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Responses of root morphology and architecture to phosphorus deficiency at seedling stage of tobacco (*Nicotiana tabacum*) growth

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Abstract

Plant root has various responses under a low phosphorus (P) status, but knowledge on enhancing root development in tobacco is limited. The aim of this study was to investigate responses of root morphology and architecture of tobacco to different P application levels (0, 0.002, 0.02, 0.2, 2 m mol L^{-1}, at early seedling stage; 0.05, 0.1, 0.2, 1.0, 10 m mol L^{-1} at later seedling stage). Two varieties, XiangYan No.4 and K326, were used in water culture with complete nutrient solution (Hoagland's solution). Results indicated that P deficiency can enhance the lateral root development of tobacco, and thus may be useful in breeding developed tobacco with stronger root system. At early growth stage (within 30 days), 5-fold diluted Hoagland nutrient solution was selected as culture solution. P deficiency of 0.02 m mol L^{-1} can enhance the development of tobacco root hairs, while P deficiency can cause decline in taproot elongation. At later seedling stage (31 days - 75 days), P deficiency concentration with 0.1 m mol L^{-1} was more likely to improve root growth under normal Hoagland nutrient solution condition, mainly by enhancement of lateral root development. In brief, compared with normal P concentration treatment (1 m mol L^{-1}), P deficient concentrations with 0.02 m mol L^{-1} and 0.1 m mol L^{-1} can effectively promote development of tobacco roots at early and later seedling stages, respectively.

Keywords: floating-seedling system, phosphorus deficiency, root morphology and architecture, tobacco seedling.
Abbreviations: P_phosphorus; TTC_triphenyltetrazolium chloride; TPF_triphenylformazan; OD_optical density.

Introduction

Phosphorus (P) is a major plant growth-limiting factor. Although abundant in soil, availability of soil P is very low, because a large proportion of soil P reserve is fixed in soil as composites bound to iron and aluminum oxides (Shen et al., 2002; Giaveno et al., 2010). Plants possess different adaptive responses to acquire poorly mobile soil resources, especially P. It is well documented that plant root can have various responses under a low P status (Zinn et al., 2009). Recently, there is more research on the responses of root development in different plants to P deficiency, especially in *Arabidopsis thaliana* (Devaiah et al., 2008) and *leguminous* plants (Zhu et al., 2010; Sbabou et al., 2010). It is reported that P stress can promote root development, mainly in aspects such as lateral roots and root hairs (Estrella et al., 2001) as well as root length (Steingrobe, 2001; Steingrobe et al., 2001). Also, the number of laterals in *Arabidopsis* are reduced when P is limited (Fitter et al., 2002). Root, as an important organ in plants, can stabilize above ground parts of plants as well as absorb nutrients from soil (Hodge et al., 2009). With respect to tobacco, root tissues can also synthesize nicotine, which belongs to a *pyridine alkaloid* principally found in *Nicotiana* species (Morita et al., 2008). Nicotine is an indispensable index for assessing the quality of tobacco leaves. Therefore, preferable growth of tobacco is closely correlated with favorable root morphology and architecture at seedling stage. Before transplanted tobacco seedlings into soil, a floating system method was generally used to cultivate tobacco seedlings in a controllable environment. Thus far, ordinary problems existing in tobacco seedlings which were transplanting into natural environment were low resistance, long cultivating period as well as less developed root system (Antonopoulos et al., 2010). When transplanted into soil, further growth of tobacco root was retarded due to remarkable differences in growth environment, so that normal growth of tobacco seedlings was adversely affected. Consequently, preferable morphology and architecture of tobacco root can lay a stable foundation for remarkable growth and strong resistance to adverse environment. It is highly meaningful to investigate how to enhance root development at seedling stage, but relevant research is limited. Thus, the goal of the present study was to investigate whether P stress can improve development of tobacco root, and recognize the discrepancy of response of root morphology and architecture to P deficiency among tobacco and other plants, aiming to provide a theoretical basis for further study of tobacco root at seedling stage.

Results

Responses of P concentration in root to P application levels at early seedling stage

P concentration in tobacco seedlings decreased with the decline of P concentration in nutrient solution (Figure 1). There were significant differences (P≤0.05) among normal P concentration (2 m mol L^{-1}) and P deficiency concentrations ranging from 0 to 0.002 m mol L^{-1}, indicating that P uptake can be influenced by P deficiency. P concentration in tobacco root was decreased under P deficiency condition.

Responses of root hair number to P deficiency at early seedling stage

Results in Figure 2 show that root hair number increased with time, and there were obvious differences in root hair number at all P treatments after 11 days. Root hair development responding to P deficiency exhibited a similar trend in two cultivars, while time and intensity of response to P deficiency differed. In contrast to XiangYan No.4 cultivar, K326 cultivar experienced a P deficiency environment and physiological reaction changed correspondingly in advance. Root hair number with 0.2 and 0.02 m mol L^{-1} P treatments was higher than other treatments when seeds sprouted after 16 days, indicating that appropriate P deficiency can induce root hair development. P concentration with 0.02 m mol L^{-1} can significantly increase root hair number compared with other treatments, demonstrating that 0.02 m mol L^{-1} P lies in the optimized P stress concentration scope which can induce root hair development.

Responses of total root length to P deficiency at early seedling stage

Results in Figure 3 show that total root length was increased with time in plants grown under all treatments. Total root length of tobacco seedlings was enhanced with increase of P concentration after 2 weeks, but exhibited negligible differences. Compared with normal P concentration, root elongation was decreased with P application level decline. In contrast to normal P concentration level, total root length cannot be induced by P deficiency. Total root length was composed of two parts, namely tap root length and lateral root root length. Before the development of lateral root, total root length was determined by tap root, while during lateral root development, the contribution ratio of laterals on total root length gradually increased with time. Compared to tobacco seedlings after 2 months, the seedlings within 1 month possessed a small number of laterals. Therefore, total root length was largely determined by tap root length.

Responses of P concentration in tissues to P application levels at later seedling stage

Results in Figure 4 show that P concentration in tobacco root was decreased with P application level decreased at later seedling stage. P concentration of tobacco root can be significantly (*P*≤0.05) affected by P application level.

Responses of root vitality to P deficiency at later seedling stage

Results in Figure 5 show that appropriate P deficiency could increase root activity. In contrast to normal P concentration

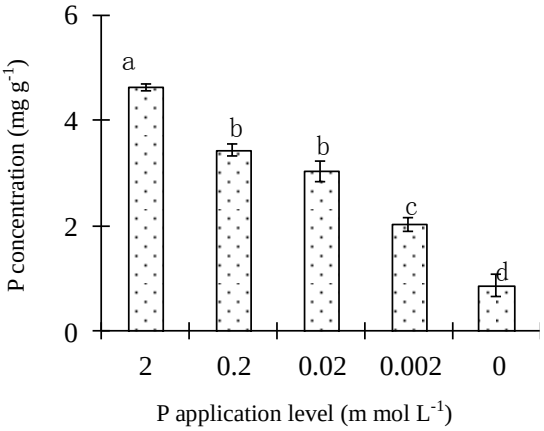

Fig 1. P concentrations in tobacco seedling at 30 days under different P application levels. Average value of P concentrations in 2 varieties was used in figures, because P concentration changes of 2 varieties under different P application levels were almost the same. Different small letters were denoting significance different according to LSD test (p≤0.05) and vertical bars indicate SE (n=4).

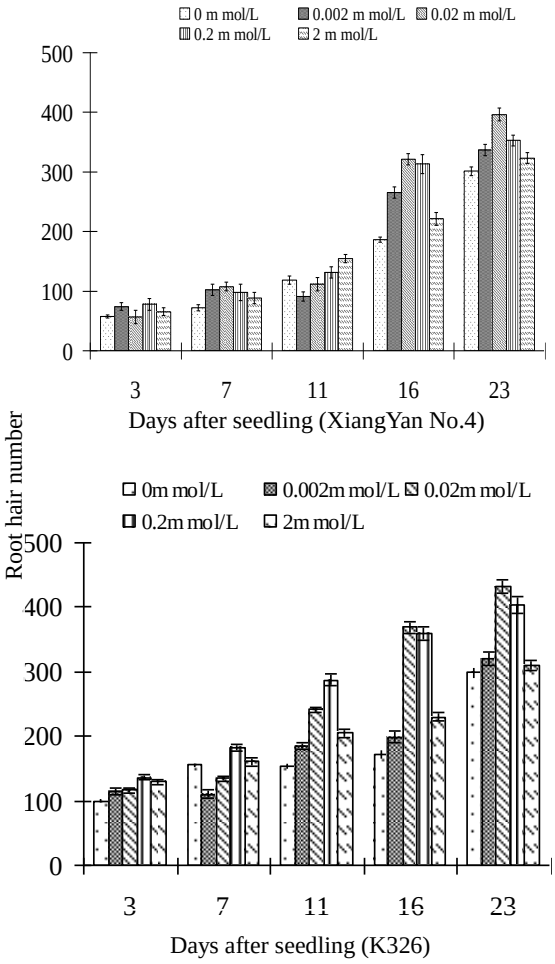

Fig 2. Root hair number of tobacco seedling under different P application levels at early seedling stage. Vertical bars indicate SE (n=4).

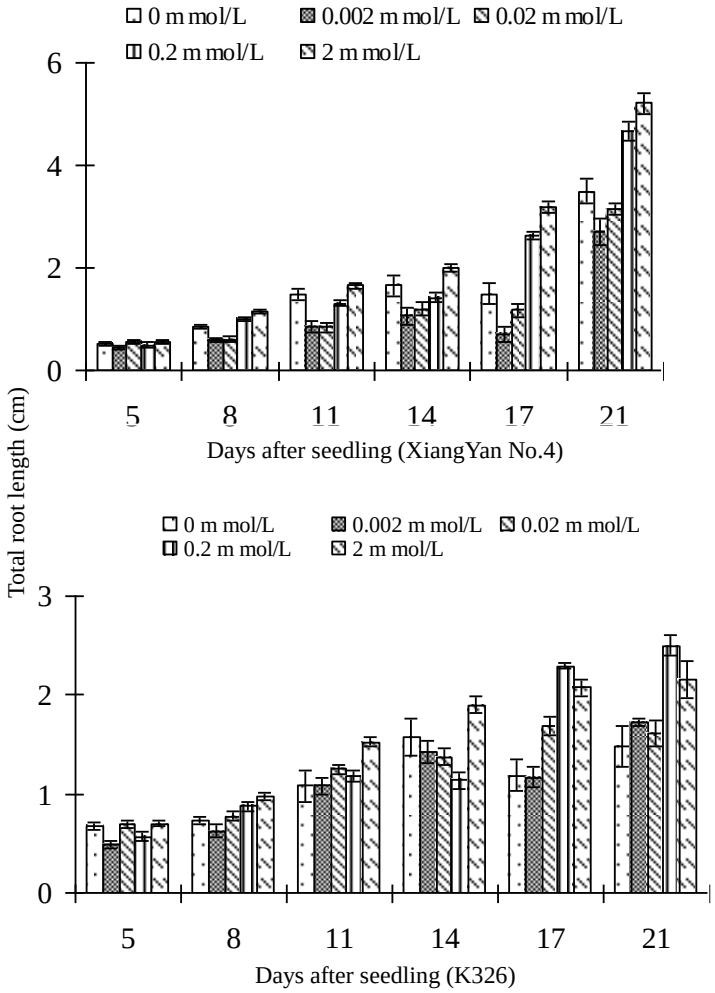

Fig 3. Total root length (cm) of tobacco seedling under different P application levels at early seedling stage. Vertical bars indicate SE (n=4).

Responses of lateral root number and total root length to P deficiency at later seedling stage

Results in Figure 6 show that lateral root number under P deficiency condition (0.2, 0.1, 0.05) was higher than normal condition (1.0 m mol L^{-1}). Lateral root number of 0.1 m mol L^{-1} P treatment was significantly ($P \leq 0.05$) higher than other treatments. In contrast to normal P treatment (1.0 m mol L^{-1}), higher P concentration (10.0 m mol L^{-1}) inhibited lateral development, indicating that lateral root development can be induced by P deficiency. Results in Figure 7 show that total root length was influenced by appropriate P deficiency. Total root length at P treatment with 0.1 m mol L^{-1} was significantly higher than other treatments. Total root length at P treatments with 1, 0.2, and 0.1 m mol L^{-1} were significantly different from 10 m mol L^{-1} P concentration treatment. Lateral root length made a great contribution to total root length in 75-day-old tobacco seedlings. P deficiency with 0.1 m mol L^{-1} concentration could enhance the development of lateral roots. Therefore, P deficiency was more likely to improve total root length of tobacco seedlings.

Discussion

P deficiency is a widespread and long-term issue in arable lands (Lambers et al., 2006; Shulaev et al., 2008). From the perspective of biological evolution, plants have various special genes responding to P stress (Hammond et al., 2004), and possess different metabolic reaction pathways to cope with P stress in soils (Lynch and Brown 2001; Kuo and Chiou 2011). Most of our understanding of plant adaptation to P deficiency comes from researches in *Arabidopsis thaliana* and *legumes*, and little information is available for tobacco plants. In addition, although much research of tobacco plants (*Nicotiana tabacum*) has been affected, there is little knowledge about the role of P applied to tobacco root growth at seedling stage. Currently, floating-seedling system has been adopted in tobacco seedling raising in China, aiming at cultivating strong tobacco seedlings, especially developed root system, which can resist adverse environment. It has been reported that P-deficiency can induce plant root development, including root hair, lateral roots and root length (Cheng et al., 2011). Therefore, it is especially meaningful to study whether P-deficiency can improve tobacco root development at seedling stage. Root system traits are quite important for plant adaptation to adverse environment (Kano et al., 2011; Secenji et al., 2010; Skaggs and Shouse 2008). Our data clearly prove that a continuous low P supply can enhance root development of tobacco, indicating that proper P deficiency is beneficial for root growth. P concentration in tobacco root was decreased with the decline of P concentration in nutrient solution at early seedling stage (Figure 1), which was consistent with the result of later seedling stage (Figure 4). Similarly, P concentration in root generally declined with the severity of P deficiency (Richardson et al., 2007). At the early seedling stage, root hair is closely related to nutrient absorption (Hodge et al., 2009; Libault et al., 2010). We found that low P supply can stimulate the development of root hair and lateral root in tobacco. The degree of root hair stimulation by P stress that we observed agrees with a similar study in *lupin* (Cheng et al., 2011). Lateral root is an important part of plant system in the acquisition of not only P, but also of other essential mineral nutrients (Hodge, 2010). When transplanting tobacco seedlings into soil, the number of laterals, to a large extent, determines the adaptation ability to adverse environment. Development of lateral root occurs later than root hair in tobacco. During the later seedling stage, lateral root development is faster than the early seedling stage (Figures 3 and 7). Besides, P deficiency can enhance tobacco root vitality (Figure 5), which was consistent with a result in rice (Li et al., 2009). Root elongation stimulated by P deficiency was observed in *Arabidopsis thaliana* (Peret et al., 2011) and *Brassica napus* (Yang et al., 2010). The total root length increased dramatically under P deficiency conditions, which was mainly due to promotion of lateral root growth (Liu et al., 2004; Liu et al., 2008). In general, total root lengths is comprised of tap root and lateral root, and a percentage of them in total root length are related to floristic composition (Chmelikova and Hejcman 2012). At early seedling stage, tap root length determines the total root length (Figure 3), while at later seedling stage, lateral root has a larger proportion of total root length (Figure 7), indicating that the contribution ratio of laterals on total root length gradually increased with growth over time. Developed root is a premise of vigorous growth of tobacco plants. It is well known that ideal tobacco root possesses short tap root length and large quantities of laterals. The tap root is too long to thrive when transplanting seedling into soil, thus the growth of tobacco is affected. Our data indicated that P deficiency can decline tap root elongation, and improve development of

tobacco laterals. Seed P content

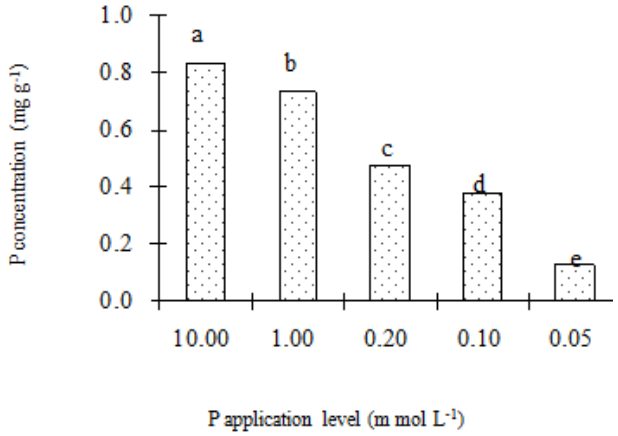

Fig 4. P concentrations in tobacco seedling tissues at 75 days under different P application levels. Average value of P concentrations in 2 varieties was used in this figure, because P concentration change of 2 varieties under different P application levels was almost the same. Different small letters denote significance different according to LSD test ($p \leq 0.05$) and vertical bars indicate SE (n=4).

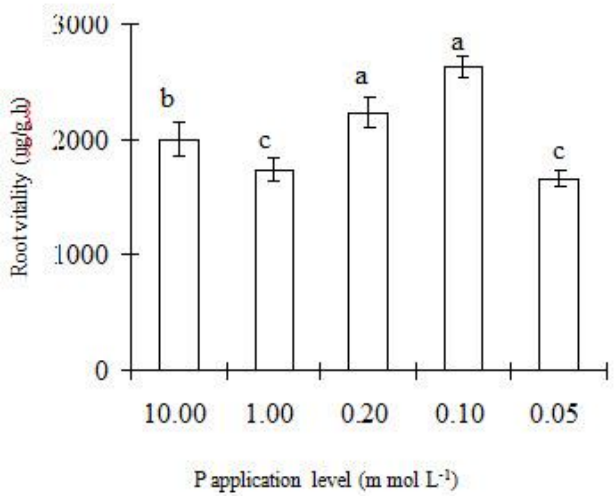

Fig 5. Root vitality (ug/g.h) at 75 days under different P application levels. Average value of root vitality in 2 varieties was used in this figure, because root vitality change of 2 varieties under different P application levels was almost the same.

may postpone the response of tobacco root to P deficiency (Pang et al., 2010). There was no significant difference in the number of root hairs (Figure 2) as well as total root length (Figure 3) within 14 days, hence seed P can satisfy the need of P by tobacco seedling. At the early seedling stage, P is absorbed by tobacco root from nutrient solution and seed. While, when seed P iss depleted, the growth of tobacco root can be affected by different P treatments.

Materials and methods

Plant cultivation

Tobacco (*Nicotiana tabacum*) varieties, K326 and XiangYan No. 4 (popular varieties in Southern China), obtained from Chinese Tobacco Company, Hunan Province, China, were used as plant materials. Seeds were surface-sterilized in 20% (v/v) Clorox® for 30 min, followed by rinsing with deionized water several times and germinated in petri dishes covered with ten layers of hospital gauze as well as two layers of filter papers and placed in the dark. The nutrient composition of the Hoagland solution was: 5 m mol L^{-1} KNO_3, 1 m mol L^{-1} KH_2PO_4, 7 m mol L^{-1} $MgSO_4$, 5 m mol L^{-1} $Ca(NO_3)_2$. $4H_2O$, 3 m mol L^{-1} Fe-EDTA, 0.5 mg L^{-1} B, 0.5 mg L^{-1} Mn, 0.05 mg L^{-1} Zn, 0.02 mg L^{-1} Cu, 0.01 mg L^{-1} Mo. The solution was replaced every 5 days and the pH value adjusted to 5.5. Plants were grown at 28/20°C day/night temperature, respectively, with a 10 h d^{-1} photoperiod with irradiance of 3330-3780 μmol m^{-2} s^{-1} in a phytotron room.

Treatment and sampling

Early seedling stage (within 30 days): seeds germinated after 3 days and transferred to culture container (35cm length, 25cm width, and 30cm height). Hoagland nutrient solution diluted 5-fold, instead of Hoagland solution, was selected to add into container to avoid seedlings being burnt by nutrient solution. For the P-deficient treatment (normal with 0.2 m mol L^{-1}, KH_2PO_4), the P concentrations were 0, 0.002, 0.02, 0.2, 2 m mol L^{-1}, respectively. In the Hoagland solution, KNO_3 could supply sufficient K so that K deficiency could not occur due to change of KH_2PO_4 concentration. Seedlings were sampled with 4 replicates at 30 days for P concentration measurement. samples at 3-23 days with 4 replicates were used for root hair number and total root length measurement. Later seedling stage (31 days – 75 days): complete Hoagland nutrient solution was added into container. In view of more nutrient requirement in older tobacco seedlings, the P treatments began under the concentrations of 0.05, 0.1, 0.2, 1.0, 10 m mol L^{-1}. Seedlings were sampled with 4 replicates at 75 days for P concentration, root vitality, lateral root number, and total root length measurement.

Sample analysis

Root hair numbers was measured under a microscope at 200 times magnification. Intact root, with laterals and root hairs, was picked with the cleaned pearlstone being removed by soaking and washing, root sample was placed in a glass rectangular dish with about 4-5-mm-deep layer of water to untangle the roots and minimize root overlap. Total root length and lateral root number were measured and counted, respectively. Measurement of root vigor was according to the triphenyltetrazolium chloride (TTC) method (Liu et al., 2008). The surface liquid of white young root was blotted with tissue paper and their fresh weights was measured. Roots with weights 0.5 g were placed in tubes, filled with 5 ml of 0.4% TTC, 5 ml phosphate buffer solution (0.1 mol L^{-1}, pH 7.5). The tubes were incubated at 37°C for up to 3 h. The chemical reaction was stopped by adding 2 ml of 15 ug L^{-1} sulfuric acid in the tubes. This step was followed by extraction with triphenylformazan (TPF), which consisted of taking the root out of the tubes and placing them in a pestle, filled with 3–4 ml of ethyl acetate and a little quartz sand and then ground. The liquid phase was removed into a test tube. Ethyl acetate was added to the 10 ml level and optical density (OD) values were recorded with a UV–vis recording spectrophotometer at 485 nm. The OD values were used to calculate equivalent TPF concentrations with which the root activity was determined for each fresh root weight as follows: root vigor (TPF ugg^{-1} FW h^{-1})=TPF reduction (ug)/fresh weight (g)/time (h) Samples

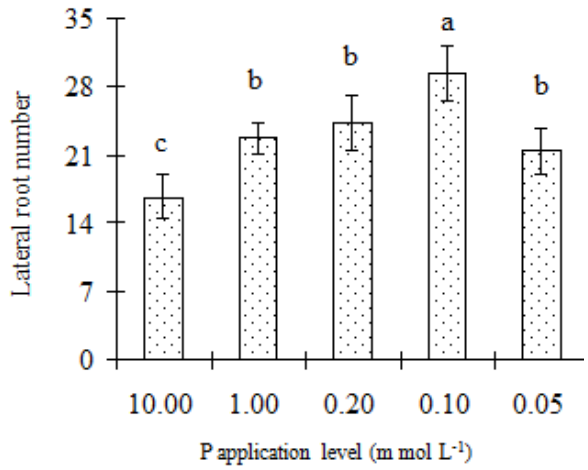

Fig 6. Lateral root number at 75 days under different P application levels. Average value of lateral root number in 2 varieties was used in this figure, because lateral root number change of 2 varieties under different P application levels was almost the same.

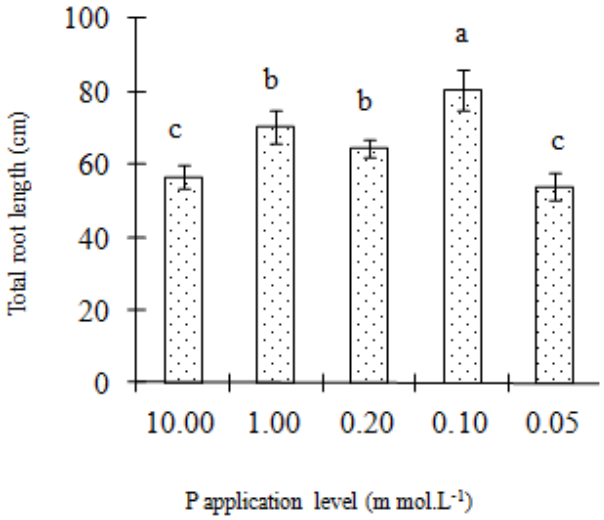

Fig 7. Total root length (cm) at 75 days under different P application levels. Average value of total root length in 2 varieties was used in this figure, because total root length change of 2 varieties under different P application levels was almost the same.

were dried at 120°C for 1 h, and then at 70°C in an air-forced oven for 72 h to determine P concentration.

Data analysis

Each determination was carried out with four replicates. The significant differences among treatments were tested by the analysis of variance and the least significant difference (LSD) at level of 5% using the SPSS software package.

Conclusions

The results showed that P deficiency can significantly improve root development of tobacco. P concentration of 0.02 m mol L^{-1} can enhance tobacco root development at early seedling stage, mainly by enhancement of root hairs. Meanwhile, 0.1 m mol L^{-1} concentration can improve the root growth at later seedling stage, principally by promotion of lateral root development.

Acknowledgements

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References

Antonopoulos DF, Melton T, Mila AL (2010) Effects of chemical control, cultivar resistance, and structure of cultivar root system on black shank incidence of tobacco. Plant Dis. 94: 613-620.

Cheng LY, Bucciarelli B, Shen JB, Allan D, Vance CP (2011) Update on white *lupin* cluster root acclimation to phosphorus deficiency update on *lupin* cluster roots. Plant Physiol. 156: 1025-1032.

Chmelikova L, Hejcman M (2012) Root system variability in common legumes in central Europe. Biologia. 67: 116-125.

Devaiah BN, Madhuvanthi R, Karthikeyan AS, Raghothama KG (2008). Phosphate starvation responses and gibberellic acid biosynthesis are regulated by the MYB62 transcription factor in *Arabidopsis*. Mol. Plant. 2: 43-58.

Estrella LH, Bucio JL, Grierson C (2001) Molecular characterization of the effects of phosphorus on the development of root hairs in *Arabidopsis*. Plant Nutr. 92: 20-21.

Fitter A, Williamson L, Linkohr B, Leyser O (2002) Root system architecture determines fitness in an Arabidopsis mutant in competition for immobile phosphate ions but not for nitrate ions. Proceedings Royal Socie London (B). 269: 2017-2022.

Giaveno C, Celi L, Richardson AE, Simpson RJ, Barberis E (2010) Interaction of phytases with minerals and availability of substrate affect the hydrolysis of inaction phosphates. Soil Biol Biochem. 42: 491-498.

Hammond JP, Broadley MR, White PJ (2004) Genetic responses to phosphorus deficiency. Ann Bot. 94: 323-332.

Hodge A, Berta G, Doussan C, Merchan F, Crespi M (2009) Plant root growth, architecture and function. Plant Soil. 321: 153-187.

Hodge A (2010) Roots: The acquisition of water and nutrients from the heterogeneous soil environment. Prog Bot. 71: 307-337.

Kano M, Inukai Y, Kitano H, Yamauchi A (2011) Root plasticity as the key root trait for adaptation to various intensities of drought stress in rice. Plant Soil. 342: 117-128.

Kuo HF, Chiou TJ (2011) The role of microRNAs in phosphorus deficiency signaling. Plant Physiol. 156: 1016-1024.

Lambers H, Shane MW, Cramer MD, Pearse SJ, Veneklaas EJ (2006) Root structure and functioning for efficient acquisition of phosphours: matching morphological and physiological traits. Ann Bot. 98: 693-713.

Li JZ, Xie Y, Dai AY, Liu LF, Li ZC (2009) Root and shoot traits responses to phosphorus deficiency and QTL analysis at seedling stage using introgression lines of rice.

J Genet Genom. 36:173-183.
Libault M, Brechenmacher L, Cheng JL, Xu D, Stacey G (2010) Root hair systems biology. Trends Plant Sci. 15: 641-650.
Liu Y, Mi GH, Chen FJ, Zhang JH, Zhang FS (2004) Rhizosphere effect and root growth of two maize (*Zea mays* L.) genotypes with contrasting P efficiency at low P availability. Plant Sci. 167: 217-223.
Liu RX, Zhou ZG, Guo WQ, Chen BL, Oosterhuis DM (2008) Effects of N fertilization on root development and activity of water stressed cotton (*Gossypium hirsutum* L.) plants. Agric Water Manage. 95: 1261-1270.
Lynch JP, Brown KM (2001). Topsoil foraging-an architectural adaptation of plants to low phosphorus availability. Plant Soil. 237:225-237.
Morita M, Shitan N, Sawada K, Montagu M, Inze D, Rischer H, Goossens A, Caldentey K, Moriyama Y, Yazaki K (2008) Vacuolar transport of nicotine is mediated by a multidrug and toxic compound extrusion (MATE) transporter in *Nicotiana tabacum*. Proc Natl Acad Sci USA. 106: 2447-2452.
Pang JY, Tibbett M, Denton MD, Lambers H, Siddique K, Bolland M, Revell CK, Ryan MH (2010) Variation in seedling growth of 11 perennial legumes in response to phosphorus supply. Plant Soil. 328: 133-143.
Peret B, Clement M, Nussaume L, Desnos (2011) Root development adaptation to phosphorus starvation: better safe than sorry. Trends Plant Sci. 16: 442-450.
Richardson AE, Hocking PJ, Simpson RJ, George TS (2007) Plant mechanisms to optimize access to soil phosphorus. Crop Pasture Sci. 60: 124-143.

Sbabou L, Bucciarelli B, Miller S, Liu JQ, Berhada F, Maltouf AF, Allan D, Vance C (2010) Molecular analysis of SCARECROW genes expressed in white lupin cluster roots. J Exp Bot. 61: 1351-1363.
Secenji M, Lendvai A, Miskolczi P, Kocsy G, Galle A, Szucs A, Hoffmann B, Sarvan E, Schweizer P, Stein N, Dudits D, Gyorgyey J (2010) Differences in root functions during long-term drought adaptation: comparison of active gene sets of two wheat genotypes. Plant Biol. 12: 871-882.
Shen H, Yan XL, Zhao M, Zheng SL, Wang XR (2002) Exudation of organic acids in common bean as related to mobilization of aluminum- and iron-bound phosphates. Environ Exp Bot. 48: 1-9.
Shulaev V, Cortes D, Miller G, Mittler R (2008) Metabolomics for plant stress response. Physiol Planta. 132: 199-208.
Skaggs TH, Shouse PJ (2008) Roots and root function: introduction. Vadose Zone J. 7: 1008-1009.
Steingrobe B (2001). Root renewal of sugar beet as a mechanism of P uptake efficiency. J Plant Nutr Soil Sci. 164: 533-539.
Steingrobe B, Schmid H, Claassen N (2001) Root production and root mortality of winter barley and its implication with regard to phosphate acquisition. Plant Soil. 237: 239-248.
Yang M, Ding GD, Shi L, Feng J, Xu FS, Meng JL (2010) Quantitative trait loci for root morphology in response to low phosphorus stress in *Brassica napus*. Theor Appl Genet. 121: 181-193.
Zhu YY, Zeng HQ, Dong CX, Yin XM, Shen QR, Yang ZM (2010) MicroRNA expression profiles associated with phosphorus deficiency in white lupin (*Lupinus albus* L.). Plant Sci. 178: 23-29.
Zinn KE, Liu JQ, Allan DL, Vance CP (2009) White Lupin (Lupinus albus) response to phosphorus stress: evidence for complex regulation of *LaSAP1*. Plant Soil. 322: 1-15.