# Analysis of the Tolerance of 22 *Malus* baccata Accessions to Alkali Stress

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Abstract. Alkali stress is an important factor that restricts the growth and yield of crops. Apple (Malus baccata Borkh.) rootstocks have attracted widespread attention because of their wide distribution and ability to exist in various forms. This study reported the tolerance of several *M. baccata* accessions to alkaline stress using the sand culture method. Nitrogen (N), phosphorus (P), and potassium (K) contents in the roots of 22 *M. baccata* accessions decreased after 40 days of alkali stress exposure. N and P contents in the leaves of most *M. baccata* seedlings decreased, whereas K content increased. In addition, the new leaf number and fresh and dry weights of the *M. baccata* accessions decreased significantly, indicating that alkali stress inhibits the growth of *M. baccata* seedlings. The 22 *M. baccata* accessions were divided into three categories: high, moderate, and low tolerance based on the cluster analysis of the resistance coefficients of six growth indices. These delineations are important for screening *M. baccata* resources. This study provides a basis for the growth of the apple industry in areas such as northwest China, which suffer from severe salinization.

Recently, the increasingly severe problem of soil alkalization has restricted the rapid development of agriculture and animal husbandry (Guo et al. 2015). Alkalized soil covers  $\sim 10\%$  of the world's arable land, particularly in China. Alkali stress is caused by alkaline salts such as NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> and is accompanied by high pH (Munns 2002). Several studies have been conducted on alkali stress because of its influence on crop growth and yield. For example, Bai et al. (2013) reported that alkali stress

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significantly reduced the yields and weights of oat grains. Wang et al. (2015) studied the physiological and molecular responses of two rice plants with different alkali resistance types to alkaline stress. The results showed that alkali-tolerant varieties maintained higher rates of photosynthesis and root system activity under alkaline stress than alkali-sensitive varieties. Therefore, studying the effects of alkali stress and resistance mechanisms in plants is crucial.

Most of the energy for life is provided by plant photosynthesis, which is sensitive to environmental changes (Bode et al. 2016). Photosynthesis depends on chloroplasts, which contain an important component called chlorophyll. A previous study reported that the contents of chlorophyll and carotenoids in barley plants are significantly reduced under alkali stress (Yang et al. 2009). In addition, alkali stress affects the ability of plants to absorb water and destroys the membrane system and chlorophyll, thereby affecting photosynthetic rate and interrupting physiological metabolism. Changes in plant biomass is also an important index that reflects growth and development. The growth and development of plant leaves and roots are substantially damaged under stress conditions. Such conditions accelerate the senescence, death, and withering of plant organs, reducing their accumulation of fresh weight and dry matter (Pearce et al. 1999). Mohsenian et al. (2012) discovered that plant height, leaf number, leaf area, and dry weight of the leaves and roots of tomatoes decreased significantly following treatment with 10 mm NaHCO<sub>3</sub>. In addition, alkali stress has an important effect on the absorption of mineral elements in plant roots. N, P, and K are essential elements for plant growth, development, and metabolism. Alkali stress reduces the utilization rate of certain trace elements, such as Fe, Zn, and Mn, leading to N and P deficiency (Guardia and Alcantara 2002; Valdez and Reed 2008). Reportedly, alkali stress can decrease K levels in plants (Gong et al. 2014).

Apple (*Malus* sp.) is one of the most important fruits worldwide because it grows in a weakly acidic or neutral environment (Zhang et al. 2016). Soil alkalization affects the growth and quality of apples. *Malus baccata* Borkh. is

Table 1. Twenty-two M. baccata accessions used in the experiment.

Code	Abbreviation	Genotype	Origin in China	
A	SAX	Shaanxi Shuiqiuzi	Tongchuan, Shaanxi	
В	NMG	Neimenggu Shandingzi	Hailaer, Neimenggu	
С	JPH	Jingpohu Shandingzi	Xingcheng, Liaoning	
D	YN	Yunnan Shandingzi	Lijing, Yunnan	
E	HLJ	Heilongjiang Shandingzi	Xingcheng, Liaoning	
F	HY	Heiyu Shandingzi	Xingcheng, Liaoning	
G	GS	Gansu Shandingzi	Pingliang, Gansu	
Н	MS	Maoshandingzi	Xingcheng, Liaoning	
Ι	JL	Jilin Shandingzi	Gongzhuling, Liaoning	
J	SX	Shanxi Shandingzi	Taigu, Shaanxi	
Κ	3#	M. baccta 3 #	Mudanjiang, Heilongjiang	
L	5#	M. baccta 5 #	Mudanjiang, Heilongjiang	
М	4#	M. baccta 4 #	Mudanjiang, Heilongjiang	
Ν	7#	M. baccta 7 #	Mudanjiang, Heilongjiang	
0	HB	Hebei Shandingzi	Changli, Heibei	
Р	YY	Yanyuan Shandingzi	Yanyuan, Sichuan	
Q	8#	M. baccta 8 #	Mudanjiang, Heilongjiang	
R	1#	M. baccta 1 #	Mudanjiang, Heilongjiang	
S	6#	M. baccta 6 #	Mudanjiang, Heilongjiang	
Т	10#	M. baccta 10 #	Mudanjiang, Heilongjiang	
U	YJI	Yongji Shandingzi	Mudanjiang, Heilongjiang	
V	YJ	Yijun Shandingzi	Yijun, Shaanxi	

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widely distributed and highly adaptable to the environment. In addition, *M. baccata* is an important source of apple rootstock. Herein, the tolerance of *M. baccata* to alkali conditions was comprehensively evaluated under alkali stress by analyzing the seedling growth and physiological indices of 22 *M. baccata* accessions (Table 1). The *M. baccata* Borkh. accessions that exhibited different alkali tolerance levels were screened to select apple rootstocks with strong alkali tolerance.

#### Materials and Methods

Plant materials and experimental design. Seedlings were used in this experiment. First, the seeds of 22 M. baccata accessions were treated with sediment (2 to 7 °C, 50 to 60 d), and the germinated seeds were sown in a  $9 \text{ cm} \times 9 \text{ cm} \times 10 \text{ cm}$  nutrient pot for sand culture, with four seeds being sown in each nutrient pot. Second, the seedlings were irrigated every 5 d with 1/2 Hoagland nutrient solution (pH = 6.0, adjusted using concentrated sulfuric acid) when two to three true leaves of the seedlings sprouted. Finally, the experiment was initiated when the seedlings had grown six to eight true leaves. A total of 120 seedlings that exhibited similar growth levels were selected from each resource and randomly divided into control (CK) and treatment (T) groups, with 60 plants in each treatment group. CK was irrigated with 1/2 Hoagland nutrient solution at pH 6.0, whereas the treatment group was irrigated with 1/2 Hoagland nutrient solution at pH 9.0 [1 mol/L of NaHCO3 and Na2CO3 (v:v, 1:1)]. The plants were watered every 3 d, and the total duration of the experiment was 40 d.

*Growth index evaluation.* Before starting the treatment, 30 plants were randomly selected to determine plant height, and the first new leaf was marked. After 40 d of treatment, the number of new leaves was investigated, and plant height was determined.

Thirty plants were randomly selected from each treatment, with each group containing six plants and five replicates of each treatment. After 40 d of treatment, the seedlings were harvested and separated into root and leaf portions to determine individual fresh weights (FWs). Finally, enzymes were deactivated by exposing tissues to  $105 \,^{\circ}$ C for 15 min. Dry weights (DWs) were measured after oven-drying the samples to a constant weight at 75  $^{\circ}$ C.

Chlorophyll, N, P, and K contents. Chlorophyll content was determined as described by Arnon (1949). Briefly, 80% acetone was used for chlorophyll extraction, and the concentrations were determined using a spectrophotometer.

The N, P, and K contents were determined as described by Lowther (1980). The solution was extracted using the  $H_2SO_4$ – $H_2O_2$  digestion method, and the N, P, and K contents were determined using a flow analyzer (Flowsys, Systea, Italy) after the solution was diluted to different concentrations.

Assays of adversity resistance coefficients and cluster analysis. Adversity resistance coefficients (ARCs) were computed based on six growth parameters: new leaf number,

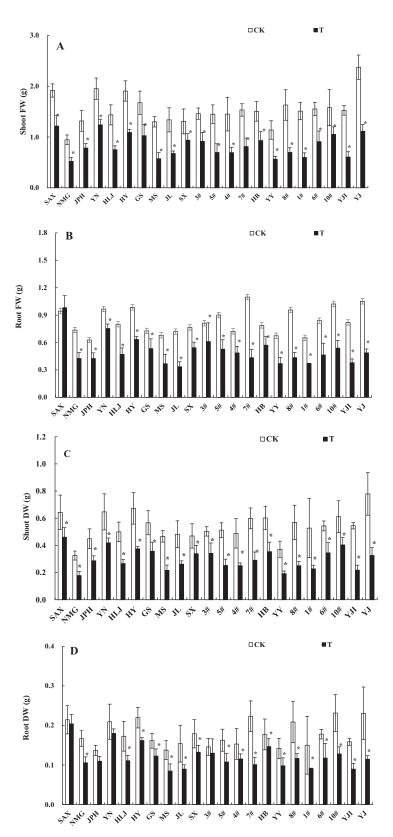


Fig. 1. Effects of alkali stress on the fresh and dry weights of the 22 *Malus baccata* accessions. (A) Shoot FW; (B) root FW; (C) shoot DW; and (D) root DW. Data are the means  $\pm$  standard errors. For each parameter, values with \* differ significantly from the control at P < 0.05 based on *t* test. CK = control; T = alkali stress; FW = fresh weight; DW = dry weight.

height increment, root FW, shoot FW, root DW, and shoot DW. Corresponding values were used in these calculations, with the ARC for each factor, which is the ratio of treatment and control values. Based on the

ARCs of the six growth parameters described previously, the 22 *M. baccata* accessions were analyzed using the Ward method.

Statistical analysis. All data were statistically analyzed using Microsoft Excel 2007 (Redmond, WA, USA) and SPSS 19.0 software (San Jose, CA, USA). Values were considered significantly different at P < 0.05.

#### Results

Growth parameters. After 40 d of exposure to alkali stress, the growth of the 22 M. baccata accessions was inhibited, but the degree of inhibition differed among the accessions. The shoot FW of the 22 M. baccata accessions decreased significantly compared with the control (Fig. 1A), with 1 # (R) and YJI (U) decreasing by 60.3% and 60.4%, respectively. However, the root FW of the treatment group with M. baccata accessions, except for SAX (A), was significantly inhibited, with a decrease of 22.0% to 60.4%. The root FW of SAX (A) increased slightly, but the difference was not significant (Fig. 1B). The shoot DW of the 22 M. baccata accessions decreased from 27.8% to 59.9%, which differed significantly from that of the control. Figure 1C shows that the shoot DW of SAX (A), JPH (C), and 3 # (K) decreased under alkali stress, but the differences were not significant compared with those of the control. The root DW of the other 19 M. baccata accessions was reduced by 14.0% to 54.8%, indicating differences in tolerance to alkali stress among the 22 M. baccata accessions.

In addition, after 40 d of alkali stress exposure, the number of new leaves of the 22 *M. baccata* accessions were significantly lower than that of the control. The decrease in the new leaf number in 7 # was the smallest, but it was significantly different from that of the control (Fig. 2A).

Compared with the control, alkali stress inhibited the growth of all the *M. baccata* accessions (Fig. 2B), and the height growth of 6 # (S) decreased by 95.2%, representing the greatest degree of alkali stress. Treatment HY (F) exhibited the lowest decrease in plant height with a difference of 29.4%, whereas the decrease in plant height of the other *M. baccata* accessions was between these two extremes. There was a significant difference in plant height increment between the control and treatment groups.

Effects of alkali stress on chlorophyll content. After 40 d of exposure to alkali stress, except for SAX (A), the contents of chlorophyll a (chl a), chlorophyll b (chl b), and total chlorophyll (chl t) in the 21 M. baccata accessions were lower than those of the control. The decrease in chl a contents in the 22 M. baccata accessions ranged from 12.6% to 52.3%; the decrease in chl a levels in GS (G) was the smallest, whereas the decrease in chl a levels in YJ (V) was the largest (Fig. 3A). The decrease in chl b varied from 13.2% to 65.5%; the decrease in chl b of 3 # (K) was the smallest, whereas the decrease in chl b of NMG (B) was the largest (Fig. 3B). In the 22 M. baccata accessions, the content of chl t decreased by 8.7% to 54.2%, among which the chl t content in GS (G) was the lowest, and that of NMG (B) was the highest (Fig. 3C).

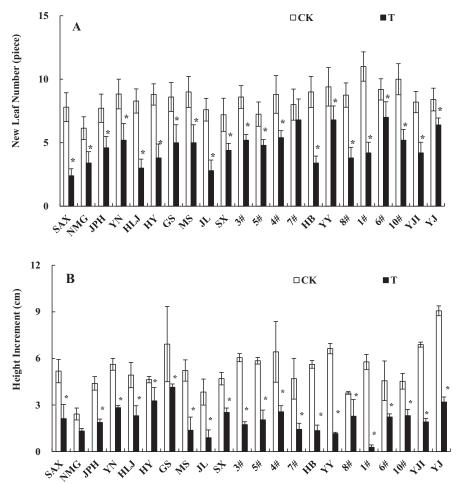


Fig. 2. Effects of alkali stress on the number of new leaves and height increment of the 22 *Malus baccata* accessions. (A) Number of new leaves and (B) height increment. Data are the means  $\pm$  standard errors. For each parameter, values with \* differ significantly from the control at P < 0.05 based on *t* test. CK = control; T = alkali stress.

Effects of alkali stress on N, P, and K content. After 40 d of alkali stress, the N content in the leaves of most of the M. baccata accessions decreased significantly, ranging from 2.4% to 38.7%, among which JL (I) showed the highest reduction. However, the decrease in N content in YJ (V) was the smallest. These results may be due to differences in alkali resistance among the 22 M. baccata accessions (Fig. 4A). Under alkali stress conditions, the root N content of the 22 M. baccata accessions decreased. However, except for 10 # (T), the N content in the roots of the other 21 M. baccata accessions decreased by 9.0% to 37.4%, which was significantly different from that in the control. The N content in the root of M. 1 # (R) decreased by 37.4% compared with that in the control, whereas that in YN (D) decreased by 9% (Fig. 4B).

After 40 d of exposure to alkali stress, the P content in the leaves of 16 *M. baccata* accessions decreased by 7.2% to 38.8%, and the difference was significant compared with the P content in the control. By contrast, the P content in the leaves of YN (D), HLJ (E), HY (F), YY (P), 8 # (Q), and YJ (V) plants increased at amounts ranging from 0.1% to 18.7% (Fig. 4C). The P content in the roots of the 22 *M. baccata* accessions decreased

from 10.9% to 50.7%, which was significantly higher than that in the leaves, and the difference was significant compared with that in the control (Fig. 4D).

Under alkali stress conditions, except for SAX (A), K content increased in the leaves of 21 *M. baccata* accessions. However, the degree of increase was different. The increase in K content in 10 # (T) was the largest, reaching 28.8%. By contrast, the increase in SX (J) was the smallest, exhibiting a decrease of 2.7% (Fig. 4E). The K content in the roots of the 22 *M. baccata* accessions decreased significantly, ranging from 8.7% to 50%; the K content in YJ (V) decreased by 8.7%, whereas that in HB (O) decreased by 50% (Fig. 4F).

ARCs and cluster analysis of alkali tolerance. The ARCs varied among the 22 *M. bac*cata accessions (Table 2), with higher values indicating greater tolerance. The ARCs of the 22 *M. baccata* accessions were low (0.49 and 0.86), indicating that the alkali resistance of the *M. baccata* accessions was low, but the alkali tolerance varied depending on the treatments. The average ARC of the six growth indices, including height increment, shoot FW, root FW, shoot DW, root DW, and new leaf number, from the 22 *M. baccata* was delineated into three groups: 1) high tolerance

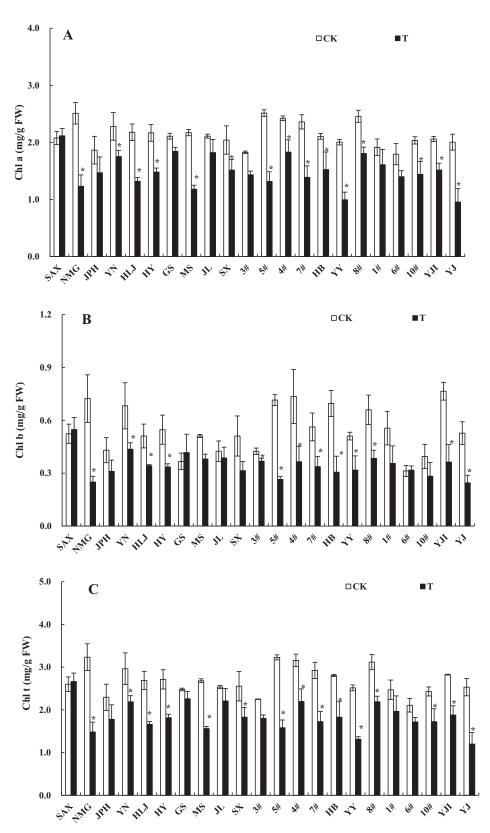


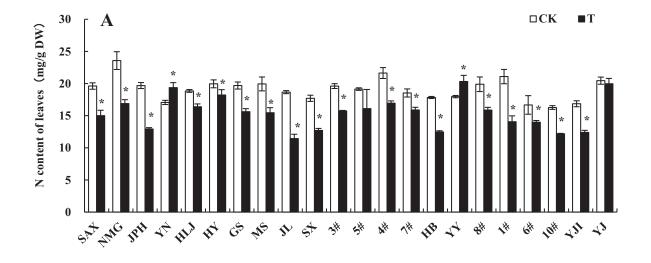
Fig. 3. Effects of alkali stress on the chlorophyll content of the 22 *Malus baccata* accessions. (A) Chlorophyll a (chl a); (B) chlorophyll b (chl b); and (C) total chlorophyll (chl t). Data are the means  $\pm$  standard errors. For each parameter, values with \* differ significantly from the control at *P* < 0.05 based on *t* test. CK = control; T = alkali stress.

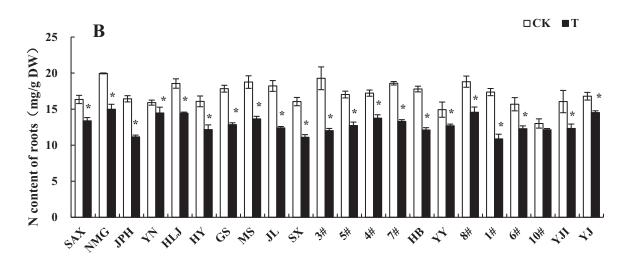
(ARC  $\ge$  0.6): SAX (A), YN (D), SX (J), GS (G), 3# (K), JPH (C), 6 # (S), and HY (F); 2) moderate tolerance (0.5  $\le$  ARC < 0.6): 4 # (M), NMG (B), 10 # (T), HB (O), 5 # (L), YY (P), HLJ (E), and 7 # (N); and 3) low tolerance

(ARC < 0.5): 8 # (Q), YJ (V), MS (H), JL (I), YJI (U), and 1 # (R).

With the ARC for the six growth indices, cluster analysis was conducted following the method described by Ward (2007). The *M*.

*baccata* accessions were also classified into three groups. The results of the two classification methods were the same, except for HY (F) and 6 # (S), which were classified in the high-tolerance group by ARC, were classified





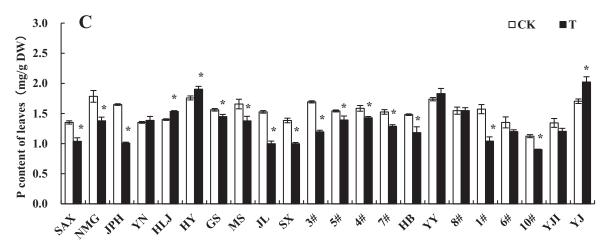
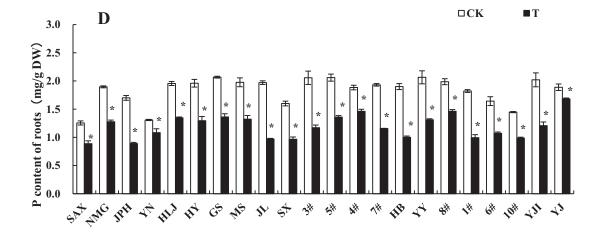


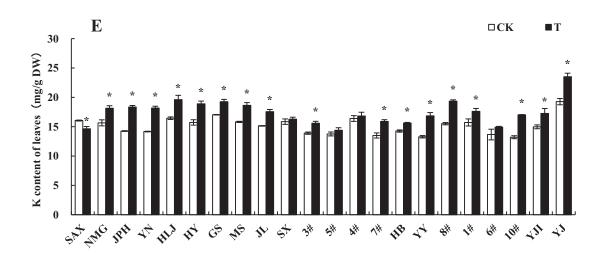
Fig. 4. Effects of alkali stress on the nitrogen (N), phosphorus (P), and potassium (K) content in the leaves and roots of the 22 *Malus baccata* accessions. (A, B) N content in the leaves and roots; (C, D) P content in the leaves and roots; and (E, F) K content in the leaves and roots. Data are the means  $\pm$  standard errors. For each parameter, values with \* differ significantly from the control at P < 0.05 based on *t* test. CK = control; T = alkali stress.

into the moderate-tolerance group, and 8 # (Q), which was classed in the low-tolerance group by ARC, was classified in the moderate-tolerance group. In addition, 5 # (L), 4 # (M), and YY (P) were categorized into the low-tolerance

group, but were categorized into the moderatetolerance group based on the ARC analysis.

Thus, the 22 *M. baccata* accessions could be categorized as follows (Fig. 5): 1) high tolerance: SAX (A), JPH (C), YN (D), GS (G), HB (O), SX (J), and 3 # (K); 2) moderate tolerance: NMG (B), HLJ (E), HY (F), 8 # (Q), 6 # (S), and 10 # (T); and 3) low tolerance: MS (H), JL (I), 5 # (L), 4 # (M), 7 # (N), YY (P), 1 # (R), YJI (U), and YJ (V).





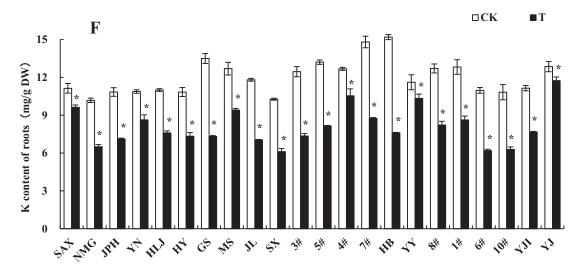


Fig. 4. Continued

#### Discussion

Plant response to alkali stress is primarily reflected in their growth, which can be used as an indicator of alkali tolerance in plants (Li et al. 2015; Yin et al. 2010). A previous study reported that alkali stress inhibits the growth of sunflower plants (Shi and Sheng 2005). Results obtained in this study showed that the growth of the *M. baccata* accessions was inhibited under alkali stress, which is consistent with the results of previous studies. Growth indices, such as increases in plant height, new leaf number, shoot FW, shoot

DW, root FW, and root DW, decreased differently in the 22 *M. baccata* accessions. These results show that the effects of alkali stress on the same index of the different *M. baccata* accessions differ, which primarily depends on the alkali tolerance of the *M. baccata* accessions. For example, after

Table 2. Adversity resistance coefficients (ARCs) and alkali tolerance of 22 Malus baccata accessions.

Code	Height increment	Shoot FW	Root FW	Shoot DW	Root DW	New leaf number	Avg ARC	Alkali tolerance
A	$0.41 \pm 0.11$	$0.64 \pm 0.14$	$1.04 \pm 0.24$	$0.72 \pm 0.11$	$0.95 \pm 0.15$	$0.31 \pm 0.08$	$0.68 \pm 0.29$ a	Н
D	$0.50\pm0.03$	$0.64\pm0.07$	$0.78 \pm 0.12$	$0.65 \pm 0.12$	$0.86 \pm 0.15$	$0.59 \pm 0.15$	$0.67 \pm 0.13$ a	Н
J	$0.54 \pm 0.02$	$0.72 \pm 0.20$	$0.71 \pm 0.21$	$0.72 \pm 0.07$	$0.74 \pm 0.12$	$0.61 \pm 0.10$	$0.67 \pm 0.08$ a	Н
G	$0.60 \pm 0.17$	$0.61\pm0.06$	$0.73 \pm 0.10$	$0.63 \pm 0.05$	$0.76\pm0.06$	$0.58 \pm 0.13$	$0.65 \pm 0.08$ a	Н
Κ	$0.29\pm0.04$	$0.63 \pm 0.17$	$0.75 \pm 0.22$	$0.68 \pm 0.15$	$0.89 \pm 0.13$	$0.60\pm0.03$	$0.64 \pm 0.20$ ab	Н
С	$0.43 \pm 0.01$	$0.60\pm0.08$	$0.68\pm0.07$	$0.64 \pm 0.11$	$0.80\pm0.07$	$0.60\pm0.09$	$0.63 \pm 0.12$ abc	Н
S	$0.49 \pm 0.10$	$0.59\pm0.09$	$0.55 \pm 0.17$	$0.64 \pm 0.09$	$0.66 \pm 0.17$	$0.76 \pm 0.13$	$0.62 \pm 0.09$ abc	Н
F	$0.71 \pm 0.18$	$0.57\pm0.06$	$0.64 \pm 0.11$	$0.56 \pm 0.11$	$0.74 \pm 0.11$	$0.43 \pm 0.17$	$0.61 \pm 0.11$ abcd	Н
М	$0.40 \pm 0.13$	$0.48 \pm 0.12$	$0.67 \pm 0.27$	$0.51 \pm 0.11$	$0.75 \pm 0.13$	$0.61 \pm 0.16$	$0.57 \pm 0.13$ abcd	М
В	$0.55 \pm 0.05$	$0.55 \pm 0.04$	$0.58\pm0.09$	$0.55 \pm 0.05$	$0.63\pm0.05$	$0.55 \pm 0.15$	$0.57 \pm 0.03$ abcd	М
Т	$0.51 \pm 0.04$	$0.67 \pm 0.23$	$0.53 \pm 0.13$	$0.66 \pm 0.12$	$0.55 \pm 0.15$	$0.52 \pm 0.14$	$0.57 \pm 0.07$ abcd	М
0	$0.24\pm0.05$	$0.62 \pm 0.18$	$0.72 \pm 0.19$	$0.59 \pm 0.16$	$0.83 \pm 0.13$	$0.38 \pm 0.11$	$0.56 \pm 0.22$ abcd	М
L	$0.35 \pm 0.10$	$0.48 \pm 0.16$	$0.59 \pm 0.13$	$0.49 \pm 0.10$	$0.66 \pm 0.14$	$0.66 \pm 0.14$	$0.54 \pm 0.12$ abcd	М
Р	$0.18 \pm 0.01$	$0.50 \pm 0.13$	$0.55 \pm 0.11$	$0.52 \pm 0.12$	$0.69 \pm 0.11$	$0.72 \pm 0.10$	$0.53 \pm 0.20$ abcd	М
Е	$0.47 \pm 0.10$	$0.52 \pm 0.09$	$0.59 \pm 0.14$	$0.53 \pm 0.08$	$0.64 \pm 0.12$	$0.36 \pm 0.09$	$0.52 \pm 0.10$ abcd	М
Ν	$0.31 \pm 0.06$	$0.53 \pm 0.13$	$0.40\pm0.09$	$0.49\pm0.08$	$0.45 \pm 0.13$	$0.85 \pm 0.16$	$0.51 \pm 0.19$ abcd	М
Q	$0.60 \pm 0.28$	$0.43 \pm 0.10$	$0.45 \pm 0.10$	$0.44 \pm 0.12$	$0.56 \pm 0.10$	$0.43 \pm 0.12$	$0.49 \pm 0.08$ abcd	L
Ŷ	$0.35 \pm 0.02$	$0.47\pm0.08$	$0.46\pm0.06$	$0.42 \pm 0.11$	$0.50 \pm 0.12$	$0.76\pm0.07$	$0.49 \pm 0.14$ abcd	L
Н	$0.26 \pm 0.12$	$0.44 \pm 0.12$	$0.54 \pm 0.20$	$0.46 \pm 0.11$	$0.62\pm0.05$	$0.56 \pm 0.16$	$0.48 \pm 0.12$ abcd	L
Ι	$0.23 \pm 0.11$	$0.50 \pm 0.10$	$0.47 \pm 0.18$	$0.54 \pm 0.12$	$0.58 \pm 0.12$	$0.37\pm0.09$	$0.45 \pm 0.13$ bcd	L
U	$0.28\pm0.03$	$0.40\pm0.09$	$0.46\pm0.02$	$0.40\pm0.08$	$0.57\pm0.08$	$0.51 \pm 0.14$	$0.44 \pm 0.10 \text{ cd}$	L
R	$0.05\pm0.03$	$0.40\pm0.10$	$0.57\pm0.07$	$0.43\pm0.14$	$0.61\pm0.10$	$0.38\pm0.13$	$0.41 \pm 0.20 \ d$	L

FW = fresh weight; DW = dry weight; ARC = treatment/control value; H = high tolerance (ARC  $\ge 0.6$ ); M = moderate tolerance ( $0.5 \le ARC < 0.6$ ); L = low tolerance (ARC < 0.5).

40 d of exposure to alkali stress, the degree of inhibition of SAX (A) was smaller than that of JL (I), indicating that the alkali tolerance of SAX (A) was stronger than that of JL (I). Alkali stress occurs at high pH, and the effect of interaction with Na<sup>+</sup> on the germination of *Leymus chinensis* seeds is greater than that of single-ion injury (Guo et al. 2010; Zhang and Mu 2009). High pH can damage root structures, affect their function, destroy ion balance, and disrupt plant cell metabolism. Therefore, to resist the harm caused by high pH, plants need to metabolize more energy and substances,

which may be a reason for the decrease in the growth index of mountain stator accessions under alkali stress.

After 40 d of exposure to alkali stress, chl a, chl b, and chl t contents in the leaves of the 22 *M. baccata* accessions decreased compared with those in the control, indicating that alkali stress can affect the chloroplast structure of *M. baccata* accessions, resulting in a significant decrease in photosynthetic pigment content. However, owing to differences in the degree of alkali tolerance of the different *M. baccata* accessions, the degree of chloroplast damage and chlorophyll content differed. Photosynthesis is an important physiological process by which plants produce organic matter and store energy, and it is the basis of material and energy metabolism. Chlorophyll is a key factor in plant photosynthesis (Li et al. 2010, 2013). However, alkali stress can precipitate  $Mg^{2+}$ , which can affect chlorophyll synthesis and simultaneously affect the assimilation and photosynthetic electron transport of CO<sub>2</sub>, hindering the photosynthesis of mountain stator leaves. As a result, the growth and development of mountain stator was inhibited under alkali stress (Shi and Zhao 1997).

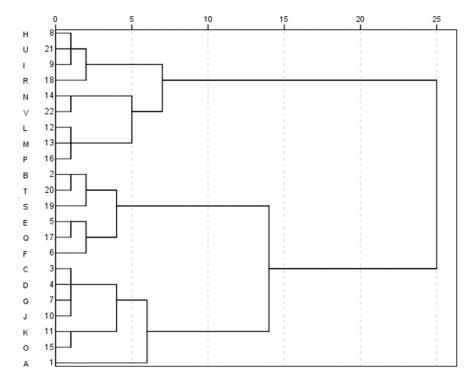


Fig. 5. Cluster analysis of alkali tolerance of the 22 *Malus baccata* accessions. Six adversity resistance coefficients, new leaf number, height increment, root dry weight (DW), shoot DW, root fresh weight (FW), and shoot FW were used to determine average linkage clustering using Euclidean's distance tests.

Plants absorb water and roots primarily via the root. If root structure and function are damaged, water and nutrient absorption will be affected, resulting in changes in physiological and metabolic activities in plants (Davies and Zhang 1991; Lopez et al. 2002). The results showed that N, P, and K contents decreased in the 22 *M. baccata* accessions, except for the N content in the roots of 10 # (T). The N, P, and K contents in the roots of the other *M. baccata* accessions were significantly lower than those in the control. Except for SAX (A), K content in the leaves increased. By contrast, N and P contents decreased, except for in individual *M. baccata* accessions.

P is required for protein, carbohydrate, and fatty acid metabolism in plants. Therefore, changes in P content are vital for plant metabolism and normal growth and development (Martinez and Lauchli 1994). Soil pH value is the main factor affecting the efficiency of P utilization (Guo et al. 2013). The primary forms of P in the soil are  $H_2PO_4^-$  and HPO<sub>4</sub><sup>2-</sup>. Under alkali conditions, the content of  $H_2PO_4^-$  decreases and that of  $HPO_4^{2-}$  increases. Therefore, the decrease in P content in the *M. baccata* accessions could be because it is easier for roots to absorb H<sub>2</sub>PO<sub>4</sub><sup>-</sup> than to absorb  $HPO_4^{2-}$ . Because of the decrease in P absorption by the roots, the amount of P transported to the leaves decreased, resulting in decreased P content. However, the increase in P content in the leaves of some treatments could be due to the varied responses and resistance of the different M. baccata accessions to alkali stress.

The decrease in N content could be due to the inhibition of the roots to absorb  $NO_3^-$  under alkali stress, resulting in decreased N content. Plants primarily rely on the transport and distribution of cations to reduce ion-induced damage. In this experiment, the increase in Na<sup>+</sup> levels and pH in the rhizosphere of mountain stator resulted in the selective absorption of cations by the roots (Khan et al. 2000; Shi and Wang 2005). Both factors could decrease the uptake of K<sup>+</sup>. The increased K content in leaves could be attributed to the physiological response mechanism of the *M. baccata* accessions to alkali stress.

When the plants were subjected to stress, the most sensitive response was their growth (Wen et al. 2018). Therefore, the identification of plant resistance using growth and morphological indices is still the commonly used method. Because the plant growth environment differentially affects the physiological and metabolic activities of plants, a single growth index may produce more one-sided results if used to determine the alkali resistance of mountain stator resources. Therefore, herein, six growth morphology indices were used to analyze and identify the alkaline resistance of mountain stator, and the average resistance coefficient of the growth morphology index was used to evaluate the alkali resistance of the stock. The alkali resistance evaluation of the 22 M. baccata accessions resulted in their classification into three categories, and objective and reasonable results were obtained. These results provide a basis for screening alkali-tolerant apple rootstocks.

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