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Induction of Root Hair Growth in a Phosphorus-Buffered Culture Solution

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Abstract

A system to control the release of phosphate in water was successfully established, based on solubility product of [Ca^{2+}] and [PO_4^{3-}] using tricalcium phosphate as P source in the hydroponic solution, and adding $CaCl_2$ for supplementing extra Ca^{2+}. The system, similar to soil solutions, was a P nutrient buffer solution with very low bioavailable P. The buffer solution induced the roots of both monocotyledon and dicotyledon species to grow abundant root hairs, 3 mm in maximum length. The monocotyledons were corn (*Zea mays* L.) (var. Yellow Rose), wheat (*Triticum aestivum* L.) (var. Yanzhong 144), *Triticale secale* L. (var. Jingsong 5), and ryegrass (*Lolium rigidum* L.) (var. Ruanni), and the dicotyledons were *Arabidopsis thaliana* L. (var. Columbia), white clover (*Trifolium repens*) (var. Kopu), Lotus (*Lotus peduncucatus* Cav. *Luliginosus* Schkuhr) (var. Grasslands Maku). For these species we proved that the root environment controls the induction of root hair formation. However, the hydroponic buffer solution failed to induce root hairs on the roots of onion (*Allium cepa* L.). Other investigators have concluded that corn does not form root hairs in hydroponics, but abundant long root hairs on corn were induced by this buffer system. The roots with abundant long root hairs are called "hedgehog roots" because they have hairs everywhere just like a hedgehog.

Key words: low-P stress, root hairs, *Arabidopsis thaliana*, *Zea mays*, *Triticum aestivum*

INTRODUCTION

Both water and phosphorus bioavailability crises limit agricultural production. Ensuring the efficient use of water and P is one of the biggest tasks for scientific researchers. Root hairs are responsible for 78% (Barber 1995) or even more of nutrient uptake from the growth media (soil or water). Root hairs are tubular-shaped tip-growing cells arising from root epidermal cells (Ridge 1996). They increase the absorptive capacity of roots by increasing the surface area. Because these non-dividing cells elongate outside the body of the root, their internal contents can be easily observed and experimentally manipulated. They have been used recently as experimental models to study biological processes (Hofer 1996). More recently, Michael (2001) reviewed the possible mechanisms for control of root hair formation. He presented evidence to support the role of ethylene in root hair formation. Schmidt (2001) concluded that both low-P and low-Fe stresses promoted root hair formation, but low-P stress had a greater effect than low-Fe stress. In a water culture, root hairs on plant roots are usually sparse or nonexistent, whilst most abundant when roots pass through larger air-filled gaps in the soil (Pitman *et al.* 1976; Weatherley 1982). In some environments, root hairs are much shorter than those of the same plants grown in soil (Barber 1995). However, in all recently reported studies, the tested plants were grown in traditional hydroponics or soil culture

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and the length of root hairs was about 0.3 to 1 mm (Barber 1995). Is this the maximum length of the hairs? Is it possible for them to grow more profusely? How do the root hairs grow on plants with their roots in a nutrient buffer system? Finding answers to these questions is the purpose of this study.

MATERIALS AND METHODS

Plant materials

Arabidopsis thaliana L. (var. Columbia), white clover (*Trifolium repens* L.) (var. Kopu), Lotus (*Lotus peduncucatus* Cav. *Luliginosus* Schkuhr) (var. Grasslands Maku), onion (*Allium cepa* L.) (var. White Lisbon) and ryegrass (*Lolium perenne* L.) (var. Ruanni) were collected from AgResearch Grasslands, New Zealand. Wheat (*Triticum aestivum* L.) (var. Yanzhong 144 and Chinese Spring) was obtained from Institute of Genetics, Chinese Academy of Sciences, China. Corn (*Zea mays* L.) (var. Yellow Rose), *Triticale secale* L. (var. Jingsong 5) were collected from Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, China.

Plant culture

Field crop plants Throughout the experiments, all plants except corn were germinated and grown in a Temperzone Controlled Environment Growth Chamber with 14 h daytime and 10 h night time, AgResearch Grasslands, New Zealand. The light intensity of the chamber was 500 microeinsteins m^{-2} s^{-1} and the temperature was 25°C. Corn seedlings were grown hydroponically in a screen house during September to October 2001 in Beijing. The basic culture solution recipe (Liu *et al.* 1998) was as follows: 2.8558 mM N as $NaNO_3$, (better: with $NaNO_3$ as N source) 1.0233 mM K as K_2SO_4, 0.9980 mM Ca as $CaCl_2·2H_2O$, 1.6458 mM Mg as $MgSO_4·7H_2O$, 89.5 μM Fe as $FeSO_4·7H_2O$ and EDTA, 9.1 μM Mn as $MnCl_2·4H_2O$, 0.5 μM Mo as $(NH_4)_6Mo_7O_{24}·4H_2O$, 0.2 μM Zn as $ZnSO_4·7H_2O$, 18.5 μM B as H_3BO_3 and 0.2 μM Cu as $CuSO_4·4H_2O$. There were three treatments: (1) the basic culture solution plus tricalcium phosphate (TCP) as the sole phosphorus source with 1 mM extra Ca as $CaCl_2$ to reduce the bioavailability of phosphorus; (2) the basic culture solution without P; and (3) control, the basic culture solution plus 0.3229 mM P as $NaH_2PO_4·2H_2O$. Plastic pots, each 1 200 mL in volume, were used for culture, and 0.5 mL 3% H_2O_2 was put into each pot every two days instead of aeration. Photos of root hairs were taken with a camera mounted on a microscope.

Model plants The *Arabidopsis* seeds were surface sterilized and spread on an agar gel plate in a 9-cm diameter dish containing the above nutrient recipe. There were three treatments: (1) the complete nutrient solution; (2) all nutrients minus available P but with 20 mg ground TCP and 1 mM extra Ca as $CaCl_2$; and (3) all nutrients but no P. We prepared the nutrient solutions and added 0.8 g agar per 100 mL culture solution. After that, we heated the solution in a microwave oven, and then placed 20 mL of this solution into each of 100×15 mm plastic Petri dishes. Two seeds were put in each dish and the dishes were sealed with parafilm. The plants were incubated at a 45° angle in the growth chamber, so that the root grew towards the bottom of the dish within the medium. This provided the growing root hair cells a homogeneous environment including temperature, humidity, nutrient concentration, mechanical impedance, etc.

Depletion curve

Plants were grown hydroponically for three weeks. The complete culture solution recipe was similar to that used for the field crop plants described above with the following minor modification in N forms: 0.7140 mM NH_4^+ as $(NH_4)_2SO_4$ and 2.1418 mM NO_3^- as $NaNO_3$. Then 16 uniform and P-starved seedlings of wheat were chosen and 2 seedlings were placed into each of eight 600 mL plastic pots: 4 pots with about 80 μM NaH_2PO_4 and 0.2 mM $CaSO_4$, and the other 4 with TCP and 0.2 mM Na_2SO_4. Also, 0.5 mL 3% H_2O_2 was placed into each pot for oxygen supply to seedlings. A peristaltic pump (CPP30, made in Chemlab, England) was used to circulate the liquid at 12 mL per minute for each pot. In order to keep the solution free of TCP particles, we put 0.5 g TCP in the tubing with glass fibers at the mouth of the tubing. The measurement was finished at the growth chamber. In order to keep the pots with the same liquid volume, the transpiration rate of the plants

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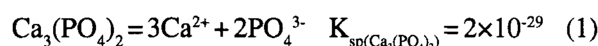
was tested before measurement. Results showed that 5 mL water was lost from each pot per hour. Therefore, 5 mL Milli Q water was added into each pot after every sampling. The measurement solution was sampled once every hour and 15 times in total. P concentrations were detected colorimetrically (Shimogawara and Usuda 1995).

RESULTS

The differences between depletion curves of soluble and Insoluble phosphates in water

Phosphate behavior in soil solution is very different from that in a culture solution (Table). In the traditional hydroponics, the P concentration is very high but variable. The diffusion constant of phosphorus in hydroponics is about 1×10^{-5} cm^2 s^{-1} (Lide 1998) but that in soil is only 5×10^{-11} cm^2 s^{-1} (Barber 1980). The former is about 200 000 times higher than the latter. Therefore, it is much easier for plants to obtain phosphorus from an hydroponic solution than from the soil. Also, a soil solution has a great buffer capability because soil is rich in potential phosphorus including organic phosphates and immobilized phosphates such as strengite ($FePO_4{\cdot}H_2O$) and hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. In fact, the concentration of total phosphorus in soil is about 200 to 500 times (Li *et al.* 1995) higher than that of bioavailable phosphorus. These organic or inorganic phosphate resources can be converted into bioavailable phosphorus via enzymatic lysis or mineralization processes. Hydroponics basically lacks a buffering capability.

Based on solubility product in chemistry, a novel hydroponic controlled release system of phosphate in water was designed. The P concentration was controlled by adjusting quantities of the companion cation of the phosphate as required. The controlled release system was built by using $Ca_3(PO_4)_2$ (TCP) as the sole phosphorus source and extra Ca^{2+} was added with $CaCl_2$ as Ca source. According to solubility products of $Ca_3(PO_4)_2$ and $CaHPO_4$, there are three very important equilibria in the solution when tricalcium phosphate is put into pure water.



$$Ca_3(PO_4)_2 = 3Ca^{2+} + 2PO_4^{3-} \quad K_{sp(Ca_3(PO_4)_2)} = 2\times10^{-29} \quad (1)$$

$$CaHPO_4 = Ca^{2+} + HPO_4^{2-} \quad K_{sp(CaHPO_4)} = 1\times10^{-7} \quad (2)$$

$$HPO_4^{2-} = H^+ + PO_4^{3-} \quad K_{a3} = 4.8\times10^{-7} \quad (3)$$

Let (1) be divided by (2) and by (3) and let pH = 7.2 to simplify the calculation, then

$$[PO_4^{3-}] = 2.63\times10^{-17} \div [Ca^{2+}]^2 \quad (4)$$

This is the mathematical relationship between $[PO_4^{3-}]$ and $[Ca^{2+}]$ and it can be as low as about 10^{-6} M when tri-calcium phosphate is added into neutral pure water. Therefore, $[PO_4^{3-}]$ will be reduced by 75% when $[Ca^{2+}]$ is doubled, or $[PO_4^{3-}]$ can be decreased by 99% when $[Ca^{2+}]$ is increased by 10-fold. This is the basic principle for adjusting the P concentration in hydroponics of the novel system. Based on this principle, we can adjust the P concentration to as low as that in a soil solution or to a P-level we desire (Fig.1).

$Ca_3(PO_4)_2$ can release P and Ca with plant absorption because there is always an equilibrium between the solid and liquid phases of $Ca_3(PO_4)_2$. An hydroponic solution with $Ca_3(PO_4)_2$ as the sole P source is a buffer system for the P nutrient. This novel hydroponic solution can

Table Differences in P concentration and its behavior between soil solution and hydroponics (Liu *et al.* 1997)

	Soil solution (a)	Hydroponics (b)	b/a
P concentration	Low (0.5-2 μM)	High (30-300 μM)	60-150
Diffusion constant (cm s^{-1})	4×10^{-11}	$5\text{-}8\times10^{-6}$	-10^5
Buffer capability	Very high	Almost none	

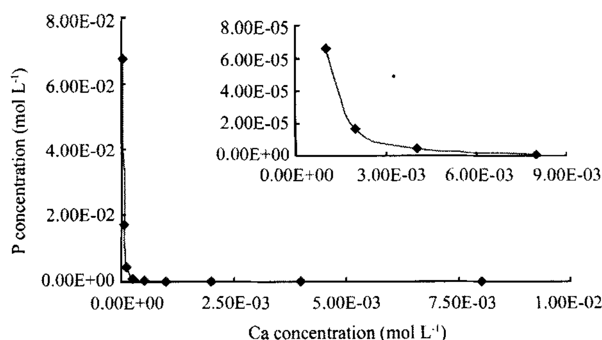


Fig. 1 Relationship between $[PO_4^{3-}]$ and $[Ca^{2+}]$ when TCP is used as the sole P source placed into pure water at pH 7.2.

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basically simulate both the P concentration and buffering capability of a soil solution. Actually, when we added about 80 μM of available P into a 600 mL plastic pot and allowed two seedlings of 21-day old wheat to take up the P for 15 h, the bioavailable P in the pot was almost as low as the minimum concentration (C_{min}) at which the wheat genotype, Yanzhong 144, was not able to extract a net amount of P from the solution. However, under identical conditions, when we used TCP as the sole P source instead of a soluble phosphate, then after 15 h there was about 20 μM of bioavailable P in the solution in the pot, even though at the outset the content of bioavailable P was only about half of that when soluble phosphate was used (Fig.2).

Formation of hedgehog roots on different species

We found repeatedly that *Arabidopsis thaliana* L. and the other tested crop plants could grow very abundant long root hairs (Fig.3) in the P nutrient buffer system. In contrast, root hairs do not develop when bioavailable P is present at a high concentration or when it is totally absent. All of the tested species of crop plants formed or failed to form root hairs under identical conditions in this study. However, the length of root hairs varied with species. *Triticale secale* L., wheat, ryegrass and Arabidopsis had much longer (about 3 mm) root hairs than white clove and lotus (around 1 mm). Some results have shown that corn is completely unable to form hairs in water (Hofer 1996). However, these same experiments proved that corn could grow very abundant and long root hairs with a very long lifespan in the novel hydroponic P nutrient buffer system. We could see the corn root hairs in about one week after placing the corn seedlings into this hydroponic system, and these roots retained their root hairs until we ended the experiment after three weeks. In contrast, even though in traditional cultural methods such as soil culture, the lifetime of root hairs is only 2 to 3 days (Barber 1995). The concentration of bioavailable P in the culture solution with TCP as the sole P source and 1 mM extra calcium ions as $CaCl_2$ was around 1.5 μM, and this level of P is very similar to that of soil solutions.

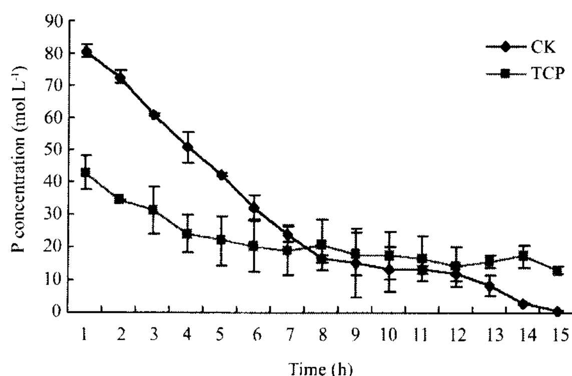

Fig. 2 A P nutrient buffer system was established with tri-calcium phosphate as the sole P source in Milli Q water (18.2 MΩ cm^{-1}). About 80 μM available P as sodium dihydrogen phosphate was used for the check (CK). Two seedlings of 21-day old wheat were put in each 600 mL plastic pot for absorbing the P. In about 15 h, the available P in the CK solution was almost as low as the minimum concentration. But when TCP was used as the P source, after 15 h about 20 μM of available P remained in the solution pot even though at the outset the available P was only half of that in the CK.

However, as all of the botany context-books have said, onions have no root hairs. They could not grow hairs either in this new system.

DISCUSSION

The control and regulation of root hair formation are well documented in literature (Michael 2001). Summarily, inorganic nutrient stress such as low-P and low-Fe and sufficient calcium ions and plant hormone like ethylene, so-called "stress-hormone", all contribute markedly to the root-hair formation (Müller and Schmidt 2004; Zhang *et al.* 2003; Ma *et al.* 2001). However, results from this study showed that maintaining a low-P signal might more profoundly affect and stimulate the plants in stress to grow uncommonly long and thick hairs because root hair density was uniquely sensitive to P-deficiency as opposed to that of other nutrients (Ma *et al.* 2001). Actually, the stressed plants might have many adaptive mechanisms such as morphology, physiology, biochemistry, genetics and even molecular biology. Root-hair formation belongs to morphological adaptability.

Fig.4 is a schematic illustration of the plants grown in different culture systems. Hydroponic solutions can be divided into two sorts: traditional culture solution and nutrient buffer solution. In terms of P, the traditional culture solution usually contains as much concentration as 30 to 300 μmol L^{-1}. That is about 60 to 150 times higher than that of soil solution (Table), but it almost has no buffer ability. Hence, in the traditional hydroponic system, it has two extremes: very high P level or no P. At the former extreme, the plants can

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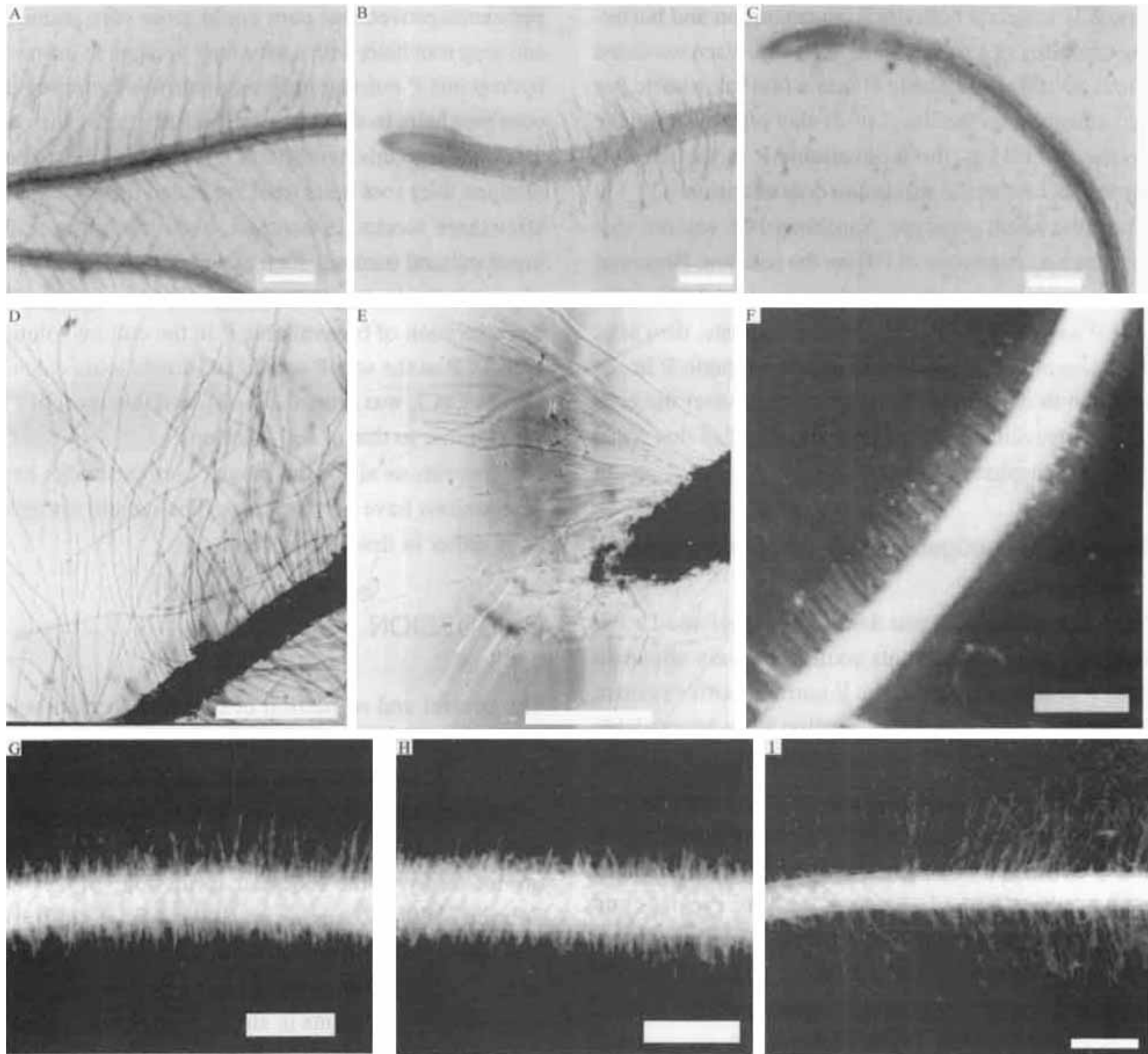

Fig. 3 The hedgehog roots of different species grown in a P-buffered nutrient solution prepared with tri-calcium and extra Ca using calcium chloride as Ca source based on solubility products of Ca^{2+} and PO_4^{3-}. A, a mature root of *Arabidopsis thialiana* L. in the buffer; B, the root tip of *Arabidopsis* grown in the buffer; C, the root tip of *Arabidopsis* grown in the CK; D, a mature root of wheat grown in the buffer; E, a root tip of wheat grown in the buffer; F, a mature root of corn grown in the buffer; G, a mature root of lotus grown in the buffer; H, a mature root of white clover grown in the buffer; I, a mature root of *Trilicale secale* L. grown in the buffer. The bar is one mm in length.

have more than sufficient P and hence, it is unnecessary for them to form root hairs. However, at the latter extreme, there is no P and hence the plants have no possibility to access P. Therefore, no *in situ* signal of phosphates to the grown plants, even if they form root hairs, they cannot take up P at all. Furthermore, they would not form any root hairs in order to save organic carbon which is very limited in this situation because of P shortage and subsequently, the photosynthetic rate is influenced as well. Therefore, Pitman and his co-workers (Pitman *et al.* 1976) are right that the plants have non-existent or very sparse root hairs when they are grown in water. However, they could grow very long and thick root hairs when the traditional culture solution was changed into a P nutrient buffer system because the buffer system could provide sustaining low-P signals to the grown plants.

Additionally, calcium is very important for root hairs. Two phosphate sources, tri-calcium phosphate in this research, and ferric phosphate in another study (data

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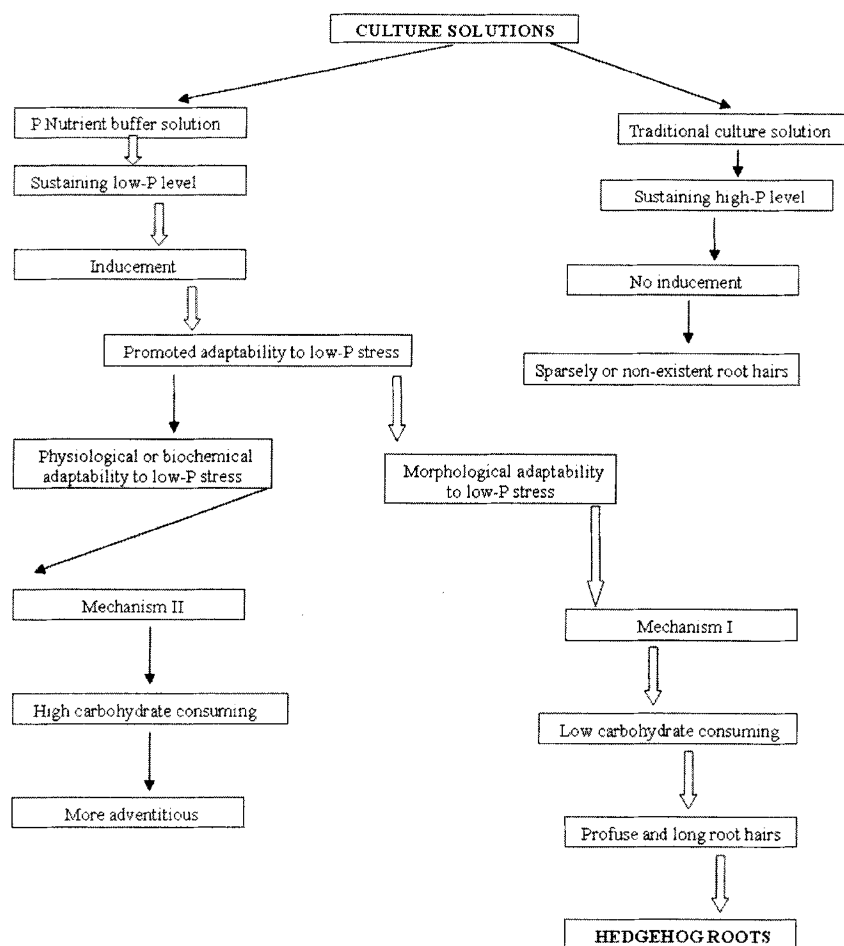

Fig. 4 Schematic illustration of formation of "hedgehog roots" when plants were grown in hydroponics with low-P but buffered solution. To low-P stress, there may be two different adaptive mechanisms in root morphology. Most of plant species including corn employ mechanism I to adapting low-P stress and develop long and thick root hairs and form hedgehog roots. However, fewer species use mechanism II to adapting the stress because they may not have the ability to form thick hairs. Obviously, mechanism I is more efficient and economic in use of carbohydrate than mechanism II.

not presented), were used for establishment of the P buffer system. The plants were able to form hedgehog roots with similar hair length in both buffer systems. However, the root hairs were much more prolific with tri-calcium phosphate as P source than that with ferric phosphate as P source.

When P is fully depleted, the grown plants cannot obtain any P from the culture solution, even though the plants could increase its absorptive area through forming root hairs. The plants did not grow root hairs and saved hydrocarbon from photosynthesis in this research. In soil solution, the condition is much different from the traditional hydroponics. There is very low concentration of phosphate but being buffered. The larger the absorptive area, the more phosphate the plants can take up. It is worthy for the plants to grow root hairs at expenditure of hydrocarbon. Therefore, what Pitman *et al.* (1976) and Weatherley (1982) described is true in traditional hydroponics. However, the tested plants formed very thick and long root hairs in the novel hydroponics with P buffer ability that could simulate the soil condition.

In terms of root-hair formation, the crop plants could be classified into two categories: having the ability to form root hairs and not having the ability to form root hairs. Among the eight crops tested, seven fell into the first category while onion fell into the second category. In this and other experiments (such as use of a root split system, and of a triad root split system) (data not presented), the onion plants failed to form hairs. This suggests that onion did not possess the genes that determine root hair formation. In other words, the onion

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genome had no gene for root hair formation to express even when the medium was very suitable for expression of such gene (s). In any case, although onion failed to produce any visible root hairs in any of the experimental conditions, one produced quite thin roots instead. Now transgene technology in both plants and animals has reached a high level of development, and possibly, the onion genome could be transformed by introducing into it the genes for root hair formation. Since all the genes in *Arabidopsis thailana* L. have been sequenced and cloned, it might be possible to introduce the *Arabidopsis thailana* root hair genes and make them expressed in onion to produce root hairs in this species.

Acknowledgements

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