interaction of year × K, year × genotype and K × genotype had significant effects on yield and quality components (Table 1).

Effects of potassium nutrition on protein and amino acid concentration of vegetable soybean

The crude protein concentration of the four vegetable soybean varieties increased over the three years with no K application (Figure 2B). Higher crude oil concentrations were found at R8 stage. In 2017, the highest average crude protein concentration with 47.3 % was found in K high-efficiency variety Line 20 at R8 stage in K0 treatment. While, in 2019 and 2020, K high-efficiency variety Line 19 had the highest average crude protein concentration with 45.9 % and 45.0 % in K0 treatment. ANOVA showed that protein concentration of vegetable soybean was affected by year, genotype, K treatment, and interaction of year × K, year × genotype, K × genotype, and year × genotype × K factors at R6 and R8 stages (Table 1).

The amino acid concentrations of vegetable soybean were affected by year, genotype and K factors (Table 2). At R6 stage, K exhibited a less effect on amino acid concentrations, only Asp and Pro were significantly affected at $P < 0.05$ and $P < 0.01$ levels. Seed total amino acid concentration was also affected by the interaction effects of year × genotype and year × K (only at R8 stage). The three-year average total amino acid concentration (TAA) in four vegetable soybean varieties revealed an increased trend from R6 to R8 stage, increased by 19.1% and 16.1% under K0 and K120 treatments, respectively. No K fertilization treatment increased TAA and total essential amino acid concentration (TEAA) of matured seed in general, but there were genotype variations and inter-annual differences (Table 2). The K
high-efficiency variety Line 19 had the highest three-year average TAA and TEAA concentrations (Figure S1). K nutrition significantly affected the concentrations of TEAA and TAA in mature vegetable soybean, but had no significant effect on TEAA/TAA ratio (Table 2).

As shown in Figure 3, Glu was the highest amino acid component in vegetable soybean seeds, accounting for 15~18% of the total amino acid content. At R6 stage, the coefficients of variation (CV) of 14 (except Glu, Cys and Phe) amino acids among vegetable soybean varieties under K0 treatment were lower than those under K120 treatment (Figure 3). In contrast, at R8 stage, CVs of 16 (except Met) amino acids under K0 treatment were higher than those under K120 treatment. The CV of Cys at R6 and R8 stages was highest among the 17 amino acids.

**Effect of K nutrition on vegetable soybean crude oil concentration**

As shown in Figure 2C, mostly, without K fertilization decreased vegetable soybean crude oil concentration over the three years with genotype variations and inter-annual differences. Year, K fertilization, genotype, and interaction of year × genotype, K × genotype, and year × K × genotype had significant influences on seed crude oil concentration at both R6 and R8 stages (Table 1). In addition, interaction of year × K also had a significant effect on mature seed crude oil concentration (P < 0.001). Without K fertilization, the three-year average crude oil concentration of four vegetable soybean genotypes were generally decreased at R6 and R8 stages. From R6 to R8 stage, the three-year average of crude oil concentration increased by 16.1~23.7 % in K0 treatment and by 16.0~28.2 % in K120 treatment. The highest crude oil concentration with 22.2~23.5 % was found in K low-efficiency variety Line 7 in K120 treatment at R8 stage in the three years.

**Effect of K nutrition on vegetable soybean mineral element concentration**

The effect of K nutrition on vegetable soybean mineral element concentration is showed in Table 3. Six mineral elements, including K, Mg, Fe, Mn, Zn and Cu, were analysed at immature and mature stages in 2017, 2019 and 2020.

Obviously, K nutrition had a significant effect on K concentration in vegetable soybean seed. Compared with K0 treatment, the three-year average K concentration in K120 treatment were generally increased at R6 and R8 stages. The seed K concentration also exhibited genotypic and inter-annual differences (Table 1). Except for year, genotype and K treatment,
interactions of year × genotype and year × K had extremely significant effects on seed K concentration ($P < 0.001$).

The effect of K nutrition on seed Mg concentration mostly presented an increased trend over the three years (Table 3). The 3-year average Mg concentrations of the four varieties at R6 and R8 stages treated with K120 were 5.2% and 7.3% higher than those treated with K0. Generally, seed Mg concentration increased with seed maturing. The highest Mg concentration with 1.79 mg g$^{-1}$ was found in K120 treatment at R8 stage in 2020 in variety Line 20. The concentration of Mg was significantly influenced by year, genotype, K treatment factors and year × genotype interaction ($P < 0.001$) (Table 1).

K application generally increased seed Fe concentration, especially at R8 stage. The 3-year average Fe concentration increased by 2.3 % and 14.4 % at R6 and R8 stage, respectively. At R6 stage, genotype difference had the greatest effect on vegetable soybean Fe concentration. At R8 stage, all the three factors and their interactions had significant effects on Fe concentration (Table 3).

Generally, K application increased Mn concentration at R6 stage. From R6 to R8, the average of the 3-year Mn concentration of the four varieties decreased by 12.5 % and 20.7 % in K0 and K120 treatment, respectively.

The concentration of Zn in vegetable soybean was affected by year, genotype, interaction of year × genotype and year × K. From R6 to R8, the 3-year average Zn concentration of the four varieties decreased by 8.3 % and 9.9 % in K0 and K120 treatment, respectively.

With K application, seed Cu concentration of vegetable soybean showed a decreased trend. From R6 to R8 stage, the 3-year average Cu concentration increased by 12.3 % and 20.6 % in K0 and K120 treatment, respectively.

**Effect of K nutrition on vegetable soybean sucrose and soluble sugar concentration**

Concentrations of sucrose and soluble sugar in immature and mature vegetable soybean were increased by K application, but there were also genotype and inter-annual differences (Table 1). The interaction of year × genotype and year × genotype × K had extremely significant effects on the concentration of sucrose and soluble sugar ($P < 0.001$).

As showed in Figure 4, no K fertilization generally decreased vegetable soybean sucrose concentration. From R6 to R8 stage, the three-year average sucrose concentration increased
by 115~156 % in K0 treatment and 113~143 % in K120 treatment. Similar trend was also found in the changes of soluble sugar concentration. Without K application, Sucrose accounted for 54.1~87.4% and 57.5~86.0% of the soluble sugar content at R6 stage in K0 and K120 treatment, respectively. At R8 stage, sucrose accounted for 74.0~94.7% and 68.7~88.0% of the soluble sugar content in K0 and K120 treatment, respectively.

**Effect of K nutrition on vegetable soybean isoflavones**

As shown in Figure 5, K application generally increased vegetable soybean isoflavones. With K application, the three-year average total isoflavone concentration increased by 9.8 % at R6 stage and 34.6 % at R8 stage. Besides, vegetable soybean isoflavones of mature vegetable soybean was significantly higher than that of immature seed. From R6 to R8 stage, the three-year average of total isoflavone concentration increased 4.8 and 6.1 times under K0 and K120 treatment, respectively. Among those, glycitin was the least changed component, the three-year average glycitin concentration increased by 52.0% and 53.5% in K0 and K120 treatment, respectively. Daidzin and genistin were the main components of six isoflavones, accounted for 68.3% and 70.0% at R6 stage and 52.9% and 52.8% at R8 stage under K0 and K120 treatment, respectively. Over the three years, Line 36 had a higher isoflavone concentration, especially at R8 stage, ranged from 1531.2 to 3292.9 μg g⁻¹ and 2619.8 to 4024.1 μg g⁻¹ at K0 and K120 treatments. Besides, Line 36 also had higher inter-annual coefficient of variations with 46.9% and 21.0% under K0 and K120 treatments, respectively (Table S1). No K application generally decreased total isoflavone concentrations of low K-efficiency vegetable soybean types.

Vegetable soybean isoflavone concentration was also influenced by genotype, inter-annual, K treatment, and their interaction factors (Table 1). Among all the six components and the total isoflavone concentration at R6 and R8 stages, glycitin and glycitrin were not significantly affected by interaction of year and K at R8 stage, and daidzein was not significantly affected by K at R6 stage.

**Discussion**

As many studies reported, K deficiency usually led to lower crop yields (Pettigrew, 2008; Lu et al., 2017). The present study also found that low content of available K in soil and non-fertilization with this nutrient significantly lowered the vegetable soybean yield, especially in low K efficiency varieties. Differences in crop potassium sensitivity due to their genotype
differences have been demonstrated (George et al., 2002; Rengel and Damon, 2008; Tsialtas et al., 2017). Therefore, the selection and breeding of low potassium tolerant varieties is an important way against severe yield reduction, caused by K deficiency (Liu et al., 2019a).

Soil potassium deficiency not only affects soybean yield, but also seriously affects its nutritional quality (Pettigrew, 2008). Protein and oil are the two most abundant nutrients in soybean. There are opposing views on the effect of potassium application on soybean protein and oil accumulation. Yin and Vyn (2003) found that due to the inversely proportional relationship between seed oil and protein, K application increased the oil content of soybean but decreased the protein content. Present study also found that the crude protein and amino acid concentrations of vegetable soybean were generally increased in no K treatment (K0), especially in mature seed, whereas the crude oil concentration was decreased. As an opposing view, in the study of Bellaloui et al. (2013), K application generally increased soybean seed protein concentration. While, Krueger et al. (2013) did not find significant changes in total seed oil and protein in response to K fertilization. Pande et al. (2014) suggested that the different results might be due to differences in experimental climatic factors or related to the content of available K in the soil. Although K deficiency exhibited positive effects on vegetable protein concentration in this study, significant genotype differences also existed over the three years. For instance, high K efficiency vegetable soybean types had relatively higher protein concentrations under no K fertilization. This provides an insight for the breeding of vegetable soybean with low K tolerance and high protein production.

Amino acids are important components of proteins. As well as crude protein in vegetable soybean, low available soil K also exhibited a positive effect on the total amino acid (TAA) concentration, total essential amino acid (TEAA) concentration and most amino acid components (except Met). The concentration of Met was less influenced by K nutrition. Met is an essential amino acid for human body, Burton et al. (1982) also recognized that the selection for high percent protein in the soybean populations did not result in large changes in Met levels in the protein. This phenomenon suggests that the seed Met concentration might be less sensitive to low K. ANOVA analysis revealed that most amino acids of immature vegetable soybean were less affected by K. As the seeds matured, the response of amino acids to K showed obvious inter-annual and genotypic differences, and the variation coefficient (CV) under no K application treatment was higher than that under normal K application treatment. Therefore, these differences of amino acids in response to the low K condition also provide an opportunity for high quality vegetable soybean breeding during K
deficiency (Khoshgoftarmanesh et al., 2010). Consistent with changes in the crude protein concentration, high K-efficiency variety Line 19 also kept higher TAA concentrations under K0 treatment among three years.

Soluble sugar (chiefly sucrose) is seemed as the main component influencing vegetable soybean eating quality at fresh edible stage (Li et al., 2012). In the present study, no K fertilization affected soluble sugar and sucrose accumulation of immature and mature vegetable soybean negatively. Without K application, the three-year average soluble sugar concentration were generally decreased. This kind of reduction might be regulated by plant phytohormones and key enzymes of sucrose metabolism induced by low K stress (Liu et al., 2017; Tu et al., 2017). Higher soluble sugar and sucrose concentrations were found in mature vegetable soybean seeds, which was consistent with the previous study (Liu et al., 2019b). The soluble sugar and sucrose concentrations were also influenced by genotype and inter-annual or the interaction of year, genotype, and K factors. Ignoring environmental factors, it is theoretically feasible to breed varieties with low potassium tolerance and high sugar content (Schneider et al., 2002; Pettigrew, 2008).

Isoflavone is a kind of plant secondary metabolite, which belongs to a group of 3-phenyl derivative synthesized by cinnamyl-CoA. Soy isoflavones, often referred to as plant “estrogens”, are found higher in soybean (Azam et al., 2019). Soybean isoflavone concentrations vary widely, which may be different depending on distinct soybean varieties, tissue types, and growth conditions (Lee et al., 2008; Bellaloui et al., 2013). Although the isoflavone concentrations of vegetable soybean was greatly affected by inter-annual variation, the present study found that the promotion effect of potassium on isoflavones was eminent. It has been reported that suitable K application has a positive effect on soy isoflavones accumulation (Vyn et al., 2002; Bellaloui et al., 2013). Present study also found that K application could promote isoflavones accumulation in immature and mature vegetable soybean in most conditions. For instance, at R6 and R8 stages, the 3-year mean total isoflavone concentration increased by 9.8 % and 34.6 % by K application, respectively. Among the selected four varieties, Line 36 had the highest mature isoflavone concentration, which was 4024 μg g⁻¹ with K application in 2017. However, isoflavones of low K-efficiency variety Line 36 was also more sensitive to no K fertilization, the three-year average total isoflavone concentration decreased by 24.3 % and 37.9 % at R6 and R8, respectively. In contrast, the isoflavone concentration of high K-efficiency variety Line 20 was less affected by no K fertilization. Therefore, the tolerance to low potassium should also be considered
when breeding high isoflavone soybean varieties. The effect of potassium fertilization on isoflavones may be due to the stimulation of some specific enzymes involved in the synthesis of isoflavones (Vyn et al., 2002). For instance, potassium has been shown to increase the activity of phenylalanine ammonia-lyase (PAL) (Li et al., 2009), a key enzyme to the phenylalanine metabolic pathway (Camm and Towers, 1973). With seed maturing, isoflavone concentrations of vegetable soybean increased dramatically (Liu et al., 2019b). The concentration of total isoflavones in mature vegetable soybean was 4.8 times and 6.1 times higher than that in immature seeds under K0 and K120 treatments, respectively. Therefore, in order to obtain vegetable soybean with high isoflavone concentration, it is necessary to ensure sufficient potassium supply, select suitable varieties with high isoflavone concentrations, and harvest them at the maturity stage.

Vegetable soybean is rich in mineral elements (Liu et al., 2019b). In the present study, six mineral elements, including K, Mg, Fe, Mn, Zn, and Cu, were analysed at R6 and R8 stages. Concentrations of these mineral elements in vegetable soybean varieties showed a trend of K > Mg > Fe > Zn > Mn > Cu at both R6 and R8 stages. The elements accumulated in crops are affected by soil types, genotype variations or environmental factors (Wang et al., 2000). The results of this study were consistent with this view. With genotype differences, K application generally demonstrated a positive effect on seed K, Mg, and Fe concentrations, but a negative effect on the Cu concentration. Pande et al. (2014) also found that suitable K fertilization will promote soybean seed Fe accumulation. The concentration of Zn was less affected by K. K deficiency generally decreased Mn concentration of immature vegetable soybean but not consistent in mature seeds. In immature vegetable soybean, concentrations of K, Zn, and Mn were greater than that of mature seeds, while the concentration of Fe was less changed between the two stages. Therefore, both immature and mature vegetable soybeans provide a good source of elements for human beings (Liu et al., 2019b). Supplementation of potassium fertilizer is helpful to the improvement of K, Mg, Fe, and Mn, in potassium-deficient soils.

Overall, the nutritional qualities of both immature and mature vegetable soybean varieties are significantly affected by K nutrition. The tolerance of the nutritional components of different vegetable soybean varieties to low K condition is distinct, it also provides a way for the breeding of low K tolerance vegetable soybean. For example, the K high-efficiency variety Line 19 is a low K tolerance but high protein variety. K high-efficiency varieties are low K tolerance varieties to isoflavones. While, K low efficiency variety Line 36 is a high isoflavone but low K sensitive variety. In terms of crop nutrient management, appropriate
potassium supplementation, such as foliar fertilizer spraying at flowering stage (Tu et al., 2017), is an important measure to avoid yield reduction and quality degradation (Vijay et al., 2016).

Conclusions

Potassium is essential for vegetable soybean yield and quality. The effect of K nutrition on the nutritional quality of vegetable soybean is a comprehensive and complex process, which is affected by genotype and inter-annual factors. There are significant differences in the overall nutritional framework between immature and mature vegetable soybean varieties, as well as in their responses to K deficiency (Figure 6). In detail, concentrations of protein, oil, amino acids, isoflavones, sucrose, soluble sugar, Fe, and Mg are higher in mature vegetable soybean. No K fertilization generally increases protein and amino acid concentrations but decreases oil, sucrose, soluble sugar, isoflavones, K, Mg, and Fe concentrations. Similar to yield, the response to low K stress among genotypes exist for nutritional components. It is feasible to breeding high yield and quality varieties against K-deficient soils.

Declaration of interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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Legend of figures

Figure 1: Monthly average temperature from May to September in 2017, 2019, and 2020.

Figure 2: Effect of K nutrition on vegetable soybean yield (A), crude protein (B), and crude oil (C) concentrations. Error bars represent the standard deviation. Significant differences between K treatments for each variety are noted with an asterisk at $P < 0.05$.

Figure 3: Boxplot shows the concentrations of 17 amino acids and total amino acid of four vegetable soybean varieties under no K (K0) and normal K (K120) conditions at immature (A) and mature (B) stages. The experiments were conducted in 2017, 2019 and 2020.

Figure 4: Effect of K application on seed sucrose (A, B and C) and soluble sugar (D, E and F) concentrations at R6 and R8 stages. significant at $P < 0.05$ level.

Figure 5: Boxplot shows concentration of six isoflavone components and total isoflavone of four vegetable soybean varieties in no K and normal K fertilization conditions. Each box contains three years’ data with three replicates.

Figure 6: Schematic diagram revealing the effect of K nutrition on vegetable soybean nutritional quality at immature and mature stages.
**Legend of tables**

**Table 1:** Analysis of variance ($P$ values) for the effect of main effects of year (Y), genotype (G), K treatment (K), and their interactions on vegetable soybean yield, protein, oil and mineral elements. The experiments were conducted in 2017, 2019 and 2020. *, ** and *** mean significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels.

| Stage | Year (Y) | Genotype (G) | K treatment (K) | Y×G | Y×K | G×K | Y×G×K |
|-------|----------|--------------|-----------------|-----|-----|-----|--------|
| Yield | R8       | ***          | ***             | *** | **  | *** | NS     |
|       | R6       | ***          | ***             | *** | *   | *** | ***    |
| Protein| R8       | ***          | ***             | *** | **  | *** | ***    |
|       | R6       | ***          | ***             | *** | NS  | *   | **     |
| Crude oil| R8      | ***          | ***             | *** | **  | *** | **     |
| Sucrose| R8       | ***          | ***             | *** | NS  | *** | ***    |
| Soluble sugar| R8    | ***          | ***             | *** | NS  | *** | ***    |
|       | R6       | ***          | ***             | *** | NS  | *   | **     |
| K     | R8       | ***          | ***             | *** | NS  | NS  | NS     |
| Mg    | R8       | ***          | ***             | *** | NS  | NS  | NS     |
| Fe    | R6       | NS           | NS              | NS  | *   | NS  | NS     |
| Mn    | R8       | ***          | ***             | *** | NS  | NS  | NS     |
| Zn    | R8       | ***          | ***             | NS  | NS  | NS  | NS     |
| Cu    | R8       | ***          | ***             | *** | NS  | NS  | NS     |
| Daidzin| R6      | ***          | ***             | *** | NS  | NS  | NS     |
|                  | R6 | R8 |
|------------------|----|----|
| Glycitin         | ***| ***|
| Genistin         | ***| ***|
| Daidzein         | ***| ***|
| Glycitein        | ***| ***|
| Genistein        | ***| ***|
| Total isoflavone | ***| ***|

* NS: Not Significant
Table 2: Analysis of variance (P values) for the effect of main effects of year (Y), genotype (G), K treatment (K), and their interactions on seed amino acids. The experiments were conducted in 2017, 2019 and 2020.
|       | Year (Y) | Genotype (G) | K treatment (K) | Y×G | Y×K | G×K | Y×G×K |
|-------|----------|--------------|-----------------|------|-----|-----|-------|
|       | R6       | R8           | R6              | R8   | R6  | R8  | R6    | R8   | R6  | R8  |
| Asp   | NS       | NS           | ***             | ***  | *   | *** | **    | ***  | NS  | *   | NS  | NS  | NS  |
| Thr   | **       | ***          | **              | NS   | NS  | **  | *     | NS   | **  | NS  | NS  | NS  | NS  |
| Ser   | ***      | ***          | *               | NS   | NS  | NS  | NS    | NS   | NS  | NS  | NS  | NS  | NS  |
| Glu   | NS       | ***          | **              | NS   | *   | **  | **    | NS   | *   | NS  | NS  | NS  | NS  |
| Pro   | *        | ***          | ***             | ***  | **  | **  | *     | **   | **  | NS  | *** | NS  | NS  |
| Gly   | ***      | ***          | **              | NS   | **  | *   | NS    | **   | NS  | NS  | NS  | NS  | NS  |
| Ala   | ***      | *            | **              | **   | NS  | *** | *     | **   | NS  | *   | NS  | NS  | NS  |
| Cys   | ***      | ***          | ***             | ***  | NS  | *   | ***   | NS   | **  | NS  | NS  | NS  | NS  |
| Val   | NS       | ***          | NS              | NS   | *   | *   | NS    | NS   | NS  | NS  | NS  | NS  | NS  |
| Met   | NS       | ***          | NS              | **   | NS  | **  | *     | NS   | NS  | *   | NS  | NS  | NS  |
| Ile   | NS       | NS           | NS              | **   | *   | **  | *     | NS   | NS  | NS  | NS  | NS  | NS  |
| Leu   | NS       | NS           | NS              | NS   | NS  | NS  | NS    | NS   | NS  | NS  | NS  | NS  | NS  |
| Tyr   | ***      | ***          | ***             | NS   | NS  | *** | NS    | NS   | *** | NS  | NS  | NS  | NS  |
| Phe   | ***      | ***          | *               | NS   | NS  | NS  | NS    | NS   | NS  | NS  | NS  | NS  | NS  |
|     | His | Lys | Arg | TEAA | TAA | TEAA/TAA |
|-----|-----|-----|-----|------|-----|----------|
| NS  | *** | *** | *** | NS   | *** | ***      |
| NS  | *** | *** | *** | NS   | **  | **       |
| NS  | *** | **  | *** | NS   | **  | NS       |
| NS  | NS  | **  | NS  | NS   | **  | NS       |
| NS  | NS  | NS  | NS  | *    | *   | * NS     |
Table 3: Effect of K application on vegetable soybean mineral elements concentration at R6 and R8 stages. The experiments were conducted in 2017, 2019 and 2020.

| Element | Stage | Year | Line 19 | Line 20 | Line 7 | Line 36 |
|---------|-------|------|---------|---------|--------|---------|
|         |       | K0   | K120    | K0      | K120   | K0      | K120   |
| R6      | 20    | 13.7±0 | 15.1±0 | 14.8±0 | 15.6±0 | 14.5±0 | 16.3±0 | 15.1±0 | 15.4±0 |
|         | 17    | 47    | 25 | 41 | 80 | .06 | .06 | 14 | 02 |
|         | 19    | 14.8±0 | 16.7±0 | 17.0±0 | 18.2±0 | 15.8±0 | 15.9±0 | 16.6±0 | 16.8±0 |
|         |       | 03    | 23 | 12 | 01 | .03 | .13 | 03 | 03 |
|         | K     | 20    | 17.8±0 | 19.1±0 | 19.0±0 | 20.6±0 | 20.6±0 | 23.3±0 | 18.1±0 | 20.6±0 |
|         |       | 37    | 55 | 13 | 10 | .10 | .43 | 19 | 03 |
|         |       | 20    | 13.6±0 | 15.2±0 | 13.6±0 | 15.3±0 | 13.5±0 | 14.5±0 | 14.0±0 | 14.8±0 |
|         |       | 19    | 01    | 22 | 08 | .18 | .09 | 05 | 29 |
| R8      | 20    | 14.7±0 | 15.4±0 | 15.4±0 | 15.8±0 | 12.8±0 | 13.6±0 | 12.9±0 | 14.3±0 |
|         | 19    | 32    | 03 | 12 | 11 | .11 | .09 | 14 | 14 |
|         |       | 20    | 17.7±0 | 21.1±0 | 21.4±0 | 22.1±0 | 17.6±0 | 19.1±0 | 17.5±0 | 19.9±0 |
|         |       | 39    | 40 | 36 | 17 | .32 | .53 | 46 | 56 |
|         |       | 20    | 1.33±0 | 1.40±0 | 1.18±0 | 1.51±0 | 1.58±0 | 1.68±0 | 1.08±0 | 1.21±0 |
|         |       | 17    | 04    | 05 | 10 | 18 | .01 | .05 | 03 | 10 |
| R6      | 20    | 1.39±0 | 1.53±0 | 1.47±0 | 1.58±0 | 1.41±0 | 1.43±0 | 1.18±0 | 1.18±0 |
|         | 19    | 02    | 03 | 02 | 02 | .06 | .01 | 02 | 04 |
| Mg      | 20    | 1.53±0 | 1.46±0 | 1.51±0 | 1.49±0 | 1.51±0 | 1.63±0 | 1.40±0 | 1.36±0 |
|         | 20    | 04    | 03 | 07 | 05 | .01 | .02 | 04 | 07 |
|         |       | 20    | 1.46±0 | 1.55±0 | 1.41±0 | 1.59±0 | 1.56±0 | 1.61±0 | 1.16±0 | 1.32±0 |
|         |       | 17    | 01    | 06 | 01 | 03 | .01 | .03 | 02 | 07 |
| R8      | 20    | 1.49±0 | 1.48±0 | 1.58±0 | 1.68±0 | 1.21±0 | 1.36±0 | 1.13±0 | 1.26±0 |
|         | 19    | 06    | 03 | 06 | 09 | .03 | .01 | 01 | 01 |
|         |       | 20    | 1.59±0 | 1.70±0 | 1.60±0 | 1.79±0 | 1.50±0 | 1.52±0 | 1.35±0 | 1.43±0 |
|         |       | 05    | 06 | 14 | 02 | .06 | .07 | 03 | 09 |
| Mn      | 20    | 16.5±0 | 18.8±0 | 19.5±0 | 20.5±0 | 24.5±0 | 32.7±0 | 19.8±0 | 21.5±0 |
|         | 17    | 62    | 38 | 24 | 06 | .19 | .57 | 44 | 87 |
| R6      | 20    | 16.1±0 | 15.5±0 | 14.8±0 | 15.1±0 | 16.8±0 | 16.1±0 | 13.2±0 | 13.9±0 |
|         | 19    | 10    | 07 | 12 | 24 | .01 | .06 | 71 | 39 |
|         |       | 20    | 23.8±0 | 18.8±0 | 19.8±0 | 18.4±0 | 21.7±0 | 24.5±0 | 16.2±0 | 18.7±0 |
|         |       | 86    | 23 | 25 | 53 | .70 | .59 | 64 | 68 |
|         |       | 20    | 21.8±0 | 19.4±1 | 15.0±1 | 12.9±0 | 16.5±1 | 14.6±1 | 11.3±0 | 13.6±0 |
|         |       | 17    | 26    | 32 | 32 | .53 | .50 | 92 | 74 |
|         |       | 20    | 13.7±0 | 11.4±0 | 8.9±0.4 | 9.4±0.3 | 14.3±0 | 14.6±0 | 12.3±0 | 12.6±0 |
|      | 17    | 17.7±1.6 | 20.2±0.5 | 20.5±1.0 | 20.7±1.4 | 20.8±1.0 | 20.9±1.6 | 20.9±1.8 | 20.9±1.9 | 20.9±1.7 | 20.7±1.7 | 20.5±1.7 |
|------|-------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Cu   | 15.3±1.9 | 9.7±0.4 | 10.2±1.0 | 10.2±1.0 | 10.2±1.0 | 10.4±1.0 | 10.5±1.0 | 10.5±1.0 | 10.5±1.0 | 10.5±1.0 | 10.5±1.0 | 10.5±1.0 |
|      | 79.8±2.8 | 79.8±2.8 | 79.8±2.8 | 79.8±2.8 | 79.8±2.8 | 79.8±2.8 | 79.8±2.8 | 79.8±2.8 | 79.8±2.8 | 79.8±2.8 | 79.8±2.8 | 79.8±2.8 |
|      | 59.4±2.3 | 59.4±2.3 | 59.4±2.3 | 59.4±2.3 | 59.4±2.3 | 59.4±2.3 | 59.4±2.3 | 59.4±2.3 | 59.4±2.3 | 59.4±2.3 | 59.4±2.3 | 59.4±2.3 |
|      | 27.8±1.2 | 27.8±1.2 | 27.8±1.2 | 27.8±1.2 | 27.8±1.2 | 27.8±1.2 | 27.8±1.2 | 27.8±1.2 | 27.8±1.2 | 27.8±1.2 | 27.8±1.2 | 27.8±1.2 |
|      | 20.8±1.0 | 20.8±1.0 | 20.8±1.0 | 20.8±1.0 | 20.8±1.0 | 20.8±1.0 | 20.8±1.0 | 20.8±1.0 | 20.8±1.0 | 20.8±1.0 | 20.8±1.0 | 20.8±1.0 |
|      | 1.5±1.0 | 1.5±1.0 | 1.5±1.0 | 1.5±1.0 | 1.5±1.0 | 1.5±1.0 | 1.5±1.0 | 1.5±1.0 | 1.5±1.0 | 1.5±1.0 | 1.5±1.0 | 1.5±1.0 |
|      | 5.8±1.0 | 5.8±1.0 | 5.8±1.0 | 5.8±1.0 | 5.8±1.0 | 5.8±1.0 | 5.8±1.0 | 5.8±1.0 | 5.8±1.0 | 5.8±1.0 | 5.8±1.0 | 5.8±1.0 |
|      | 2.1±1.0 | 2.1±1.0 | 2.1±1.0 | 2.1±1.0 | 2.1±1.0 | 2.1±1.0 | 2.1±1.0 | 2.1±1.0 | 2.1±1.0 | 2.1±1.0 | 2.1±1.0 | 2.1±1.0 |
|      | 8.2±1.0 | 8.2±1.0 | 8.2±1.0 | 8.2±1.0 | 8.2±1.0 | 8.2±1.0 | 8.2±1.0 | 8.2±1.0 | 8.2±1.0 | 8.2±1.0 | 8.2±1.0 | 8.2±1.0 |
|      | 2.5±1.0 | 2.5±1.0 | 2.5±1.0 | 2.5±1.0 | 2.5±1.0 | 2.5±1.0 | 2.5±1.0 | 2.5±1.0 | 2.5±1.0 | 2.5±1.0 | 2.5±1.0 | 2.5±1.0 |
|      | 5.4±1.0 | 5.4±1.0 | 5.4±1.0 | 5.4±1.0 | 5.4±1.0 | 5.4±1.0 | 5.4±1.0 | 5.4±1.0 | 5.4±1.0 | 5.4±1.0 | 5.4±1.0 | 5.4±1.0 |
|      | 3.9±1.0 | 3.9±1.0 | 3.9±1.0 | 3.9±1.0 | 3.9±1.0 | 3.9±1.0 | 3.9±1.0 | 3.9±1.0 | 3.9±1.0 | 3.9±1.0 | 3.9±1.0 | 3.9±1.0 |
|      | 0.6±1.0 | 0.6±1.0 | 0.6±1.0 | 0.6±1.0 | 0.6±1.0 | 0.6±1.0 | 0.6±1.0 | 0.6±1.0 | 0.6±1.0 | 0.6±1.0 | 0.6±1.0 | 0.6±1.0 |
|   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| 20 | 114.6± | 116.3± | 103.0± | 136.8± | 110.9± | 118.0± | 92.7±6.7 | 120.8± |
Figure 2

Legend:
- Green: 56 413
- Orange: 56 4126
- Red: 56 4125
- Blue: 56 4123

(a) 2017
(b) 2019
(c) 2020

Lines 18, 20, 25, 28, and 38.

Graphs showing the comparison of different years and lines with error bars.
Figure 5
