

Elymus dahuricus H⁺-PPase *EdVP1* enhances potassium uptake and utilization of wheat through the development of root system

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Abstract

We investigated the differences of K acquisition and utilization, morphological and physiological characteristics of roots and grain yield between *Elymus dahuricus* H⁺-PPase (*EdVP1*) transgenic wheat and wild type wheat under low K stress. The results showed that, the grain yield and K economic utilization index (KUI-E) in wild type wheat were only 61.14% and 50.20% of those in *EdVP1* transgenic wheat. *EdVP1* increased the free IAA accumulations in roots, which may play a key role in the development of root system. The total root length, total root surface area, root tips and total root volume in transgenic wheat were 2.26, 2.23, 2.34 and 2.00 times as high as those in wild type wheat, respectively. Excretion H⁺ and cation exchange capacity (CEC) of roots, which were enhanced in transgenic wheat, were positively correlated with K content. The exudate of organic acid in transgenic wheat was 2.22 times as high as that in wild type wheat, leading to the strong K activation of transgenic wheat. Therefore, we assume that well-developed rootsystem containing prosperous root morphology, high excretion H⁺ and CEC of roots and strong excretion ability of organic acids improved K acquisition and utilization efficiency in *EdVP1* transgenic wheat.

Keywords: Wheat, *EdVP1* gene, K efficiency, root system

1. Introduction

Potassium plays an important role in many physiological processes of plants, such as enzyme activation, protein synthesis and photosynthesis (Römheld and Kirkby, 2010). However, there are large numbers of K-deficient farmlands throughout the world. For example, 75% of the paddy soils in China are K deficient, as well as 67% of the wheat fields in Southern Australia. In addition, there are

more and more reports showing that K deficiency exists because of the export of agricultural products and leaching of K (Rengel and Damon, 2008). In the past few decades, sustainable K management has been ignored to some extent (Basak and Biswas, 2009). There are large numbers of above ground crops taken away from fields for many other uses every year. For these dry matters respectively contain about 60, 75

and 14 million tons of potassium (K) nitrogen (N) and phosphorus (P), nutrition will be lost in soils by taking crops from fields. The supply and output of N and P are nearly in balance, whereas the supply of K is much lower (only 35% of output) (Smil, 1999). With the increase of nitrogen and phosphorus fertilizer inputs and intensive cropping, the lack of potassium has become the most factor limiting wheat yields (He *et al.*, 2012; Niu *et al.*, 2013; Rafique *et al.*, 2012). K-fertilizers should be added to soil to compensate for the lack of potassium. The potassium consumption of China ranks second after USA and potassium ore is non-renewable resource (FAI, 2007). Wheat is a main grain crop, whose planting area, total output and total trade volume rank first in all types of crops. Therefore the wheat producers attach great importance to wheat improvement all around the world. As a result, it is particularly important to improve K use efficiency on increasing wheat production.

The processes of K uptake from soil and transportation in plants are mainly achieved by active transportation. H⁺-PPase is an H⁺ transport enzyme, which is different from H⁺-ATPase. In the vacuole membrane, H⁺-PPase can link free energy with H⁺ transmembrane transportation produced by inorganic pyrophosphate (PPi) hydrolysis. Meanwhile, H⁺-PPase can build electrochemical gradient across the vacuolar membrane to provide the driving force for the secondary active transportation of various solutes (such as cation, anion, amino acid and carbohydrate) molecular transmembrane movement (Wu *et al.*, 2001). Obermeyer *et al.* (1996) found that H⁺-PPase also participate in K⁺ transportation to vacuoles. *Arabidopsis* H⁺-PPase *AVP1* facilitates the auxin fluxes that regulate root growth (Li *et al.*, 2005). The size of the root system, the physiology of uptake and the ability of plants to increase K solubility in the rhizosphere by exudation of organic compounds are considered as mechanisms of uptake efficiency (Steingrobe and Claassen, 2000; Steingrobe *et al.*, 2000; Rengel and Damon, 2008). In recent years, H⁺-PPase gene cloned from *Arabidopsis* and *Hordeum vulgare* have been proved to play an important role in

improving the salt drought tolerance of transgenic plants (Liu *et al.*, 2005; Park *et al.*, 2005). However, there is little research involving the function of *Elymus dahuricus* H⁺-PPase (*EdVPI*) in the transgenic plants.

Meanwhile, *Elymus dahuricus* belongs to *Elymus* of *Gramineae* and has remarkable tolerance to various stresses. *EdVPI* had high similarity with H⁺-PPases of other plant species including wheat and so on (minimal homology was 86%). As a result, *EdVPI* may be used as an effective gene resource to improve wheat resistance to low K stress. However, the function and mechanism of *EdVPI* in promoting wheat K uptake and tolerant ability under low K supply are still unclear. It is necessary to determine K utilization characteristics and some special physiological processes involving in the K uptake of *EdVPI* transgenic wheat. Accordingly, the objectives of this study were to: (1) evaluate whether *EdVPI* can improve the wheat tolerance to low K stress; (2) figure out the mechanism of *EdVPI* in promoting wheat K uptake and tolerant ability under low K stress.

2. Materials and Methods

2.1. Plant materials

Wheat (*Triticum aestivum* L.) cultivars (cvs.) Yangmai 12 was used to obtain transgenic lines. *Elymus dahuricus* H⁺-PPase *EdVPI* gene was transferred by *Agrobacterium tumefaciens*-mediated methods. The results of PCR showed that the *EdVPI* transgenic wheat was obtained. Six independent transgenic wheat lines (EV48, EV52, EV56, EV66, EV76 and EV88) were used in this study. Wild type wheat (Yangmai 12) was used as control plant.

2.2. Soil

Soil samples (0-20cm depth) used in root box and pot experiment are categorized as Ferrisols and Udic Argosols, respectively. Ferrisols were obtained from State Experimental Station of Yingtan Agro-

Ecosystem, Jiangxi, China (116°55'E, 28°15'N). While Udic Argosols were obtained from Yangzhou, Jiangsu, China (32.42°N, 119.11°E). pH, organic matter content, available N, available P, exchangeable K, non-exchangeable K and total K of Ferrisols are 4.83, 16.84 g kg⁻¹, 14.73 mg kg⁻¹, 27.94 mg kg⁻¹, 87.14 mg kg⁻¹, 198.47 mg kg⁻¹ and 9.76 g kg⁻¹, respectively. pH, organic matter content, available N, available P, exchangeable K, non-exchangeable K and total K of Udic Argosols are 6.5, 8.64 g kg⁻¹, 85.7 mg kg⁻¹, 4.4 mg kg⁻¹, 67 mg kg⁻¹, 489 mg kg⁻¹ and 14.73 g kg⁻¹, respectively. Soil samples were air-dried and passed through a 2-mm sieve.

2.3. Culture experiment

After the immersion of 0.9% NaClO₃ for 24h under 25 °C, the white seeds were washed by distilled water and then put into sterilized sands. When the seedlings grew to about 7 cm, we chose neat ones, washed them by distilled water, and then transferred them to the nutrient solution (pH 6.5) after removing endosperms. The concentration of K⁺ was 0.01mmol L⁻¹ in the nutrient solution. The nutrient solution was as follows (mmol L⁻¹): Ca(NO₃)₂•4H₂O 1.0×10⁻³, MgSO₄•H₂O 1.0×10⁻³, NaH₂PO₄ 2.5×10⁻⁴, NH₄NO₃ 1.0×10⁻³, CaCl₂ 1.5×10⁻³, Fe-EDTA 1.0×10⁻⁴, MnSO₄•H₂O 1.0×10⁻⁶, ZnSO₄•7H₂O 1.0×10⁻⁶, CuSO₄•5H₂O 5.0×10⁻⁷, (NH₄)₆ Mo₇O₂₄•4H₂O 5.0×10⁻⁹, H₃BO₄ 1.0×10⁻⁶, K₂SO₄ 5.0×10⁻³. Volume of culture box was 35L. 50 seedlings were fixed in foam board by sponge in each box. Nutrient solution was ventilated 12h by pumps every day and replaced every three day. Each time, we used 0.1 mmol L⁻¹ HCl or NaOH to make the pH value of the solution to 6.5. The entire experiment was carried out in a growth chamber with a day/night regime of 16/8 hours, temperature of 25/18 °C and relative humidity of 70%. The photosynthetic active radiation during the daytime was 30000 lux. After 30 days, we selected the neat wheat to determine the plant nutrients, root parameters, free IAA of root, CEC and H⁺ secretion of root. Each index was determined for three times.

2.4. Root exudates collection and soil K activation test

Some neat wheat was chosen from the culture experiment after 30 days, and then put four seedlings to a brown bottle with 250 ml distilled water. Each treatment was replicated three times. The seedlings were fixed by sponge in the brown bottles. Every 24 h, we collected the distilled water in the brown bottles and put 250 ml fresh distilled water into the brown bottles. The collections continued for 3 days. The entire experiment was carried out in a growth chamber with the same conditions as the culture experiment.

The collections of the root exudates for three days were combined into a brown bottle. The collections of the root exudates were concentrated to 5 ml by rotary vacuum evaporator under 40 °C and the concentrated root exudates were stored in the freezer at -20 °C. The concentrated root exudates were used for the soil K activation test and the determination of organic acids content.

In soil K activation test, we put 3.75 g concentrated root exudates to 2.5 g soils. Soil samples were air-dried and passed through a 2-mm nylon sieve. Each treatment was replicated three times. The entire experiment was carried out in a growth chamber with temperature of 25 °C and relative humidity of 70%. After 15 days, soil exchangeable K content of each treatment was determined.

2.5. Root box experiment

The root box of 20 cm in length, 12 cm in width and 20 cm in height, was used in this experiment. There was an intermediate chamber of 2 cm width in each root box. Root box was made of two stage PVC plate. There was a nylon mesh with the pore size of 30 μm between each room in the root box. We put 5 kg soils with fertilizers into each root box. The fertilizers were as follows: 5.455 g Ca (NO₃)₂•4H₂O, 3.05g MgSO₄•7H₂O, 0.0365 g H₃BO₃, 0.057 g CuSO₄•5H₂O, 0.2025 g MnCl₂•4H₂O, 0.0135 g Na₂MoO₄•2H₂O, 0.132 g ZnSO₄•7H₂O and 1.105 g Ca (H₂PO₄)₂•H₂O.

After immersing in 0.9% NaClO₃ for 24h under 25 °C, the white wheat seeds were washed by distilled water and then put into sterilized sands. When the seedlings grew to about 7 cm, we chose neat ones, washed them by distilled water, and then transferred them to the root boxes after removing endosperms. Each box contained three seedlings and each treatment was replicated three times. The entire experiment was carried out in a growth chamber with the same conditions as the culture experiment. We irrigated the wheat with distilled water once every day. Soil moisture was controlled within 85% of the maximum field moisture capacity by weighing method. After 30 days, we took out the wheat from soils carefully, shook off soils from roots and then mixed them with the soils in the intermediate chamber. The mixed soils were called rhizosphere soils. The content of exchangeable K and non-exchangeable K in rhizosphere soils were determined.

2.6. Pot experiment

The pot used was a cylinder with diameter of 25 cm and height of 35 cm. Root bag was a cylinder with diameter of 10 cm and height of 30 cm, which was made of 30 μm-nylon meshes. Each root bag was put in the middle of each pot. Each pot totally contained 19 kg soils with 3 kg soils in the root bag. The fertilizers used in each pot at the beginning of the experiment were as follows: 4.226 g urea, 1.676 g Ca (H₂PO₄)₂•H₂O, 1.900 g CaCl₂, 11.590 g MgSO₄•7H₂O, 0.139 g H₃BO₃, 0.217 g CuSO₄•5H₂O, 0.770 g MnCl₂•4H₂O, 0.051 g Na₂MoO₄•2H₂O and 0.502 g ZnSO₄•7H₂O. 4.226 g urea was used at elongation and heading stage, respectively. Each root bag contained six neat seedlings and each treatment was replicated three times. The entire experiment was carried out in the same conditions as the root box experiment. When the wheat became mature, we shook off soils from roots and then mixed them with the soils in the root bag. The mixed soils were called rhizosphere soils. Grain yield and K content in each part of the plant were determined.

2.7. Chemical analysis and parameter calculation

Soil pH, organic matter content, available N, available P, exchangeable K and non-exchangeable K were determined by the methods as described by Lu (2000). K content in plant was determined by the method as described by Mills and Jones (1996), which involved digesting plant in a mixture of H₂SO₄ and H₂O₂. Potassium concentration in the digested solution was determined by a flame photometer. The plant K acquisition and utilization parameters were calculated as follows (Yang *et al.*, 2010):

K biological utilization index

$$(KUI-B)(g^2 g^{-1}) = \text{dry weight per plant (g)} / \text{K concentration in shoots (g g}^{-1}\text{)} \quad (1)$$

K economic utilization index

$$(KUI-E) (g^2 g^{-1}) = \text{grain yield per plant (g)} / \text{K concentration in shoots (g g}^{-1}\text{)} \quad (2)$$

The whole roots were scanned by EPSON scanner and the root parameters were analyzed by WinRHIZO. Root CEC was determined using the method as described by Wu and Hendershot (2009):

$$\text{Root CEC} = C \times V \times 100 / \text{DRW} \quad (3)$$

where C represents the concentration of KOH used for titration, V represents the volume of KOH used for titration and DRW represents the root dry weight of plant used for experiment. The content of free IAA in roots was determined by enzyme-linked immune technology as described by Fallik *et al.* (1989). The organic acid contents in root exudates were determined by HPLC Agilent 1100 and DAD detector with wavelength of 210 nm. Root excretion H⁺ was determined using the method as described by Britto and Kronzucker (2008):

$$\text{Root excretion H}^+ (C \times V_1) / V_2 \times \text{DRW} \times T \quad (4)$$

where C represents the concentration of NaOH used for titration, V_1 represents the volume of NaOH used for titration, V_2 represents the volume of absorption

liquid used for titration, DRW represents the dry root weight of plant used for experiment and T represents the absorption time.

Table 1. K accumulated content (KA), K biological utilization index (KUI-B) and root to shoot ratio in wild type and *EdVPI* transgenic wheat lines under low K stress (0.01mmol L⁻¹ K).

Genotypes	Root free IAA content (ng g ⁻¹ FW)	RL (m)	RA (cm ²)	RT	RV (cm ³)	RD (mm)
Transgenic type	41.42±4.28**	4.21±1.20*	25.15±5.99*	794±209.80*	0.12±0.02*	0.19±0.02
Wild type	23.76±2.39	1.86±0.54	11.26±4.04	339±154.54	0.06±0.02	0.19±0.02

Results are means±SD, n=6. * and ** denote significant differences at $p < 0.05$ and 0.01 , respectively.

2.8. Statistical analysis

T-test was used to test the significance of the type differences and the least significant difference (LSD) was computed. For statistical analysis of data SPSS window version 17 (SPSS Inc., Chicago, USA) and Microsoft Excel (Microsoft Corporation, USA) were used. The relationships among root excretion H^+ , root CEC and KA in wheat were computed by the OriginPro 8.1 (Origin Inc., Chicago, USA).

3. Results

3.1. K acquisition and utilization in low-K solution

Under low-K condition, K accumulation (KA) and K biological utilization index (KUI-B) were significant different between *EdVPI* transgenic wheat and wild type wheat (Table 1). KA in plants refers to the total K absorption of unit plant. The KA in *EdVPI* transgenic wheat was significantly higher than that in wild type wheat ($p < 0.01$) (Table 1). The KA in wild type wheat

was only 50.45% of that in *EdVPI* transgenic wheat. KUI-B refers to biomass yield produced by unit content of K in shoots (Equation 1). *EdVPI* gene enhanced the KUI-B of wheat significantly ($p < 0.05$) (Table 1). The KUI-B in wild type wheat was only 63.20% of that in *EdVPI* transgenic wheat. Moreover, the root to shoot ratio was enhanced in *EdVPI* transgenic wheat, which was 1.16 times as high as that in wild type wheat under low K stress ($p < 0.05$) (Table 1). So the relatively developed root system may enable K absorption ability and utilization efficiency of wheat.

3.2. Root responses to low K stress

Under low-K condition, the root morphology character and root free IAA content were different between *EdVPI* transgenic wheat and wild type wheat (Table 2). The total root length, total root surface area, root tips and total root volume of *EdVPI* transgenic wheat were significantly higher than those of wild type wheat ($p < 0.05$). Nevertheless the average root diameter had no significant difference between *EdVPI* transgenic wheat and wild type wheat (Table 2). Under low-K condition, the total root length, total root surface area,

root tips and total root volume in transgenic wheat were 2.26, 2.23, 2.34 and 2.00 times as high as those in wild type wheat, respectively. *EdVPI* gene significantly enhanced the root free IAA in transgenic wheat. The content of root free IAA in wild type wheat was only

57.36% of that in transgenic wheat. *EdVPI* gene can enhance free IAA accumulations in root and improved the root development, especially the total root length, total root surface area, root tips and total root volume.

Table 2. Root free IAA content, total root length (RL), total root surface area (RA), root tips (RT), total root volume (RV) and average root diameter (RD) in wild type and *EdVPI* transgenic wheat lines under low K stress (0.01 mmol L⁻¹ K)

Genotypes	Root free IAA content (ng g ⁻¹ FW)	RL (m)	RA (cm ²)	RT	RV (cm ³)	RD (mm)
Transgenic type	41.42±4.28**	4.21±1.20*	25.15±5.99*	794±209.80*	0.12±0.02*	0.19±0.02
Wild type	23.76±2.39	1.86±0.54	11.26±4.04	339±154.54	0.06±0.02	0.19±0.02

Results are means±SD, n=6. * and ** denote significant differences at $p < 0.05$ and 0.01 , respectively.

H⁺ can be secreted by the plant root in the uptake of K. The amount of root excretion H⁺ can reflect the ability of absorbing potassium from the environment. The root excretion H⁺ was closely related to potassium absorption ability of wheat. Under low K stress, K accumulation of wheat had a significant positive correlation with root excretion H⁺ ($r = 0.87^{**}$, Figure 1(A)). The root excretion H⁺ of *EdVPI* transgenic wheat was ranged from 0.94 to 1.10 with an average of 1.01 mmol L⁻¹ g⁻¹ DRW h⁻¹. While the root excretion H⁺ of wild type wheat was ranged from 0.83 to 0.90 with an average of 0.87 mmol L⁻¹ g⁻¹ DRW h⁻¹ (Figure 1(A)). Thus *EdVPI* gene can enhance root excretion H⁺ of wheat.

Root CEC has an important effect on the mineral nutrient elements uptake of plant. Under low K stress, K accumulation of wheat had a significant positive correlation with root CEC ($r = 0.93^{**}$, Figure 1(B)). This showed that with the increase of root CEC, the K uptake of wheat will increase. This result was similar

to the previous researches (Ozolina *et al.*, 2010; Wu and Hendershot, 2009). The root CEC of *EdVPI* transgenic wheat ranged from 0.98 to 1.14 with an average of 1.10 me 100g⁻¹. While the root CEC of wild type wheat ranged from 0.71 to 0.74 with an average of 0.72 me 100 g⁻¹ (Figure 1(B)). Therefore, *EdVPI* gene can enhance root CEC of wheat.

3.3. K activation in rhizosphere

In the root box experiment, soil exchangeable K in rhizosphere had no significant difference between *EdVPI* transgenic wheat and wild type wheat, while soil non-exchangeable K in rhizosphere differed between the two genotypes on Ferrisols ($p < 0.01$) (Table 3). The results of pot experiment on Udic Argosols were quite similar with the above ones. In rhizosphere, soil non-exchangeable K in *EdVPI* transgenic wheat was only 64.54% and 78.71% of that in wild type wheat on Ferrisols and Udic Argosols, respectively.

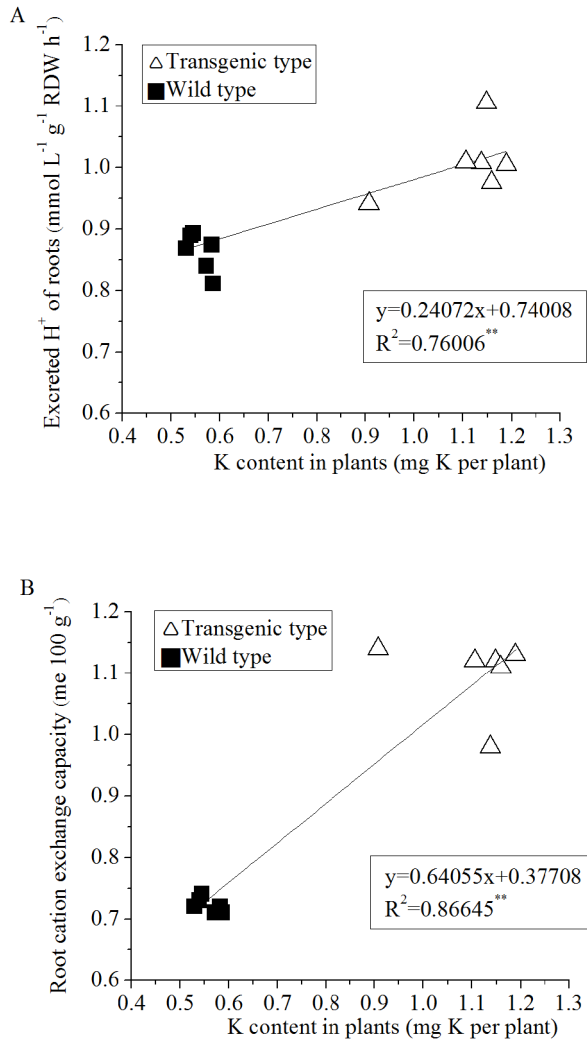


Figure 1: **A)** The relationship between K content in plants and root excretion H⁺ in wild type and *EdVP1* transgenic wheat lines under low K stress; **B)** The relationship between K content in plants and root cation exchange capacity in wild type and *EdVP1* transgenic wheat lines under low K stress (0.01mmol L⁻¹ K ; RDW=root dry weight).

This showed that the roots of *EdVPI* transgenic wheat can effectively transform soil non-exchangeable K in rhizosphere to exchangeable K to meet the plant requirement on potassium. Root exudates change the physical and chemical properties of soil through various means and then change soil mineral element form in rhizosphere (Xie *et al.*, 2012). The soil K activation abilities between *EdVPI* transgenic wheat and wild type wheat were significantly different on

both Ferrisols and Udic Argosols ($p < 0.01$). The leaching amount of soil exchangeable K in root exudates of *EdVPI* transgenic wheat were 1.62 and 2.09 times as high as those in wild type wheat on Ferrisols and Udic Argosols, respectively. The strong soil K activation ability of root exudates in *EdVPI* transgenic wheat root exudates played an important role in its effective utilization of soil non-exchangeable K.

Table 3. Soil exchangeable K (K_{exe}), non-exchangeable K (K_{non}) in rhizosphere and soil K activation ability of root exudates (KAE) of wild type and *EdVPI* transgenic wheat lines on Ferrisols and Udic Argosols.

Soil types	Genotypes	K_{exe} (mg kg^{-1})	K_{non} (mg kg^{-1})	KAE (mg kg^{-1})
Ferrisols	Transgenic type	97.63±4.81	106.64±1.25**	247.83±11.29**
	Wild type	97.61±1.84	165.23±1.66	152.69±11.01
Udic Argosols	Transgenic type	52.23±10.42	343.47±24.68**	257.68±14.76**
	Wild type	59.67±3.83	436.39±12.13	123.43±9.23

Results are means±SD, n=6. * and ** denote significant differences at $p < 0.05$ and 0.01 , respectively.

Root exudates are the important markers of nutrition efficiency genotypes. Low molecular weight organic acids are an important ingredient in root exudates. The low molecular weight organic acids can not only improve the biological activity of soil, but also promote the release of mineral potassium (Grierson, 1992; Tuason and Arocena, 2009; Fujii *et al.*, 2012). Under low K stress, *EdVPI* transgenic wheat and wild type wheat secreted five kinds of organic acids, including oxalic acid, malic acid, tartaric acid, citric acid and acetic acid (Figure 2). The amount of oxalic acid, tartaric acid, malic acid, acetic acid and citric acid in the root exudates of *EdVPI* transgenic wheat were 35.13, 0.19, 14.11, 0.46 and 0.28 mg g^{-1} RDW, respectively. While the amount of oxalic acid, tartaric

acid, malic acid, acetic acid and citric acid in the root exudates of wild type wheat were 13.16, 0.04, 9.33, 0.07 and 0.03 mg g^{-1} RDW, respectively. Oxalic acid and malic acid, which were 98.15% and 99.38% of *EdVPI* transgenic and wild type wheat root exudates respectively, were the main organic acids. The content of organic acids in *EdVPI* transgenic wheat root exudates was 2.22 times as high as that in wild type wheat. Oxalic acid, malic acid, tartaric acid and citric acid in *EdVPI* transgenic wheat root exudates were 2.67, 1.51, 4.76 and 9.19 times as high as those in wild type wheat, respectively. This showed that organic acid secretion ability of *EdVPI* transgenic wheat was significantly higher than that of wild type wheat ($p < 0.01$).

The strong organic acid secretion ability of *EdVPI* transgenic wheat may be an important reason for its effective activation of soil potassium.

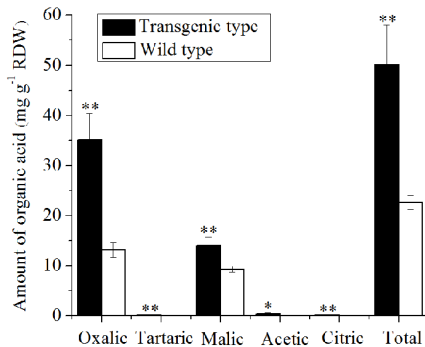


Figure 2. The kind and content of organic acids in root exudates of wild type and *EdVPI* transgenic wheat lines under low K stress (0.01 mmol L⁻¹ K; RDW=root dry weight; Bars represent means±SD; n=6; * and ** denote significant differences at $p < 0.05$ and 0.01, respectively).

3.4. Grain yield and K utilization on Udic Argosols

The grain yield in transgenic lines was significantly higher than that in wild type plants. The grain yield of transgenic lines was 2.06 to 1.24 times as high as that of wild type plants. However, there were significant differences among the different transgenic lines. For instance, EV56 had the highest grain yield, which was about 1.66 times as high as that of EV52.

Significant differences in KUI-B and KUI-E were observed between transgenic lines and wild type plants on Udic Argosols. KUI-B and KUI-E were higher in transgenic lines than those in wild type plants. The KUI-B in wild type wheat was only 33.38% to 48.43% of that in transgenic lines. The KUI-E in wild type

wheat was only 39.34% to 70.04% of that in transgenic lines. However, different transgenic lines had different KUI-B and KUI-E on Udic Argosols. Among all transgenic lines, EV66 had the highest KUI-B, while EV52 had the lowest one. While EV56 had the highest KUI-E, while EV88 had the lowest one.

4. Discussion

Horn *et al.* (2006) reported that K efficient genotypes had the characteristics of high K absorption ability and K utilization efficiency. In this study, *EdVPI* gene can not only enhance K acquisition ability of wheat, but also promote K utilization efficiency of wheat significantly. KA in plants represents plant K absorption ability (Shehu *et al.*, 2010). KA in *EdVPI* transgenic wheat was significantly higher than that in wild type wheat, which showed that the K acquisition ability of *EdVPI* transgenic wheat was much higher. Potassium utilization index (KUI) considers biomass and utilization efficiency, which can better reflect the nutrient efficiency (Yang *et al.*, 2010). KUI includes K biological utilization index (KUI-B) and economic potassium utilization index (KUI-E). In the same external conditions, the K utilization efficiency is basically controlled by genetic traits of crops (Fageria *et al.*, 2001). In this study, the KUI-B and KUI-E of *EdVPI* transgenic wheat were significantly higher than those of wild type, which indicated that the K utilization efficiency of *EdVPI* transgenic wheat was much higher.

The over expression of *AVPI* can make cells acidification, which facilitates the auxin fluxes (Li *et al.*, 2005). In this experiment, the content of free IAA was significantly enhanced in *EdVPI* transgenic wheat. In this view, *EdVPI* transgenic lines had more advantages on auxin regulating responses to K deficiency. What's more, auxins have the best-known effects on root development among phytohormones (Casson and Lindsey, 2003; Smet *et al.*, 2006). *EdVPI* transgenic lines had more advantages on auxin fluxes,

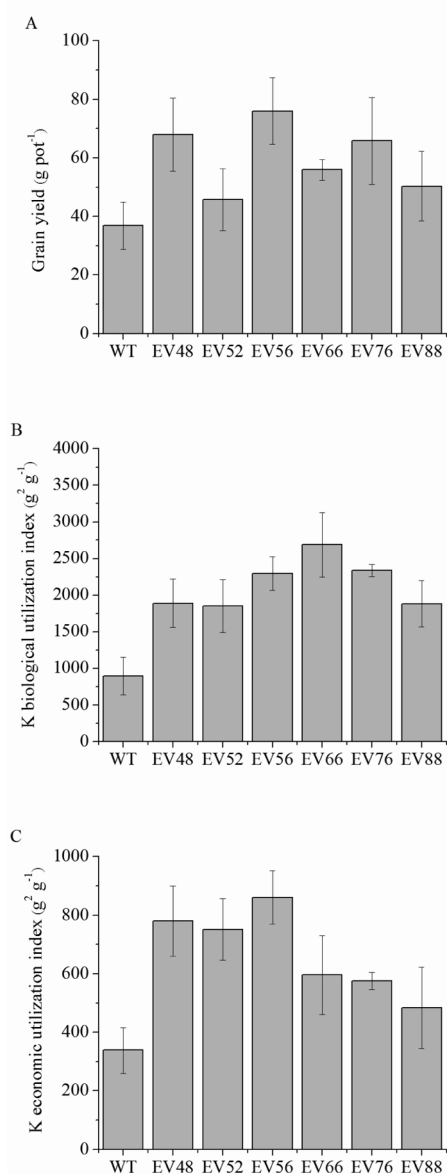


Figure 3: A) Grain yield; B) K biological utilization index (KUI-B); C) K economic utilization index (KUI-E) of wild type and *EdVP1* transgenic wheat lines under Udic Argosols (Bars represent means \pm SD; n=6).

which may make great contributions to root development. The results showed that *EdVP1* gene could promote the root growth of wheat, especially the total root length, root surface area and root tips. Root morphology character is closely related to plant K uptake efficiency, especially the root length, root surface area and root tips (Hassan and Arshad, 2010). The main root morphological characteristics of potassium efficient genotypes were good root morphology and distribution, higher root to shoot ratio, more root tips, larger root surface area and so on (Farmaha *et al.*, 2012; Wang and Chen, 2012). The total root length, root surface area and root tips of *EdVP1* transgenic wheat were significantly higher than those of wild type wheat. This makes *EdVP1* transgenic wheat intercept K^+ from the medium effectively and promotes the K absorption under low K stress.

In the vacuole membrane, H^+ -PPase produces energy through splitting PPi into Pi and releasing H^+ . This process can uptake other ions into cells (Gaxiola *et al.*, 2012). Britto and Kronzucker (2008) reported that K^+ uptake rate of barley was significantly positively correlated with root excretion H^+ rate. This experimental results showed that, K accumulation of wheat had a significant positive correlation with root excretion H^+ ($r = 0.87^{**}$). Moreover root excretion H^+ of *EdVP1* transgenic wheat was significantly higher than that of wild type wheat. This is because that *EdVP1* gene makes H^+ -PPase split PPi and release H^+ , which makes the root excretion H^+ of *EdVP1* transgenic wheat increase. The absorption of K^+ is an active transportation (Hafsi *et al.*, 2011). Root respiration releases CO_2 into soil solution, in which CO_2 and H_2O change into H_2CO_3 . Ion exchange happens between K^+ on the surface of the soil particle and H^+ on the cell surface (Wani and Datta, 2007). Then H^+ gets into soil solution and K^+ enters the roots. Therefore, *EdVP1* gene can not only provide energy for K^+ transportation, but also enhance root excretion H^+ and promote the absorption of K^+ .

Generally, with the increase of root CEC, the mineral nutrient elements uptake of plant will increase (Zhang *et al.*, 2010). CEC of root has a significant effect on

K uptake efficiency. Higher CEC of root greatly facilitates the efficient absorption of K^+ (Ozolina *et al.*, 2010; Wu and Hendershot, 2009). This experimental results showed that, K accumulation of wheat had a significant positive correlation with root CEC ($r = 0.93^{**}$). This showed that CEC of root had a direct impact on K absorption capacity of wheat. The root CEC of *EdVPI* transgenic wheat was significantly higher than that of wild type wheat. This may be due to the higher adsorption of K^+ in root "Dunant space" of *EdVPI* transgenic wheat. K^+ accumulation in the free space can not only contribute to root indirect absorption of K^+ , but also play an important role in K^+ transportation to shoot (Hafsi *et al.*, 2011).

The soil K activation test conducted on Ferrisols and Udic Argosols showed that soil K activation abilities of *EdVPI* transgenic wheat root exudates were much higher than those of wild type wheat, which promoted soil non-exchangeable K in rhizosphere to change in to soil exchangeable K in order to meet the plant needs for potassium. In particular nutrient stress condition, root will secrete specific substances into the rhizosphere to promote the activation of soil nutrients, which will promote element uptake and improve the utilization efficiency of these elements through increasing their solubility and mobility (Jones *et al.*, 2003; Erro *et al.*, 2010). It will cause plant physiological disorders and enzyme activity decrease under low K stress. At the same time, the amount of root exudates has greatly increased, especially the simple organic compounds (Fujii *et al.*, 2012). These organic compounds, especially organic acids, can activate soil mineral K, thus improve plant adaptability to the environment of K stress (Zhang *et al.*, 2010; Yavorskii *et al.*, 2009). This experiment results showed that, *EdVPI* transgenic wheat and wild type wheat secreted five kinds of organic acids, including oxalic acid and malic acid as the main components. The content of organic acids in *EdVPI* transgenic wheat root exudates was 2.22 times as high as that in wild type wheat. Oxalic acid in *EdVPI* transgenic wheat root exudates was 2.67 times as high as that in wild type wheat. Root secretion of organic acids activates potassium

in soils to meet plant requirements on nutrients through protons involvement in the replacement and hydrolysis or organic anion chelation (Carvalhais *et al.*, 2011). The complex and hydrolysis ability of oxalic acid are quite strong. Thus oxalic acid has strong destruction decomposition on soil minerals, such as feldspar, kaolinite and so on (Jones *et al.*, 2003). Therefore, root organic acid secretion ability of *EdVPI* transgenic wheat is much higher than that of wild type wheat, which maybe an important reason for effective K utilization on Ferrisols and Udic Argosols.

It is either higher K acquisition ability (the main factor of K external use efficiency) or greater K internal use efficiency in dry matter or grain yield that plays a key role in the better adaptation of crops to low K (Yang *et al.*, 2003). *EdVPI* gene improved K acquisition ability and K utilization efficiency of wheat, leading to the high grain yield in transgenic lines under low-K condition.

5. Conclusion

Under low K stress, *EdVPI* gene promoted the accumulation of free IAA in wheat root, which may play a key role in the development of root system. *EdVPI* enhanced the amount of root excretion H^+ and root CEC, leading to the higher K accumulation ability in *EdVPI* transgenic wheat. What's more, the root of *EdVPI* transgenic wheat released more organic acids, making great contributions to the activation of soil K. Due to the combination of physiological processes above, *EdVPI* gene significantly promote K absorption and utilization of wheat under low-K condition, thus enhanced the grain yield of wheat. In a word, *EdVPI* gene could be an alternative and viable resource to improve wheat tolerance ability to low-K condition.

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