Nutritional requirements and precise fertilization of wine grapes in the eastern foothills of Helan Mountain

Tingting Jiang[1], Pengke Yan[1,2], Tinghui Ma[3], Rui Wang[1,4]

(1. *College of Agronomy, Ningxia University, Yinchuan 750021, China*;
2. *College of Resources and Environment, Northeast Agricultural University, Harbin 150030, China*;
3. *Institute of Agricultural Resources and Environment, Ningxia Academy of Agriculture and Forestry Sciences, Yinchuan 750002, China*;
4. *Ningxia Grape and Wine Research Institute, Yinchuan, Ningxia 750021, China*)

Abstract: Cabernet Sauvignon grapes in the wine-producing area of Helan Mountain, East Ningxia, China, were the research object in this study. The dissection of the roots and branching stems method was used to explore the dynamic changes in the nitrogen, phosphorus, and potassium nutrient requirements of wine grapes over a number of growth stages. The results showed that over the whole growth period, the nitrogen content of the roots was the highest during the leaf-expansion stage and lowest during the turning-color stage, and that the nitrogen content of the leaves and fruit showed a downward trend as growth progressed. The nitrogen content of the secondary branches was the lowest during the fruit expansion stage and the highest during the leaf-expansion stage; and the phosphorus content of the roots was the highest during the leaf-expansion stage and lowest during the fruit expansion stage. The phosphorus content of the trunk and primary branches showed a trend of "rising-falling-rising". The phosphorus content of the leaves and secondary branches was the lowest during the turning-color stage, whereas the phosphorus content of the fruit was at its highest during this stage. The potassium contents of the secondary branches and fruit showed a downward trend, but the potassium content of the leaves was highest during the fruit expansion stage and lowest in the nutrient return stage. Over the whole growth period, the accumulation of nitrogen, phosphorus, and potassium in wine grapes was 129.92 kg/hm^2, 41.51 kg/hm^2, and 189.47 kg/hm^2, respectively, the total requirements for N, P_2O_5, and K_2O were 262.38 kg/hm^2, 288.15 kg/hm^2, and 569.04 kg/hm^2, respectively, and the reasonable nutrient requirement ratio was 1.00:1.10:2.17.

Keywords: wine grapes, macronutrients, nutritional diagnosis, fertilizer requirements, the eastern foot of Helan Mountain
DOI: 10.25165/j.ijabe.20221504.5406

Citation: Jiang T T, Yan P K, Ma T H, Wang R. Nutritional requirements and precise fertilization of wine grapes in the eastern foothills of Helan Mountain. Int J Agric & Biol Eng, 2022; 15(4): 147–153.

1 Introduction

The excellent terrain conditions of the wine-producing area on the eastern foothills of Helan Mountain in Ningxia, China, have led to the production of unique, high-quality wines[1,2]. The flourishing production area at the eastern foot of Helan Mountain has become a hot spot for the international wine industry[3]. The grape planting area in the region covers 40 700 hm^2, of which wine grapes account for 35 300 hm^2 and "Cabernet Sauvignon" is the main cultivar[4]. However, associated problems have appeared. Previously, fruit farmers lacked theoretical guidance on fertilization management and did not apply the appropriate amount of fertilizer, resulting in uneven nutrition in vineyards and reduced fertilizer utilization, which affected the healthy development of the Ningxia wine grape industry.

Nitrogen, phosphorus, and potassium are known as the "three elements of plant nutrition" and are the indispensable material basis for growth and development, yield formation, and the quality improvement of wine grapes[5,6]. The natural conditions, and cultivation and management measures in different production areas vary, which means that the nitrogen, phosphorus, and potassium contents in wine grapes are also different. For example, research in the former Soviet Union has shown that after fertilization over nine consecutive years the optimal amounts of nitrogen, phosphorus, and potassium for grapes were 60 kg/hm^2, 60 kg/hm^2, and 120 kg/hm^2, respectively. Whereas France produces high-end wines and the amount of fertilization in the vineyards was controlled at 60 kg/hm^2, 60 kg/hm^2, and 120 kg/hm^2, respectively[7]. Bell et al.[8] carried out a field study about the effect of nitrogen supply on the vegetative and reproductive growth of Cabernet Sauvignon under low nitrogen state conditions and concluded that the optimal nitrogen application rate was 100 g/plant. However, under the condition of drip irrigation to the gravelly limestone soil found on the eastern foothills of Helan Mountain in Ningxia, it is still not clear what the dynamic changes in nitrogen, phosphorus, and potassium nutrient requirements are for wine grapes.

The types and quantities of nutrient elements contained in various wine grape organs during each growth period vary and the demand for nutrients is also significantly different[9]. The nutritional diagnosis and analysis of the dynamic changes in various mineral nutrients can reveal the fertilizer requirements for wine grapes[10,11]. At present, many different methods are used to nutritionally diagnose fruit trees across the globe. For example,

Received date: 2021-08-20 **Accepted date:** 2022-05-08
Biographies: Tingting Jiang, MS, research interests: plant nutrition and utilization of agricultural resources, Email: Jiangtingting0321@126.com; **Pengke Yan**, PhD, research interests: resource utilization of agricultural waste, Email: yanpengke1993@163.com; **Tinghui Ma,** MS, research interests: plant nutrition and agricultural waste resource utilization, Email: ruoqian1@126.com.
***Corresponding author: Rui Wang,** PhD, Professor, research interests: Engaged in research on the utilization of agricultural resources. College of Agronomy, Ningxia University, Yinchuan 750021, China. Tel: +86-15909617205, Email: Email：amwangrui@126.com.

Mostashari et al.[12] used component nutrition diagnosis (CND) and diagnosis and recommendation integrated system (DRIS) diagnostic methods to determine the fertilization standard for grapes, and Portugal uses a DRIS analysis of petioles and has developed specific nutritional diagnostic criteria based on soil, climate, and cultivar variability[13]. However, these two diagnostic methods are based on leaf analyses and do not consider other organs. Furthermore, these diagnostic methods can only determine the order of fertilizer needs, but not the amount of fertilizer that needs to be applied[14].

The traditional diagnosis methods rely on a partial diagnosis, such as a leaf assessment, which has limitations. Root dissection and micro-loss sampling of various organs during the whole growth period can comprehensively, dynamically, and accurately produce a precise nutritional diagnosis of wine grapes, which has important guiding significance for promoting the scientific and precise fertilization of wine grapes in Ningxia.

2 Experimental procedures

2.1 Experimental site

This study was conducted at the Lilan Winery Base (38°16′38″N, 105°58′20″E) in Yongning County, Ningxia, China, from April to October, 2019. The region has a temperate arid continental climate with an altitude of about 1050 m, there is little rain all year round, and the average annual expected volume is about 200 mm. The average annual temperature is 8.8 ℃, the temperature difference between day and night is 10 ℃-15 ℃, the annual temperature is ≥10°C, the accumulated temperature can reach 3000 ℃, and the annual sunshine hours are above 2800 h. The frost-free period is 150-170 d and water rapidly evaporates. The soil parent material in this experimental area is mainly diluvial, and the soil types are aridisol order, sierozem soil types, and gravelly sierozem soil subtypes. The soil texture is loamy sandy soil. The basic chemical properties are shown in Table 1.

Table 1 Basic chemical properties of the vineyard soil

Soil depth/cm	pH	Total salt/g·kg^{-1}	SOM/g·kg^{-1}	TN/g·kg^{-1}	TP/g·kg^{-1}	AN/mg·kg^{-1}	AP/mg·kg^{-1}	AK/mg·kg^{-1}
0-20	8.54	0.65	8.29	0.51	0.25	39.60	39.68	110.40
20-40	8.50	0.44	5.01	0.39	0.20	28.35	25.18	89.37
40-60	8.60	0.43	3.49	0.31	0.20	23.80	17.27	81.80

2.2 Research methods

The research object was 7-year-old Cabernet Sauvignon, which was planted in a north-south direction. The shaping method was long tip tilting on the shelf, the plant row spacing was 0.6 m×3.5 m, the planting density was 4760 plants/hm^2, and the drip irrigation quota was 3000 m^3/hm^2. Vineyards with similar site conditions were chosen and five plots were set up. Vineyards with comparable site conditions were chosen and each contained five plots. Each plot had a total of 100 wine grapes plants at the germination stage. A fertilization ditch was dug 30 cm away from the root of the grape plant. It had a length and width of 30 cm and a depth of 60 cm. All the fertilizer was mixed with the excavated soil and then the soil was backfilled into the ditch. The amount of organic fertilizer was 4.5 t/hm^2 (Organic matter ≥45%, N-P_2O_5-K_2O: 2.5-1.0-1.5), and the amounts of actual fertilizer added were N 225 kg/hm^2, P_2O_5 195 kg/hm^2, K_2O 300 kg/hm^2.

During the germination stage (April 13), the leaf-expansion stage (April 28), the flowering stage (May 28), the fruit expansion stage (July 12), the turning-color stage (August 11), the mature stage (September 25), and the nutrient return stage (October 25), seven plant samples were collected along with a soil sample collected during the germination stage.

The plant samples were divided into roots, stems (main trunk, primary branches, and secondary branches), leaves, and fruits according to their organs (Figure 1). Five trees were selected for sampling each time by the arithmetic difference method, and the sampled soil depth was 0-60 cm. The coarse roots, medium roots, and fine roots were mixed together as the root samples, and the aboveground vegetation sampling method included 1/2 of the main trunk. Five entire branches from the sampling section were removed from each tree and each tree was divided into three layers. Then 200 leaves at different heights were taken and mixed together to form the leaf samples, and five bunches of fruits of similar quality were mixed together as the fruit samples. The soil samples were collected at a distance of 30 cm from the tree root, and were divided into three layers (0-20 cm, 20-40 cm, 40-60 cm) according to human disturbance and natural soil conditions. A total of 15 soil samples were collected.

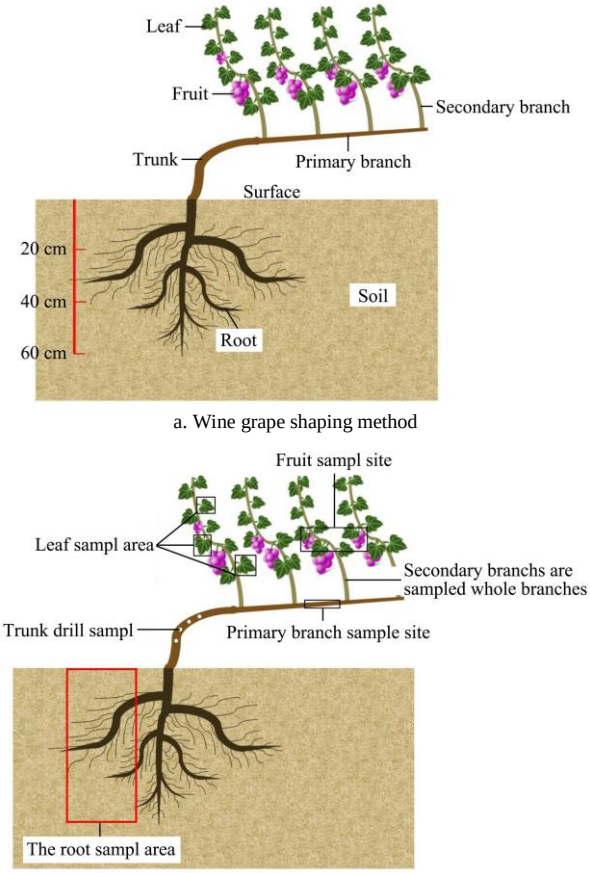

Figure 1 Wine grape shaping method and sampling sites of various organs

2.3 Measurement instruments and methods

2.3.1 Volume determination

The wine grape plants are coaxially branched and the branches were classified into the main stem, first-level branches, and second-level branches. Each level of branch was approximately regarded as being a truncated cone. At each sampling, the diameters of all marked grape trunks and half of the first-level

branches were measured with a Vernier caliper and recorded as *d*, and the length was measured with a tape measure and recorded as *L*. The volume $V = \pi(d/2)\times 2L$, which represents a cylinder and truncated cone of equal height. When the cylinders and truncated cones are of equal height, and the radius of the base of the cylinder is the middle term of the arithmetic difference between the radii of the two bases of the truncated cone, then their volumes are the same.

2.3.2 Density determination

A triangular flask was filled with water and tightly plugged. Any water attached to the flask was wiped off with clean filter paper and then it was weighed as M_1. The sample weight was M_2. The sample was placed in the triangular flask, which was then tightly stoppered and cleaned using filter paper. The flask plus sample was then weighed as M_3 (in order to prevent bubbles on the surface of the sample, the sample was soaked in a surfactant so that the effect on ρ is negligible), and the volume of the sample was calculated from $V=(M_1+M_2-M_3)/\rho_{\text{water}}$, where ρ_{water} is the density of water, in the standard state, $\rho_{\text{water}} = 1$ g/mL, and the density calculation formula for the sample is: $\rho = M_2/V$ [15].

2.3.3 Quality determination

The dry matter mass of the trunk and primary branches for the whole tree = $V\cdot\rho$, the dry matter mass of secondary branches = the mass of a single branch × the number of secondary branches, the dry matter mass of leaves = weight of 100 leaves/100 ×the number of leaves, the fruit dry matter mass = 100-grain weight/100 × number of fruits, root dry matter mass = root-shoot ratio × ground dry matter, and mass = root-shoot ratio ×(main stem dry matter mass + first-level branch dry matter mass + second-level branch dry matter mass + dry matter mass of leaves + dry matter mass of fruits)[16].

The total amount of dry matter produced at each stage = the overall dry matter mass of the stage – the dry matter mass of the previous stage.

2.3.4 Plant samples analysis

The collected plant samples were rinsed with distilled water and placed in an oven for 0.5 h at 150 ℃ to deactivate the enzymes. Then they were dried at 70 ℃ and crushed to determine the nitrogen, phosphorus, and potassium contents of the plants. The plant nitrogen, phosphorus, and potassium samples were digested with H_2SO_4-H_2O_2, total nitrogen content was measured by the micro-Kjeldahl method, total phosphorus content was measured by vanadium molybdenum yellow colorimetry, and total potassium content was measured by the flame photometer method[17].

2.3.5 Soil samples analysis

The collected soil samples were air-dried, ground, and passed through 1 mm and 0.25 mm sieves to determine pH, total salt, organic matter, total nitrogen, total phosphorus, alkali hydrolyzed nitrogen, available phosphorus, and available potassium according to Bao[17]. Soil pH (water-soil ratio 5:1) was measured with a PHS-25 (PHS-25, LeiCi, China) precision pH meter, total salt (water-soil ratio 5:1) was measured with a conductivity meter, organic matter was measured by the potassium dichromate volumetric method-external heating method, total nitrogen was measured using the Kay nitrogen determination method, total phosphorus was determined by the molybdenum-antimony anti-colorimetric method, alkaline hydrolysis nitrogen was determined by the alkaline hydrolysis diffusion method, available phosphorus was determined by the molybdenum-antimony anti-colorimetric method, and quick-acting potassium was determined by flame photometry.

2.3.6 Determination of macroelements accumulation

The accumulation of N in a certain organ during a certain growth period = the N fertilizer content of a certain organ in this growth period – the N fertilizer content of the organ in the previous growth period.

The accumulation of P in a certain organ during a certain growth period = the P fertilizer content of a certain organ in this growth period – the P fertilizer content of the organ in the previous growth period.

The accumulated amount of K in an organ during a certain growth period = the content of K fertilizer in an organ during this growth period – the content of K fertilizer in the organ in the previous growth period.

2.3.7 Determination of fertilizer requirements

N demand = nitrogen accumulation in each organ during each growth stage / nitrogen utilization rate (N utilization rate is calculated at 35%).

P_2O_5 demand = 2.29 × phosphorus accumulation in each organ during each growth stage / phosphorus utilization rate (the utilization rate of P_2O_5 is calculated as 25%).

K_2O requirement = 1.20 × potassium accumulation in each organ during each growth stage / potassium utilization rate (K_2O utilization rate is calculated as 45%) [18].

2.4 Data analysis

Excel 2007 was used to organize the data and graphs, SPSS 21.0 software was used for the analysis of variance, and the LSD method ($p < 0.05$) was used for multiple comparisons. The data in the table are the means ± standard error.

3 Results

3.1 Dry matter quality of various organs during the growing period for wine grapes

It can be seen from Table 2 that the wine raisin quality significantly increased over the whole growth period. The dry matter quality of the roots, main stems, and first-level branches was lowest at the germination stage (1628.64 g/plant, 1178.75 g/plant, and 275.39 g/plant, respectively, and the dry matter qualities were greatest during the nutrient return period (7717.13 g/plant, 1333.29 g/plant, and 344.29 g/plant, respectively), which increased by 373.84%, 13.11%, and 25.02%, respectively, compared to the germination stage. The dry matter quality of each organ significantly increased during the fruit expansion stage, and the fruit expansion stage qualities increased by 76.18%, 4.50%, 7.99%, 67.06%, 130.89%, and 48.91%, respectively compared to the flowering stage.

3.2 Cumulative change law for the quality of wine raisins

Table 3 shows that the dry matter accumulation in the wine grape roots was greatest during the expansion period, accounting for 44.00% of the entire growth period, and the accumulation of nutrients was lowest during the nutrient return stage, accounting for 27.39% of the entire growth period. Dry matter accumulation by secondary branches, primary branches, and the main stem over the whole growth period accounted for 91.23%, 4.68%, and 2.09% of the total dry matter accumulation by stems, respectively. Leaf dry matter accumulation over the whole growth period was 2522.26 kg/hm^2, and the dry matter accumulations during each growth stage were in a descending order for flowering stage, mature stage, turning-color stage, fruit expansion stage, leaf expansion stage, and nutrient return stage. The largest proportion occurred during the flowering stage (27.60%), and the accumulation of nutrients during the return stage was lowest at

3.92%. Dry matter accumulation by the fruit over the whole growth period was 1696.46 kg/hm^2, of which the greatest accumulation occurred during the fruit expansion stage followed by the turning-color stage, and the mature stage was the lowest.

Table 2 Dry matter quality of each organ during the growth period of wine grapes (g plant^{-1})

Growth stage	Root	Stem			Leaf	Fruit
		Trunk	First branch	Second branch		
GS	1628.64 ±6.00 g	1178.75 ±4.71 g	275.39 ±0.66 g	-	-	-
LS	1797.85 ±6.74 f	1184.52 ±4.26 f	278.00 ±0.72 f	57.85 ±0.14 f	159.86 ±1.19 f	-
FS	2910.87 ±12.36 e	1209.78 ±6.99 e	287.20 ±0.54 e	679.29 ±2.81 e	855.89 ±2.54 e	-
ES	5128.4 ±21.02 d	1264.26 ±5.53 d	310.14 ±0.95 d	1568.38 ±7.81 d	1274.51±4.28 d	815.77 ±2.88 c
TS	6529.62 ±23.87 c	1297.26 ±5.81 c	327.66 ±0.76 c	2342.25 ±9.92 c	1801.32 ±5.28 c	1406.92 ±4.46 b
MS	7579.13 ±27.63 b	1325.83 ±6.11 b	340.87 ±0.70 b	2925.21 ±12.51 b	2423.27 ±7.15 b	1696.46 ±5.29 a
NRS	7717.13 ±26.29 a	1333.29 ±6.22 a	344.29 ±0.69 a	3012.43±10.80 a	2522.26 ±6.86 a	-

Note: Abbreviations: GS: germination stage; LS: leaf-expansion stage; FS: flowering stage; ES: expanding stage; TS: turning-color stage; MS: maturation stage; NRS: nutrient reflux stage, different letters in the same column indicate that the difference is significant (p<0.05), the same below.

Table 3 Dry matter accumulation of wine grapes during each growth stage (kg hm^{-2})

Growth stage	Root	Stem			Leaf	Fruit
		Trunk	First branch	Second branch		
GS		-	-	-	-	-
LS	169.21 ±1.28 e	5.77 ±0.78 e	2.61 ±0.11 f	57.85 ±0.24 f	159.86 ±2.06 e	-
FS	1113.02 ±9.73 c	25.26 ±2.74 d	9.20 ±0.32 d	621.44 ±4.62 c	696.03 ±2.34 a	-
ES	2217.53 ±14.99 a	54.45 ±2.53 a	22.94 ±0.71 a	889.09 ±8.66 a	418.62 ±4.37 d	815.77 ±4.98 a
TS	1401.22 ±4.94 b	33.00 ±0.48 b	17.52 ±0.11 b	773.87 ±3.66 b	526.81 ±1.73 c	591.15 ±2.75 b
MS	1049.51 ±6.52 d	28.57 ±0.53 c	13.21 ±0.10 c	582.96 ±4.49 d	621.95 ±3.23 b	289.54 ±1.43 c
NRS	138.00 ±2.32 f	7.46 ±0.18 e	3.42 ±0.01 e	87.22 ±2.97 e	98.99 ±0.49 f	-

3.3 Dynamic analysis of nitrogen, phosphorus, and potassium contents in wine grapes

3.3.1 Dynamic analysis of nitrogen in wine grapes

Figure 2 shows that the nitrogen contents of the leaves and secondary branches significantly changed. From the leaf-expansion stage, the nitrogen content of the leaves decreased slowly, but the nitrogen content of the secondary branches slightly increased. The nitrogen content of the branches was lowest during the expansion stage, at 4.8 g/kg, and the nitrogen content during the open-leaf stage was highest, at 21.41 g/kg. The nitrogen content of the main stem and first-level branches over the whole growing period showed small fluctuations in the range of 2.62-8.07 g/kg, and both reached their lowest values during the expansion stage (2.62 g/kg and 3.43 g/kg, respectively). The nitrogen content of the roots showed a trend of "rising-falling-rising" throughout the whole growth period, reaching a peak value of 12.96 g/kg during the leaf-expansion stage, and was lowest at 6.82 g/kg during the turning-color stage. The nitrogen content of the fruit showed a downward trend from the expansion stage to the mature stage, and the maximum content during the expansion stage was 13.83 g/kg.

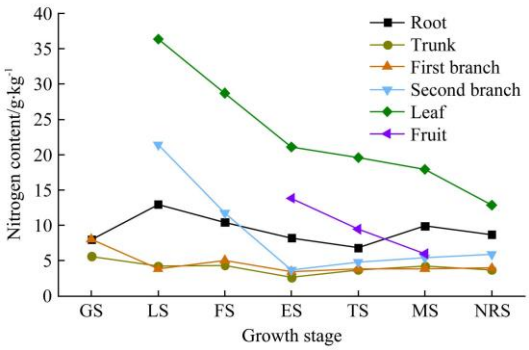

Figure 2 Dynamic changes to nitrogen content in various organs at the different growth stages

3.3.2 Dynamic analysis of phosphorus in wine grapes

Figure 3 shows that the phosphorus contents of the leaves and secondary branches over the whole growth period significantly changed during the early stage. It significantly decreased from the leaf expansion stage and reached its lowest value during the turning-color stage. The phosphorus contents were 2 g/kg and 1.43 g/kg, for the leaves and secondary branches, respectively. The phosphorus content of the trunk and first-level branches during the whole growth period showed similar changes and were in the range 0.56-3.28 g/kg. The difference was that the phosphorus content in the trunk was lowest during the expansion stage at 0.56 g/kg, whereas the phosphorus content in the first-level branch was lowest during the mature stage at 0.69 g/kg. The phosphorus content of the roots gradually increased from the germination stage, reached a peak at the leaf expansion stage, and then gradually decreased and reached its lowest value during the fruit expansion stage (2.82 g/kg). The phosphorus content of the fruit significantly increased from the fruit expansion stage to the turning-color stage where it reached 4.06 g/kg. It was lowest at the mature stage (1.45 g/kg).

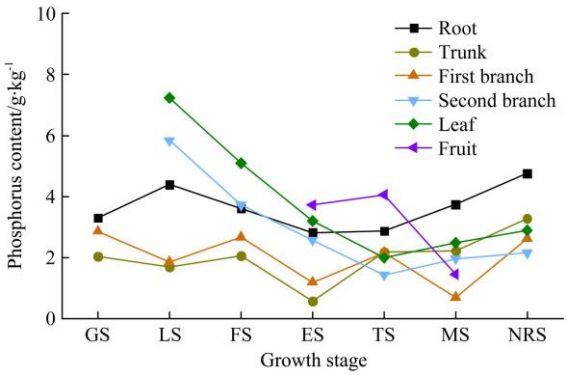

Figure 3 Dynamic changes to phosphorus content in various organs at the different growth stages

3.3.3 Dynamic analysis of potassium in wine grapes

Figure 4 shows that the potassium contents of the roots, main stems, and primary branches across the whole growth period varied from 4.11 to 11.26 g/kg, and that the potassium content of the roots was highest during the nutrient return stage (8.67 g/kg) and lowest during the leaf expansion stage (4.11 g/kg). The main stem potassium content was highest during the leaf expansion stage (7.07 g/kg) and lowest during the germination stage (5.13 g/kg). The potassium content of the first-level branches was highest during the fruit expansion stage (11.26 g/kg), and lowest during the mature stage (5.59 g/kg); the potassium content of the secondary branches significantly decreased from the leaf expansion stage and slightly after the flowering stage; and the potassium content of the leaves slightly decreased from the leaf expansion stage, reached its lowest value during the flowering stage, then increased slightly, but began to slowly decrease after reaching its peak during the expansion stage. The potassium content of the fruit gradually decreased from the expansion stage to the mature stage.

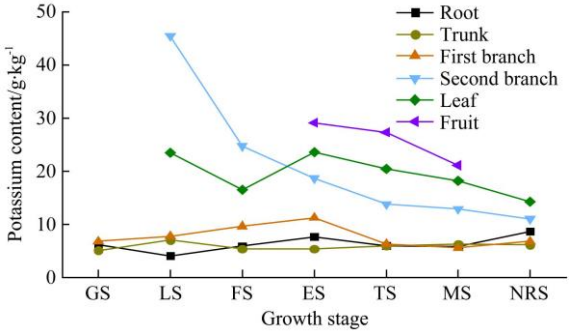

Figure 4 Dynamic changes to potassium content in various organs at the different growth stages

3.4 Accumulation of nitrogen, phosphorus, and potassium in wine grapes

It can be seen from Table 3 that nitrogen accumulation by the roots was greatest during the flowering stage and lowest during the nutrient return stage, while nitrogen accumulation in the stem was greatest during the flowering stage and lowest during the nutrient return stage. Overall, accumulation was low during the germination stage and greatest during the fruit expansion stage, accounting for 8.68% of the whole growth period. Nitrogen accumulation was lowest during the mature stage, accounting for 1.33% of the whole growth period.

The phosphorus accumulations in each organ were in a descending order for root, leaf, stem and fruit, while the phosphorus accumulations in roots at each growth stage were in a descending order for expansion stage, color-turning stage, flowering stage, mature stage, leaf development stage, nutrient return stage and germination stage. Phosphorus accumulation in stems was greatest during the flowering stage, and lowest during the nutrient return stage. Phosphorus accumulation in the leaves was also greatest during the flowering stage and lowest during the nutrient return stage followed by the maturity stage.

The potassium accumulations in each organ were in a descending order for stem, leaf, fruit and root. The roots accumulated the most potassium during the expansion stage and the least during the leaf-expansion stage, and the potassium accumulations during each growth stage by the stem were a descending order for expansion stage, flowering stage, turning-color stage, mature stage, leaf development stage and nutrient return stage. Potassium accumulations in the leaves over the whole growth period were highest during the flowering stage and lowest during the nutrient return stage, which were in a descending order for fruit expansion stage, color-turning stage, and mature stage.

Table 4 Nitrogen, phosphorus, and potassium accumulation in various organs at the different growth stages (kg·hm^{-2})

Growth stage	Root			Stem		
	Nitrogen	Phosphorus	Potassium	Nitrogen	Phosphorus	Potassium
GS	-	-	-	-	-	-
LS	2.19 ±0.02 e	0.74 ±0.01 d	0.07 ±0.01 f	1.27 ±0.01 e	0.35 ±0.00 d	2.63 ±0.02 e
FS	11.61 ±0.10 b	4.01 ±0.04 b	6.63 ±0.06 c	7.49 ±0.07 a	2.39 ±0.02 a	15.60 ±0.13 b
ES	18.21 ±0.12 a	6.25 ±0.04 a	17.05 ±0.12 a	3.48 ±0.04 c	2.34 ±0.02 b	17.16 ±0.18 a
TS	9.56 ±0.03 d	4.02 ±0.02 b	8.41 ±0.03 b	3.90 ±0.01 b	1.22 ±0.01 c	11.00 ±0.05 c
MS	10.40 ±0.06 c	3.93 ±0.02 c	6.19 ±0.04 d	3.35 ±0.03 d	1.21 ±0.01 c	7.78 ±0.06 d
NRS	1.20 ±0.02 f	0.06 ±0.01 e	1.20 ±0.02 e	0.56 ±0.01 e	0.22 ±0.01 e	1.03 ±0.03 f
Total	53.17	19.01	39.55	20.05	7.72	55.267
Growth stage	Leaf			Fruit		
	Nitrogen	Phosphorus	Potassium	Nitrogen	Phosphorus	Potassium
GS	-	-	-	-	-	-
LS	5.82 ±0.07 d	1.16 ±0.01 d	3.76 ±0.05 e	-	-	-
FS	2.00 ±0.07 e	3.55 ±0.01 a	11.51 ±0.04 a	-	-	-
ES	8.82 ±0.09 c	1.34 ±0.01 c	9.88 ±0.10 d	11.28 ±0.07 a	3.03 ±0.02 a	23.76 ±0.15 a
TS	10.31 ±0.03 b	1.05 ±0.00 e	10.76 ±0.04 c	5.59 ±0.03 b	2.40 ±0.01 b	16.16 ±0.08 b
MS	11.15 ±0.06 a	1.54 ±0.01 b	11.31 ±0.06 b	1.73 ±0.01 c	0.42 ±0.00 c	6.11 ±0.03 c
NRS	1.27 ±0.01 f	0.29 ±0.00 f	1.41 ±0.01 f	-	-	-
Total	38.1	8.93	48.63	18.60	5.85	46.03

3.5 Analysis of the law of nutritional requirements for wine grapes

Table 5 shows that the demand for and the proportions of N, P_2O_5, and K_2O in wine grapes are different during each stage. The total demands for N, P_2O_5, and K_2O were 262.38 kg/hm^2, 288.15 kg/hm^2, and 569.04 kg/hm^2, respectively, and the total ratio was 1.00:1.10:2.17. Wine grapes have the greatest demand for N, P_2O_5, and K_2O during the expansion stage (83.58 kg/hm^2, 89.93 kg/hm^2, and 203.55 kg/hm^2, respectively). Each accounted for 31.85%, 31.21%, and 35.77% of the total demand during the

entire growth period, respectively. The N demand was in a descending order for fruit expansion stage, turning-color stage, mature stage, flowering stage, leaf development stage, nutrient return stage, and germination stage; the P_2O_5 demand was in a descending order for fruit expansion stage, flowering stage, turning-color stage, mature stage, leaf development stage, nutrient return stage, and germination stage; and the K_2O demand was in a descending order for fruit expansion stage, turning-color stage, flowering stage, mature stage, leaf development stage, nutrient return stage, and germination stage. The ratio for $N:P_2O_5:K_2O$ changed with the growth stage. The ratio for N first decreased and then increased, and the ratios for P and K both increased at first, but then decreased.

Table 5 Nutritional requirement characteristics during each growth stage

Growth stage	N/kg ·hm^{-2}	P_2O_5/kg ·hm^{-2}	K_2O/kg ·hm^{-2}	$N:P_2O_5:K_2O$
GS	-	-	-	-
LS	18.56 ±0.20 e	15.61 ±0.14 e	19.38 ±0.24 e	1.18:1.00:1.24
FS	42.20 ±0.48 d	69.05 ±0.49 b	101.22 ±0.11 c	1.00:1.63:2.40
ES	83.58 ±1.90 a	89.93 ±0.62 a	203.55 ±1.65 a	1.00:1.08:2.44
TS	58.72 ±0.20 b	60.33 ±0.28 c	138.99 ±0.60 b	1.00:1.03:2.37
MS	53.26 ±0.32 c	49.27 ±0.28 d	94.17 ±0.57 d	1.08:1.00:1.91
NRS	6.06 ±0.08 f	3.96 ±0.14 f	11.73 ±0.18 f	1.53:1.00:2.96
Total	262.38	288.15	569.04	1.00:1.10:2.17

4 Discussion

4.1 Dynamic analysis of the macronutrient requirements for wine grapes

Appropriate amounts of nitrogen fertilizer at the appropriate stage can coordinate the vegetative and reproductive growth of wine grapes, and promote leaf tip germination[19,20]. Metay et al.[21] believed that the nitrogen that accumulates in the tree body is gradually absorbed by the roots from the germination stage and is transferred to the new tissues. It then migrates from the vegetative growth areas to the reproductive growth areas after flowering. The vegetative organs provide nutrition for the reproductive organs from the leaf development stage to the expansion stage and the nitrogen contents of leaves and fruits gradually decrease during the reproductive stage as the fruit expands. At the mature stage, the nitrogen returns and is stored in the roots and stems, which increases their nitrogen contents. This study found that the root nitrogen content of wine grapes across the whole growth period reached a peak during the leaf development stage, was lowest during the turning-color stage, and gradually increased at the mature and nutrient return stages. The stem contents were lowest during the expansion stage. The content showed a linear downward trend.

Phosphorus transfers energy in the metabolic process, and participates in the control of carbohydrate metabolism and the transport of carbohydrate substances[22]. Schreiner et al.[23] suggested that early phosphorus was mainly used for the vegetative growth of wine grapes, the differentiation of leaf and flower buds, and the formation of new tissues. In this study, the phosphorus content of wine grape leaves and secondary branches gradually decreased during the leaf development stage. After reaching its lowest point, the phosphorus content of the leaves increased. In contrast, the phosphorus content of the fruit showed an opposite trend. It was greatest during the turning-color stage, which was consistent with the results reported by Schreiner et al. The slow movement of phosphorus means that the phosphorus content in secondary branches and leaves gradually decreases. As the fruit expands, the accumulation of dry matter increases and phosphorus gradually migrates to the fruit. After the turning-color stage, a large amount of phosphorus accumulates in the fruit, and this accumulation continues until the fruit matures. During this stage, the phosphorus gradually migrates to the growth point and perennial organs, which means that the phosphorus content of each organ gradually rises.

The growth, development, and wine quality of grapes are all affected by potassium[24]. Poni et al.[25] suggested that potassium is highly mobile in the tree and rapidly migrates to the site of vigorous metabolism after being absorbed by the root system. This study found that the potassium contents of the roots, trunks, and primary branches significantly changed over the whole growth period. The overall values were 4.11-11.26 g/kg, and the potassium content of the fruit showed a downward trend. The potassium content of the leaves decreased from the leaf expansion stage, increased during the expansion stage, and then began to decrease. The reason is that before the mature stage, the tree body continues to absorb the potassium necessary for growth, and the potassium in the leaves, roots, and stems migrates to the fruit. At the mature stage, potassium flows back and is stored in roots and stems, which decreases the potassium content in leaves and the increases the potassium contents of roots and stems.

4.2 Determination of the fertilization requirements during the different growth stages of wine grapes

Fertilizer utilization by wine grapes is directly affected by the nitrogen, phosphorus, and potassium ratio. This study found that the total demands for N, P_2O_5, and K_2O were 262.38 kg/hm^2, 288.15 kg/hm^2, and 569.04 kg/hm^2, respectively, with a ratio of 1.00:1.10:2.17. During the expansion stage, the demand for N, P_2O_5, and K_2O all reached their maximums, accounting for 31.85%, 31.21%, and 35.77% of the total demand, respectively. This shows that the expansion stage is when the nitrogen, phosphorus and potassium are most efficiently used in wine grapes. During the expansion stage, the volume of the various wine grape organs increase, the cells grow and divide, dry matter contents gradually increase, and the demand for nitrogen rises. Phosphorus controls the metabolism of carbohydrates and their movement in fruit trees. As the fruit expands, dry matter accumulation, carbohydrate levels, and P_2O_5 demand increase. The increase in potassium accumulation during the wine grapes ripening process promotes the synthesis[26], migration, and transformation of protein and sugar in the fruit. Therefore, after the fruit expands, the quality of the fruit gradually improves with the change in fruit color, and the demand for K_2O in the fruit decreases.

5 Conclusions

On the relatively poor alkaline calcareous soil found across the eastern foothills of Helan Mountain in Ningxia, the demand for N during the grape vegetative growth stage is high, whereas the demand for P and K gradually increases during the later stages of expansion, especially the demand for K during the mature stage. The fruit expansion stage is the stage when the nutritional requirements of wine grapes are the greatest. The requirements for N, P_2O_5, and K_2O over the whole growth period of 7-year-old Cabernet Sauvignon are 262.38 kg/hm^2, 288.15 kg/hm^2, and 569.04 kg/hm^2, respectively. The most suitable N, P and K ratio is 1.00:1.10:2.17.

Acknowledgements

This work was supported by Ningxia Key Research and Development Project (2020BCF01003) and Science Technology Innovation Guidance Project of Ningxia Academy of Agriculture and Forestry Sciences (NKYZZ-J-19-04) and Ningxia Natural Science Foundation (2020AAC02011). The authors also acknowledge their colleagues for their comments regarding this study and the journal's editors and anonymous reviewers for their critical reviews and comments regarding this manuscript.

[References]

[1] Li E, Liu A, Bo X, Liu, Y. Yeast species associated with spontaneous wine fermentation of Cabernet Sauvignon from Ningxia, China. World Journal of Microbiology and Biotechnology, 2011; 27(10): 2475–2482.

[2] Qi Y, Wang R, Qin Q, Sun, Q. Soil affected the variations in grape and wine properties along the eastern foot of Helan Mountain, China. Acta Agric Scand Sect B-Soil Plant Sci, 2019; 69(6): 494–502.

[3] Liu N, Song Y Y, Qin Y, Gong X, Liu Y L. Chromatic characteristics and anthocyanin compositions of cabernet sauvignon wines: Influence of indigenous Saccharomyces cerevisiae strains in Ningxia, China. Food Science and Biotechnology, 2015; 24(6): 1973–1978.

[4] Li J. Variety selection in the wine grape industry at the eastern foot of Helan Mountain: Importance in development. Sino-Overseas Grapevine & Wine, 2013; 4: 58–59. (in Chinese)

[5] Amtmann A, Armengaud P. Effects of N, P, K and S on metabolism: New knowledge gained from multi-level analysis. Current Opinion in Plant Biology, 2009; 12(3): 275–283.

[6] Fang Y, Schreiner R P, Qian M C. Soil nitrogen, phosphorus, and potassium alter *β*-damascenone and other volatiles in pinot noir berries. American Journal of Enology and Viticulture, 2018; 69(2): 157–166.

[7] He P C. Grapeology. Acta Horticulture, 2005, 32(4): 694. (in Chinese)

[8] Bell S J, Robson A. Effect of nitrogen fertilization on growth, canopy density, and yield of *Vitis vinifera* L. cv. Cabernet Sauvignon. American Journal of Enology and Viticulture, 1999; 50(3): 351–358.

[9] Leibar U, Pascual I, Aizpurua A, Morales F, Unamunzaga O. Grapevine nutritional status and K concentration of must under future expected climatic conditions texturally different soils. Journal of Soil Science and Plant Nutrition, 2017; 17(2): 385–397.

[10] Goodarzi K, Farahi M H, Kavoosi B. Relationship of Nutritional Balance Index (NBI) with Yield and Quality Indices of Grapevine (*Vitis vinifera* L.). Acta horticulturae, 2012; 931: 259–266.

[11] Martin I, Benito A, Romero I, Dominguez, N, Garcia-Escudero, E. Preliminary diagnosis and recommendation integrated system norms for leaf nutrient diagnosis of tempranillo grapevine in the Rioja Appellation. Communications in Soil Science and Plant Analysis, 2013; 44(1-4): 655–667.

[12] Mostashari M, Khosravinejad A, Golmohammadi M. Comparative study of DOP and CND methods for leaf nutritional diagnosis of vitis vinifera in Iran. Communications in Soil Science and Plant Analysis, 2018; 49(5): 576–584.

[13] Carneiro A, Pereira O, Cunha M, Queiroz J. The diagnosis and recommendation integrated system (DRIS) – First aproach for the establishment of norms for vineyards in Portugal. Ciência E Técnica Vitivinícola, 2016; 30(2): 53–59.

[14] Fan G Y. Preliminary study on suitable value of mineral element content and leaf nutrition diagnosis of 'Fuji' apple leaves in western Liaoning. PhD dissertation. Chinese Academy of Agricultural Sciences, 2014. (in Chinese)

[15] Zhong G L. A rapid method for measuring the volume of plant organs. Plant physiology Communication, 1990; 5: 58–64. (in Chinese)

[16] Ma Q L, Wang J H, Zhang D M, Wu C R. Probe on the drought resistance mechanism of zizyphus jujube. Journal of Gansu Forestry Science and Technology, 2000; 25(1): 20–23. (in Chinese)

[17] Bao S D. Soil agrochemical analysis. China Agriculture Press, 2000. (in Chinese)

[18] Yan P K, Yu R, Wang D Q, Hou T, Guo P F, Zhou X R, et al. Annual dynamic change of N, P, and K contents as fertilizer requirement of jujube tree (*Zizyphus jujube* 'Tongxinyuanzao') in Ningxia. Journal of Fruit Science, 2020; 37(1): 77–87. (in Chinese)

[19] Bell S J, Henschke P A. Implications of nitrogen nutrition for grapes, fermentation and wine. Australian Journal of Grape & Wine Research, 2010; 11(3): 242–295.

[20] Gonzalez-Santamaria R, Ruiz-Gonzalez R, Nonell S, Garde-Cerdan T, Perez-Alvarez E P. Influence of foliar riboflavin applications to vineyard on grape amino acid content. Food Chemistry, 2018; 240: 601–606.

[21] Metay A, Magnier J, Guilpart N, Christophe A. Nitrogen supply controls vegetative growth, biomass and nitrogen allocation for grapevine (cv. Shiraz) grown in pots. Functional Plant Biology, 2015; 42(1): 105–114.

[22] Sharma S B, Sayyed R Z, Trivedi M H, Gobi T A. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus, 2013; 2(1): 587. doi: 10.1186/ 2193-1801-2-587.

[23] Schreiner R P, Osborne J. Defining phosphorus requirements for pinot noir grapevines. American Journal of Enology and Viticulture, 2018; 69(4): 351–359.

[24] Villette J, Cuéllar T, Verdeil J L, Delrot S, Gaillard I. Grapevine potassium nutrition and fruit quality in the context of climate change. Frontiers in Plant Science, 2020; 11.

[25] Poni S, Quartieri M, Tagliavini M. Potassium nutrition of Cabernet Sauvignon grapevines (*Vitis vinifera* L.) as affected by shoot trimming. Plant & Soil, 2003; 253(2): 341–351.

[26] Verdeil J L, Pascaud F, Torregrosa L, Thibaud J B, Gaillard I, Sentenac H, et al. A grapevine Shaker inward K+ channel activated by the calcineurin B-like calcium sensor 1-protein kinase CIPK23 network is expressed in grape berries under drought stress conditions. Plant Journal, 2010; 61(1): 58–69.