Bangladesh J. Bot. **45**(5): 979-985, 2016 (December)

CITRIC ACID EXUDATION AND ROOT GROWTH OF MAIZE SEEDLINGS (*ZEA MAYS* L.) UNDER P-DEFICIENT CONDITION

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Key words: Phosphorus deficient, *Zea mays*, Root growth, Citric acid exudates

Abstract

Citric acid exudation and root growth under phosphorus-deficient condition were studied of the seedlings of maize grown in solution culture and rhizobox. Phosphorus deficiency resulted to increase citric acid excretion from P-deficient maize roots with concomitant decrease in pH of growth medium. The length of primary, lateral, total roots, roots hairs and number of lateral roots as well as roots hairs and root meristem volume increased of P-deficient maize seedlings.

Introduction

An important input to agricultural systems is phosphorus and the issues associated with Pi availability and acquisition by plants represents problems of global proportions (Stutter *et al.* 2012). Under Pi deficient conditions, however, plants have adopted several strategies to enhance Pi acquisition and reduce the need for Pi to act on multiple levels, including morphological and biochemical changes, transcriptional activation and physiological responses (Czarnecki *et al.* 2013).

Root exudation of low-molecular weight organic acids, mainly citric acid is enhanced in many plant species under P-deficiency (Grierson 1992). Under Pi-deficiency, white lupin increased the formation of cluster or proteoid roots (Wasaki *et al.* 2003), released H^+ and caused an increase in exudation of citrate (Zhang *et al.* 2004, Kania *et al.*2003, Neumann *et al.*1999 and Dinkelaker *et al.* 1989).

The increased exudation of citrate from whole root systems of white lupin under phosphorus deficiency was observed. Shen *et al.* (2004) reported that the pH of the root exudates collected from the plants fed with 10 µg P/g soil was significantly lower than that from plant receiving 200 µg P/g soil. The citrate concentration in the root was higher in the plant receiving 10 µg P/g soil than that of the plant receiving 200 µg P/g soil. There was a highly negative relationship of pH value of exudates with citrate concentration.

Modification of the root growth is a well-documented response to P-starvation (Lynch 1995) . Lakshmi and Narayanan (1988) recorded higher primary and secondary root length in groundnut grown in sand culture without phosphorus. In rice, P-deficiency increased root fineness or enhanced the root growth (Wissuwa 2003). Ma and Liang (2004) observed that the root systems grew faster, root axes became smaller, number and density of lateral roots was increased following phosphorus deficiency stress.

The information about Pi-deficiency on citric acid exudation and root growth morphology in maize is rare. So the present investigation was initiated to study the citric acid exudation and root length and number, and root hair length and number and overall root growth of maize under phosphorus deficient condition.

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Materials and Methods

Zea mays L. var. barnali (Maize, 2n = 2x = 20) was used as plant material. The seeds were surface satirized by soaking the seeds in 4% sodium hypochlorite solution for one min, followed by washing 7 to 8 times in tap water running and three times in distilled water. The seeds of maize were obtained through the courtesy of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Bangladesh.

Plants were grown in solution culture for citric acid exudation study. According to Hewitt, (1996) the –P nutrient solution consisted of 1.25 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.5 mM $MgSO_4$, and trace elements, in mg/l : Fe (as FeEDTA) 4.6; B 0.05; Mn 0.05; Zn 0.05; Cu 0.02; Mo 0.01. In the + P nutrient solution 0.25 mM KH_2PO_4 was added. Pi-containing solution (+P) was used as control and Pi free solution was used as treatment (–P). Plants were subjected to Pi-deficiency treatment for 4 days prior to collection of samples and three replicates were taken for each treatment. The pH of + P and – P nutrient containing the seedlings were recorded every day up to 4 days of treatment. Two +P plants and two phosphorus deficient plants were placed in separate beakers containing 50 ml of distilled water. Three replicates were used for each treatment. The beaker containing plants was shaken by a shaker for two hrs for collecting the root exudates. The exudates were collected and stored at -14^oC for further analysis. Fifty ml of exudates was evaporated almost to dryness using a rotary evaporator (Heto Birkerod, 01PF 623, No. 8011). The residue was taken in 1 ml of deionized water. The amount of citric acid was measured enzymatically in the exudates (Anon. 1989). Fresh weight of the root was recorded after collection of root exudates.

Seedlings were grown in rhizobox to study the root growth according to Sarker and Karmoker (2009) at 2, 4, 6 and 8 days of treatment (Fig. 1).

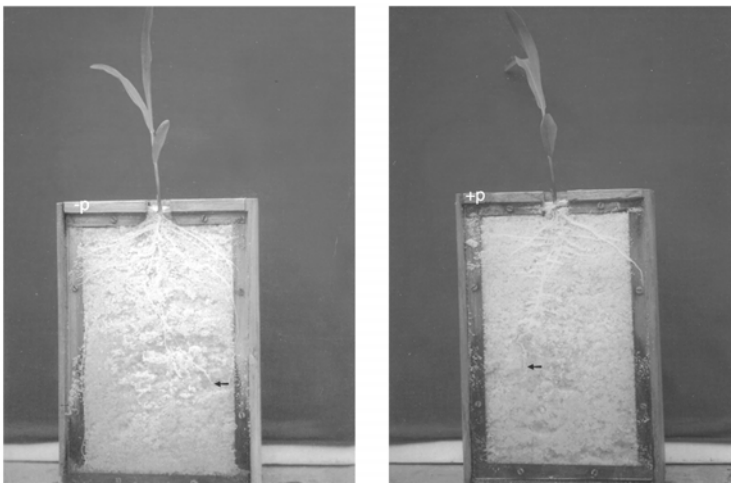

Fig. 1. The effect of P-deficiency on the root growth of 8-day-old maize seedling grown in rhizobox with phosphorus (+P) and without phosphorus (–P)

A transparent sheet was placed on the lid of the rhizobox. The roots of the seedlings in the rhizobox were traced on the transparent sheet at 2, 4, 6 and 8 days of germination. The length of the traced primary root, length and number of traced lateral roots were measured and recorded.

The rate of growth of roots from 2 to 8 days of germination was calculated from the data collected from the length of the traced in the transparent paper.

For measurement of length and number of roots hairs eye-piece graticule was placed on transparent lid of rhizobox along the root where root hairs zone was situated. Root hairs were counted through eye-piece graticule at 10x magnification within 1 cm root hairs zone section following 2, 4, 6 and 8-days of phosphorus deficiency treatment. This figure was then transformed into number of hairs/mm. The length of root hair was expressed as mm/hair.

Using the formula $1/3\ \pi r^2 h$ where the length of meristem (h), radius of the meristem (r) suggested by Rost and Baum (1988) the volume of primary root meristem was measured**.** Three replicates were used for all observations and in each observation standard error of mean was calculated.

Results and Discussion

Phosphorus deficiency gradually decreased the pH of the nutrient solution except an initial increase at the first 1 day of treatment (Table 1). Anuradha and Narayanan (1997) reported that the pH of the medium decreased due to P-deficiency in horse gram, sesame and pearl millet.

Phosphorus deficiency caused a 3.4-fold increase in citric acid content in the root exudates of maize seedlings at day 4 of phosphorus starvation (Table 2). This result was supported by Shen *et al.* (2004) who found that phosphorus deficiency increased exudation of citrate from whole root system of *Lupinus albus*. Furthermore, phosphorus deficiency resulted in an increase in citric acid exudation from the root system in purple lupin (Ligaba 2004), white lupin (Zhu *et al.* 2005, Penaloza *et al.* 2002, Tian *et al.* 2001), rape (Hoffland *et al.* 1989) and in alfalfa (Lipton *et al.* 1987). Zhang *et al.* (1997) reported that phosphorus deficient rape plant exuded citric acid which was 12 times higher than that of the control plant. P-deficient lupin exuded 65-fold more organic acid (87% citric acid) than that of P-sufficient control plants (Neumann *et al.* 1999). An increased exudation organic acid in response to P-deficiency was associated with a drop of pH in the nutrient solution.

Table 1. The effect of phosphorus deficiency on the changes in pH of the root medium. Each value is the mean of three replicates ± standard error of mean.

Duration of P-deficiency treatment (days)	pH value of the root medium	
	+ P solution	– P solution
0	5.30 ± 0.01	5.34 ± 0.01
1	5.60 ± 0.10	5.66 ± 0.10
2	6.10 ± 0.04	5.96 ± 0.02
3	6.35 ± 0.10	5.05 ± 0.03
4	6.20 ± 0.05	4.70 ± 0.10

Table 2. The effect of 4 days phosphorus deficiency on the exudation of citric acid by root systems of maize seedlings. Otherwise as in Table 1.

Treatment	Citric acid content, mg/g fresh root
+ P	0.071 ± 0.01
- P	0.241 ± 0.03

Phosphorus deficiency increased citric acid excretion from P-deficient maize roots with concomitant decrease in pH of growth medium (Tables 1, 2). Earlier, it was suggested that P-deficiency-induced release of citric acid in rhizosphere caused mobilization of phosphate from Ca-phosphate and iron-phosphate in soil (Hinsinger and Gilkes (1997) and also prevented excessive accumulation of citric acid in the cytoplasm which is important for survival of plants (Neumann *et al.* 1999).

Phosphorus deficiency caused an increase in the primary root length of maize from 6.7 to 50.6% from 2 to 8 days of treatment (Fig. 2a). Similarly, the total lateral roots length of P-deficient maize increased from 19.9 to 55.0% from 2 to 8 days of treatment (Fig. 2b). The total root length of maize was increased from 11 to 54.8% at 2 to 8 days of phosphorus deficiency treatment (Fig. 2c). Phosphorus deficiency resulted in an increase the number of lateral roots in the maize from 23.0 to 56.5% from 2 to 4 days of treatment and the stimulatory effect was maintained up to 8 days of treatment (Fig. 2d).

Earlier, Anuradha and Narayanan (1997) showed that the length of the primary and secondary roots of horse gram increased due to phosphorus deficiency treatment. Under phosphorus deficiency, the total root length and number of lateral roots of tolerant rice cultivars increased more than that of sensitive cultivars (Li *et al.* 2004). Low phosphate availability favoured lateral root growth over primary root growth, through increased lateral root number and length (Williamson *et al.* 2001).

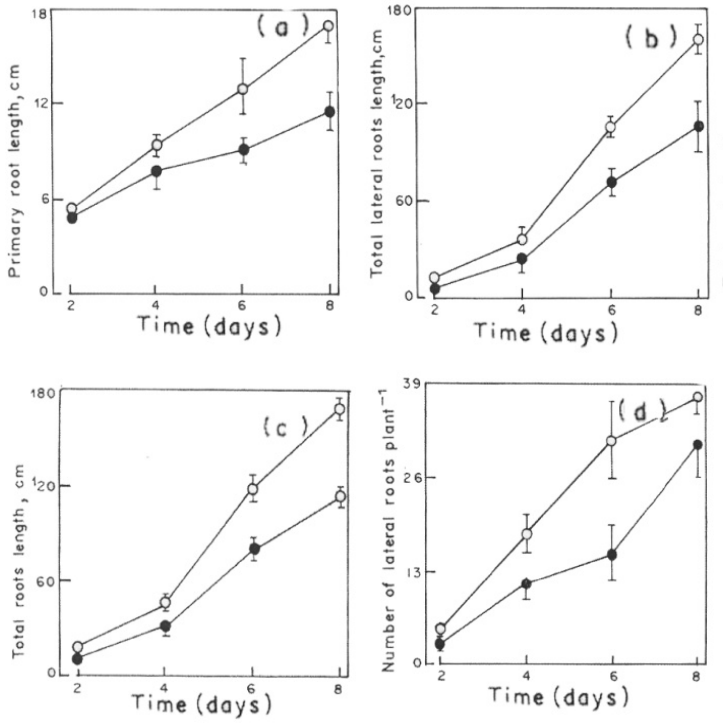

Fig. 2. Effects of phosphorus deficiency on the primary root length (a), total lateral roots length (b), total roots length(c), and number of lateral roots (d), of 8-day-old intact maize seedling grown in rhizobox. Each value is the mean of three replicates (n = 3) and vertical bars indicate ± standard error of mean. Symbols: --● -- = Control, --o-- = Pi-deficient.

The root meristem volume of maize was increased from 49.5 to 16.7% following 2 to 8-day of phosphorus deficiency application (Fig. 3a). Ma and Liang (2004) observed that increased root meristem volume was in agreement with the increased root length of the phosphorus deficient plant. The length of the root hair of maize was increased from 28.5% to 2.3-fold from 2 to 8-day of P-deficiency treatment (Fig. 3b). Similarly, the number of root hairs of maize was increased from 29.5 to 54.0% from 2 to 8 days of phosphorus deficiency stress and this stimulation was sustained up to 8 days of treatment (Fig. 3c). Fohse *et al.* (1991) reported that the length and number of root hairs increased in P-deficient plants.

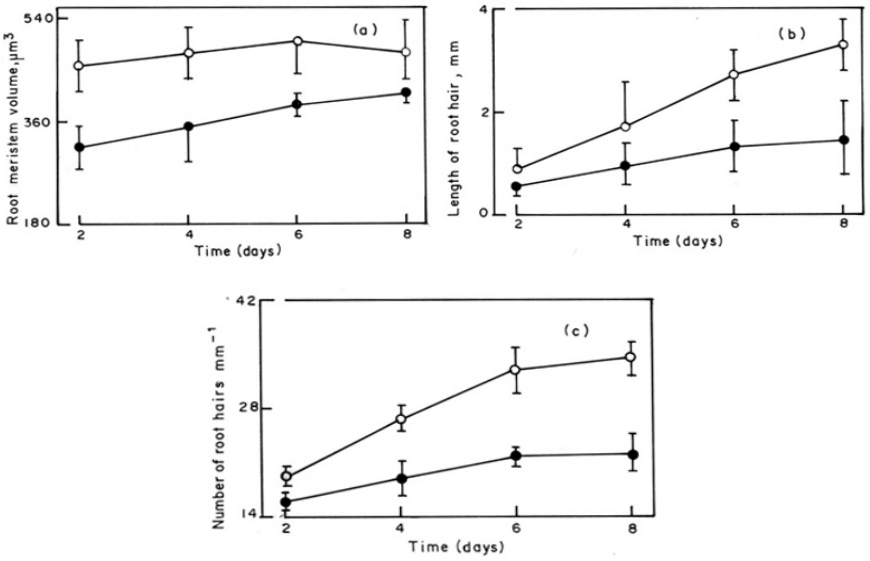

Fig. 3. Effects of phosphorus deficiency on the root meristem volume (a), length of root hair (b) and number of root hairs (c) of 8-day-old intact maize seedling grown in rhizobox. Otherwise as in Fig. 1.

The increase in the total root length and number, the length and number of root hairs might result in an increase in root surface area under phosphorus deficient condition. An increase in root surface area in phosphorus-deficient plant might be considered as a strategy for enhancing phosphorus acquisition from P-deficient soil. P-deficiency-induced increase in root meristem volume is an agreement with the increase root length.

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(Manuscript received on 11 October, 2015; revised on 14 March, 2016)