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Root-exuded malic acid versus chlorophyll fluorescence parameters in four plant species under different phosphorus levels

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Abstract

The amount of root-exuded malic acid and chlorophyll fluorescence parameters (the minimum chlorophyll fluorescence; the maximum quantum yield of photosystem II) in four plant species (*Broussonetia papyrifera, Morus alba, Orychophragmus violaceus* and *Brassica napus*) at different phosphorus levels was studied. A linear equation presents the minimum chlorophyll fluorescence (Fo) or the maximum quantum yield of photosystem II (Fv/Fm) and the amount of root-exuded malic acid. *Broussonetia papyrifera* and *Orychophragmus violaceus* easily adapted to a low-phosphorus environment. A low-cost method was used for assessing plant adaptability to a low-phosphorus environment when no chlorophyll fluorescence instrument was available.

Keywords: Adaptability, chlorophyll fluorescence parameters, malic acid, low phosphorus environment

1. Introduction

Malic acid is one of the most common low molecular weight organic acids in root exudates, root-exuded organic acids are important mechanism in response to environment stress (Jones, 1998). Recently, many studies revealed that the amount of root-exuded organic acids increases under nutrient stress to increased nutrient availability (Dakora and Phillips, 2002).

Phosphorus (P) is an important factor that can limit plant growth and development; the amount of available P directly determines plant growth condition (Broadley *et al*., 2002). Many studies indicate that the low molecular weight organic acids significantly increase under P deficiency. These organic acids complex with metallic cations to form organ-metallic complexes to overcome P deficiency (Hoffland *et al*., 2006).

Chlorophyll fluorescence kinetics technology is called for fast and undamaged probe for studying plant photosynthetic characteristics. This technology is applied in plant stress physiology, pollution determination, remote sensing and others (Ogren, 1990 ; Qiu and Liu, 2000; Meroni, 2009). It is an ideal method for the study of plant physiology, such as nutrients stress (Lu and Zhang, 2000; Lippemeier *et al.*, 2003).

This study determined the amount of root-exude malic acid in different plant species at P-sufficient, P-low and P-deficient levels. The chlorophyll fluorescence parameters were examined. A new method to evaluate plants adaptability to a low-P environment through the amount of root-exuded malic acid was proposed.

2. Materials and Methods

2.1. Solution culture experiment

Seeds of the four plant species (two Moraceae plants: *Broussonetia papyrifera*, *B. papyrifera; Morus alba, M. alba*; two Cruciferous plants: *Orychophragmus violaceus, O. violaceus; Brassica napus, B. napus*) were germinated in plastic pots for 15 days. The seedlings were washed with distilled water and carefully transferred to plastic tubes filled with Hoagland nutrient solutions (Hoagland and Arnon, 1950). The pH was adjusted to 8.0 by KOH. After 30 days, the plants were transferred to a modified Hoagland nutrient solution with different P levels for treatment, as modified by KH_2PO_4: 0.5 mM P (P-sufficient), 0.1 mM P (P-low), and without P (P-deficient). Root exudates were collected after 30 days treatment durations.

2.2. Collection, separation, purification and analysis of root exudates

Collection, separation, purification of malic acid in root exudates is the method of Wang (2007). The amount of the malic acid was analyzed by reversed-phase high-performance liquid chromatography (Cawthray, 2003).

2.3. Chlorophyll fluorescence measurements

Chlorophyll fluorescence is measured by an imaging pulse-amplitude-modulated fluorometer (Imaging PAM; Heinz Walz, Effeltrich, Germany), which applied the same array of blue light-emitting diodes (LEDs) (peakwavelength, 470nm) for fluorescence excitation, actinic illumination, and saturating light pulses. Plants were dark-adapted for 30 min prior to measurement, and the upper middle fully expanded leaves of four plant species were chosen and measured (Fu *et al.*, 2012). The minimum Chlorophyll fluorescence (Fo) was determined using a measuring beam, whereas the maximum Chlorophyll fluorescence (Fm) was recorded after exposure to a 0.8 s saturating light pulse (6000 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The maximum quantum yield of PSII (Fv/Fm) was calculated as (Fm – Fo)/Fm.

3. Results

3.1. Amount of root-exuded malic acid in four plant species at different P levels

As shown in Figure 1, the amount of malic acid in four plant species root exudates increased as the P level decreased after treatment of 30 days. The amount of root-exuded malic acid at the P-deficient level is significantly higher than that at the P-sufficient and P-low levels. For the two Moraceae plants, the amount of *B.papyrifera* root-exuded malic acid was remarkably higher than that in *M.alba* in all three P levels ($p<0.01$). The amount of malic acid in *B.papyrifera* root exudates was 1-folder greater than that in *M.alba*. Root-exuded malic acid increased up to 60% for *B.papyrifera* and 86% for *M.alba*, with the P-sufficient level sample for the same species acting as control. For the two Cruciferous plants, malic acid in *O.violaceus* was higher than that in *B.napus*. Increased in root-exuded malic acid was 79% for *O.violaceus* and 81% for *B.napus*, with the P-sufficient level sample for the same species acting as control. There was no significant difference in the two Cruciferous plants at the P-sufficient and P-low levels, while there was a difference at the P-deficient level ($p<0.05$).

3.2. Variation of chlorophyll fluorescence parameters

Figure 2 showed the variation of chlorophyll fluorescence parameters Fv/Fm and Fo at the three P levels. Fv / Fm decreased, Fo increased as the P level decreased in four plant species. For the two Moraceae plants, there was no difference at the P-sufficient level and P-low level; Fv / Fm was greater in *B.papyrifera* than in *M.alba* at the P-deficient level ($p<0.05$). There was no significant difference in Fo in the two Moraceae plants at all three P levels. For the two Cruciferous plants, there was no difference in Fv / Fm at all three P levels, Fo was greater in *O.violaceus* than in *B.napus* at the P-low level and P-deficient level ($p<0.05$)

3.3. Amount of root-exuded malic acid versus chlorophyll fluorescence parameters

As shown in Table 1, a linear equation can simulate the relationship between the amount of root-exuded malic acid and chlorophyll fluorescence parameters (Fv/ Fm, Fo). The determination coefficient (R^2) ranged from 0.785 to 0.992, R^2 of *B.papyrifera, O.violaceus*, *B.napus* was greater than 0.99, except *M.alba.*

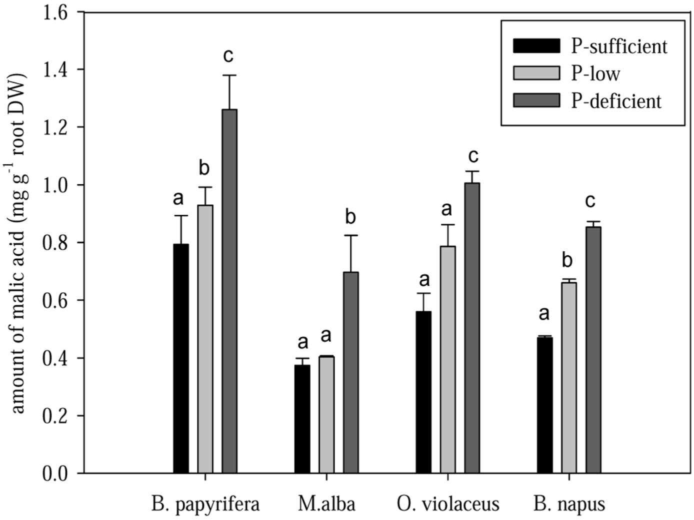

Figure 1. The amount of root-exuded malic acid in four plants under different phosphorus levels. Note: The histogram with bars indicated the means ± SE. Different letters indicate significant differences at the $p<0.05$ level, according to one-way ANOVA and t-test.

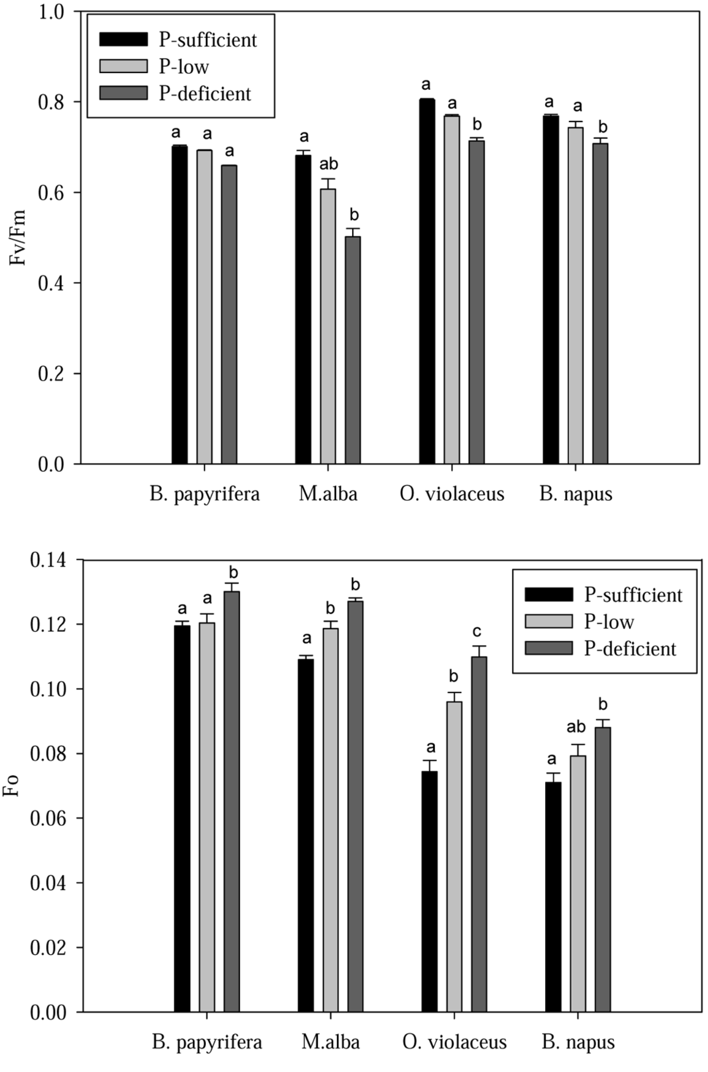

Figure 2. The effect of different phosphorus levels on chlorophyll fluorescence parameters of four plants. Note: The histogram with bars indicated the means ± SE. Different letters indicate significant differences at the p<0.05 level, according to one-way ANOVA and t-test.

Table 1. Regression equations between the amount of root-exuded malic acid and chlorophyll fluorescence parameters. Note: ** indicates significant difference at $p < 0.001$; * indicates significant difference at $p < 0.005$. a: the value of Fv / Fm, b: the value of Fo, x: the amount of root-exuded malic acid.

Plant species	chlorophyll fluorescence parameters	Regression equation$^{\#}$	Determination coefficient (R^2)	Number of samples (N)
B.papyrifera	Fv / Fm	a= -10.869x + 8.4284**	0.9908	15
	Fo	b=0.0239x+0.0995**	0.9563	
M. alba	Fv / Fm	a = -1.863x + 1.603*	0.8840	15
	Fo	b=0.0462x+0.0955*	0.7854	
O. violaceus	Fv / Fm	a= -4.8213x + 4.4567**	0.9827	15
	Fo	b=0.0797x+0.0311**	0.9827	
B. napus	Fv / Fm	a= -6.2747x + 5.3018**	0.9916	15
	Fo	b=0.0436+0.0507**	0.9582	

4. Discussion

Root-exuded organic acids are the main mediators of Krebs cycle, these organic acids are associated with respiration (Jones, 1998). As chlorophyll fluorescence parameters correspond to the properties of the photosynthetic characteristics, chlorophyll fluorescence technology is an effective method for studying plant stress physiology (Krause and Weis, 1991; Bolhar-Nordenkampf *et al*., 1989; Sayed, 2003).

Study results showed that the amount root-exuded malic acid increased with the decreased of P content in all four plant species (Figure 1), which is consistent with the results of previous studies (Jones, 1998; Egle *et al*., 2003). Corrales *et al*., (2007) shown that higher organic acids concentrations were detected in the rhizosphere of P-efficient than of P-inefficient maize in sand culture, P efficiency seemed due to enhanced P acquisition rather than to an enhanced P use efficiency. These organic acids mobilized P from insoluble phosphoric compounds such as Ca-P and Fe-P to overcome P deficiency in calcareous soils and acid mineral soils. Therefore, *B. papyrifera* and *O. violaceus* was convenient to obtain a great amount of available P under P deficiency from rhizosphere soil via complexing with root-exuded organic acids.

The chlorophyll fluorescence parameters had significant difference in P supply and P deficiency. P deficiency treatment made the maximum quantum yield of PSII, the maximum chlorophyll fluorescence decrease and the minimum chlorophyll fluorescence increase in Maize (*Zea mays* L.) leaves (Li *et al*., 2004) and citrus leaves (Guo *et al*., 2002). Similar results (the minimum chlorophyll fluorescence increased and the maximum quantum yield of PSII decreased) presented in the four experimental plants (Figure 2). P deficiency had small destroy in PS II reaction centers in *B. papyrifera* and *O. violaceus* unlike in *M.alba* and *B.napus*. This conclusion is similar to the variation of the net photosynthetic rate: *B. papyrifera* is higher than *M.alba* (Wu *et al*., 2009), and *O.violaceus* is higher than *B. napus* (Wu *et al*., 2007). This finding indicates that *B.papyrifera* and *O.violaceus* have a stronger adaptability to a low-P environment than *M.alba* and *B.napus*.

As shown in Table 1, a linear equation can present the relationship between root-exuded malic acid and Fv/Fm, Fo. Photosynthesis and respiratory metabolism adaptability to a low-P environment occurred in *B.papyrifera* and *O.violaceus*. Therefore, the amount of root-exuded malic acid from plants at different P levels should be determined. This study was successful in presenting a low-cost methodology for assessing plant adaptability to a low-P environment in the absence of a chlorophyll fluorescence instrument.

5. Conclusions

Increasing in root-exuded malic acid was important mechanisms in response to P deficiency, while chlorophyll fluorescence parameters responded the degree of P stress deficiency. Compared with M. alba (*B. napus*), in response to P deficiency, the root of *B. papyrifera* (*O. violaceus*) actively released great amount of malic acid, while the maximum quantum yield of PSII and the minimum chlorophyll fluorescence decreased and increased significantly, respectively. A linear correlation between the amount of root-exuded malic acid and the minimum chlorophyll fluorescence (the maximum quantum yield of photosystem II) was accordingly obtained. Therefore, a low-cost method can be alternatively evaluated the plant adaptability to a low-phosphorus environment when no chlorophyll fluorescence instrument was available.

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