The Journal of Animal & Plant Sciences, 25(4): 2015, Page: 1049-1054
ISSN: 1018-7081

PHOSPHATE SOLUBILIZING BACTERIA IN COMBINATION WITH PRESSMUD IMPROVE GROWTH AND YIELD OF MASH BEAN

M. T. H. Niazi[1], Saif-ur-Rehman Kashif[2], H. N. Asghar*[1], M. Saleem[1], M. Y. Khan[1] and Z. A. Zahir[1]

[1]Institute of Soil & Environmental Sciences, University of Agriculture Faisalabad. Pakistan
[2]University of Veterinary and Animal Sciences, Lahore-Pakistan
*Corresponding author's email: naeemasghar@yahoo.com

ABSTRACT

Phosphorus (P) is an important macronutrient for growth of plants and is often provided to crops by the application of inorganic sources; 80 percent of that cannot be utilized by crops due to immobilization and precipitation. In such situation, phosphate solubilizing bacteria (PSB) may play a major role in improving P availability to plants by dissolving insoluble and fixed soil P. Moreover, phosphatase activity of these PSB could further enhance the P availability from organic sources, if supplemented with organic amendments. A pot experiment was conducted to evaluate the potential of PSB containing phosphatase activity with and without organic amendment (pressmud) for improving growth and yield of mash bean. Mash bean seeds were inoculated with 4 different well characterized PSB strains [PS-01 (*Burkholderia* sp.), PS-12 (*Bacillus* sp.), PS-32 (*Pseudomonas* sp.) and PS-41 (*Flavobacterium* sp.)]. The inoculated seeds were sown in potted soil amended with and without press-mud. Results showed that combined use of PSB strain (PS-01) and PrM caused significant increase in yield (38 and 69%), P content in root (1.3 and 3.3 fold) and in shoot (32 and 136%) of mash bean as compared to sole use of PS-01 and PrM, respectively. This approach could be very effective to enhance the phosphorous availability to plants, plant growth and yield.

Keywords: phosphorous, phosphatases, rhizobacteria

INTRODUCTION

Phosphorus (P) is a most important growth-limiting nutrient, and is different from the case of nitrogen; there is no large distinctive source that can be naturally managed for crops accessibility (Sharma *et al.* 2013). It plays important role in nearly all phases of plant cycle including root growth, photosynthesis, anthesis, seed production and maturation. Its deficiency causes stunted growth and severe yield losses. Its concentration in soil solution is very low, because soluble forms of P are fixed by soil solid phase, making less than 0.01% of total P available to plants. Phosphorous is therefore, one of the least mobile nutrients in soil (Balemi and Negisho, 2012). Many agricultural soils surround huge deposition of total P, generally from 200 to 5000 mg P kg^{-1} of soil by mean value of 600 mg P kg^{-1} of soil and its accumulation based on frequent use of inorganic fertilizers and sludge from the treated wastewaters. Much of the P which is present in soil and provided to the crops through inorganic fertilizers become unavailable through precipitation by reacting with Fe^{3} and Al^{3+} in acidic and with Ca^{2+} in calcareous soils, respectively (Abbasi *et al*. 2015). Different genera of bacteria have ability of mineralizing and solubilizing P pools in soil and its bioavailability is considerably promoted by these bacteria. Solubilization of rock phosphates through different microbes is a low grade process but receiving greater attention for sustainable agriculture. This process not only compensates the input of high cost fertilizers but it can also enhance the mobilization of insoluble P already added to soil from the fertilizers. Such group of bacteria is termed as phosphate solubilizing bacteria (PSB) and inoculation with PSB as bio-fertilizers enhances P accumulation and biomass production of plants (Abbasi *et al*. 2015). To compensate the P deficiency, PSB can play an important role for supplying available P to plants in sustainable and environment friendly manners as these bacteria excrete organic acids that break up phosphatic minerals by solubilizing the inorganic form of P (Khan *et al.* 2006). Moreover, these PSB having phosphatase activity could also increase P accessibility to plants from organic P sources in soil by mineralization (He *et al.* 2004).

On another hand, soil organic matter (SOM) is a key component of the soil system that facilitates many important soil physical, chemical and biological processes and it has been decreased below 1% in many areas of Pakistan (Niaz *et al.* 2007). Use of the organic amendments in field improves the SOM status, microbial activity and biomass as compared to conventional fertilizers (Ghimire *et al.* 2014). Surely, PSB improve plant growth and yield when supplemented with chemical fertilizers. However, if PSB are used in combination with organic amendments, they could not only enhance the crop yield but also can help in preventing fertilizer run-off, leaching of nutrients, retaining more moisture and improving plant growth (Saxena *et al.* 2013). Press-mud is a main by-product of sugar cane industry, rich in nutrients required for plant growth especially P and could be used as potential organic amendment to enhance the fertility status of soil and improve the plant growth and yield (Sarker *et al.* 2013). Nowadays, PrM is being used as a low cost input for nutrient supply to crop plants and it can significantly enhance the availability of P, K and Ca contents for different crops like soybean, maize and wheat and can improve their growth and yield (Datta and Gupta, 1983; Muhammad and Khattak, 2009). By realizing the need of PSB to enhance the accessibility of P to plants in soil from organic and inorganic sources, a pot trail was conducted to evaluate PSB containing phosphatase activity alone as well as in combination with PrM to improve the growth and yield of mash bean.

MATERIALS AND METHODS

A pot experiment was conducted in the wire house under ambient temperature and light to evaluate phosphate solubilizing bacteria (PSB) containing phosphatase activity with and without pressmud (PrM) for improving growth and yield of mash bean crop. For this purpose, four PSB strains already tested for P solubilization and mineralization in our previous experiments were selected (Hussain *et al.* 2013a). The selected PSB strain *i.e.* PS-01 (*Burkholderia* sp.), PS-12 (*Bacillus* sp.), PS-32 (*Pseudomonas* sp.) and PS-41 (*Flavobacterium* sp.) were used alone as well as in combination with PrM to improve growth and yield of mash bean. The experiment was conducted by following the completely randomized design with two factors and three replication of each treatment. The inocula of selected PSB strains were prepared by taking 100 mL of sterilized glucose peptone broth in four conical flasks having 250 mL volume. Each conical flask was inoculated with respective bacteria and incubated at 28 ± 1 ºC in a shaking incubator at 100 rpm for 24 hours. Inoculum with population density of 10^7-10^8 CFU mL^{-1} of each strain was used for seed inoculation. The inoculum of each PSB strain was injected into sterile peat (100 mL kg^{-1}) and was incubated for 24 hrs at 28 ± 1°C before using it for seed coating. For seed inoculation, seed dressing was carried out with inoculated peat mixed with clay and 10% sugar solution. In case of the un-inoculated control, the seeds were coated with the same but autoclaved inoculum suspension (Shaharoona *et al*., 2006). The seeds were surface sterilized with ethanol and sodium hypochlorite before inoculation. Inoculated seeds of mash bean were sown in pots filled with sandy clay loam soil amended with and without PrM (organic amendment) at the rate of 15 mg ha^{-1}. Polyethylene sheets were used for lining of pots and 10 kg soil amended with and without PrM was used to fill pots. The soil and PrM used were analyzed for various physico-chemical properties. Soil was sandy clay loam having EC 1.45 dS m^{-1}, pH 7.6, saturation percentage 39.5%, organic matter 0.57%, available phosphorous 7.4 mg kg^{-1} soil and extractable potassium 130 mg kg^{-1} soil. Pressmud used in experiment had organic matter 12.8%, pH 6.5, EC 3.2 dS m^{-1}, total phosphorous 0.7% and total potassium 0.97%. Pots were irrigated with tap water before sowing and at field capacity five inoculated seeds of mash bean were sown per pot, keeping control treatments where un-inoculated seeds were sown in soil amended with and without PrM. Three plants were maintained in each pot by thinning after one week of germination. At harvest, data regarding growth and yield parameters was recorded. Root and shoot samples of plants were analyzed for P contents. Wolf (1982) method was followed for the digestion of plant sample. For this purpose, 0.1 g oven dried and ground plant samples were taken in a digestion tube and 2 mL of concentrated sulfuric acid was poured into it. The samples were left over night at room temperature. Then 1 mL 35% extra pure H_2O_2 was poured into each digestion tube along the sides and the tubes were rotated. The tube was heated up to 350 ^{0}C after mounting it in a digestion block for 20 minutes. Then the tubes were removed, cooled and added with 1 mL of H_2O_2 and again heated for 20 minutes. Same procedure was continued until the material became colorless. Fifty milliliter volume of the colorless extract was made by using distilled water and stored for the determination of P contents. Phosphorous was measured by spectrophotometer (Nicolet Evolution 300, Thermo Electron Corporation, England) at 430-nm wavelengths by using KH_2PO_4 as standards (Olsen and Sommers, 1982). Data were analyzed statistically by using computer based statistical software Statistix-8.1 (Analytical Software, Tallahassee, USA) and means were compared by Duncan's Multiple Range Test at 5% probability level (Duncan, 1955).

RESULTS

In present study, PSB strains containing phosphatase activity showed positive effect on growth and yield of mash bean either used with and/or without PrM. Results showed that sole application of PSB significantly improved shoot length, root length, root and shoot fresh and dry weight, yield per plant and P contents in root and shoot as compared to un-inoculated control. But further improvement in growth parameters and yield was observed by combined application of PSB strains and PrM.

Data (Table 1) showed that inoculation of PSB strain (PS-01) without PrM amendment, improved the shoot length up to 29.2% compared to un-inoculated control while this increase was up to 16.9 % more compared to treatment where only PrM was used as an organic amendment. Combined application of PrM and PSB strain (PS-01) significantly improved shoot length up to 23.5 % compared to sole inoculation of PSB strain

(PS-01) and 44.4% as compared to sole use of PrM. Data (Table 1) showed that 13.44% increase in shoot fresh weight was observed by sole inoculation of PSB strain (PS-01) as compared to un-inoculated control without PrM amendment but inoculation of PSB strain (PS-01) with PrM significantly increased the shoot fresh weight up to 7.30% as compared to sole inoculation of PSB strain (PS-01) and 15.35% compared sole application of PrM. Data (Table 1) showed that shoot dry weight was improved up to 13.1% by sole inoculation of PSB strain (PS-01) as compared to un-inoculated control without PrM. Inoculation of PSB strain (PS-01) with PrM improved the shoot dry weight up to 15.5% compared to sole inoculation of PSB strain (PS-01) and up to 15% as compared to sole application of PrM.

Table 1. Effect of PSB inoculation and PrM on shoot parameters of mash bean.

Treatments	Shoot length (cm)		Shoot fresh weight (g)		Shoot dry weight (g)	
	Without PrM	With PrM	Without PrM	With PrM	Without PrM	With PrM
Control	28.50 g	31.5 f	5.80 h	6.12 e	1.45 f	1.53 e
PS-12	34.16 e	40.83 b	6.29 d	6.83 b	1.57 d	1.71 b
PS-32	32.33 f	37.00 d	6.06 e	6.77 b	1.51 e	1.69 b
PS-01	36.83 d	45.50 a	6.58 c	7.06 a	1.64 c	1.76 a
PS-41	34.26 e	38.16 c	6.19 e	6.75 b	1.52 e	1.69 b

Means sharing the same letter(s) do not differ significantly at $p \leq 0.05$

Data (Table 2) showed that increased in root length was observed up to 30.30% by sole inoculation of PS-01 as compared to un-inoculated control without PrM. But further improvement in root length up to 64.30% was observed by inoculation of PS-01 with PrM as compared to uninoculated control. Data (Table 2) showed 17.20% increase in root fresh weight by sole inoculation of PS-01 as compared to un-inoculated control without PrM. However, combined use of PS-01 with PrM improved root fresh weight as compared to sole inoculation of PS-01 and increase in root fresh weight was up to 47.54% as compared to un-inoculated control without PrM. Data (Table 2) showed that maximum increase in root dry weight was observed by combined use of PSB strain (PS-01) and PrM as compared to sole inoculation of PS-01. Inoculation of PSB strain (PS-01) in combination with PrM increased the root dry weight up to 72% as compared to un-inoculated control without PrM amendment.

Table 2. Effect of PSB inoculation and PrM on root parameters of mash bean.

Treatments	Root length (cm)		Root fresh weight (g)		Root dry weight (g)	
	Without PrM	With PrM	Without PrM	With PrM	Without PrM	With PrM
Control	13.16 h	14.58g	1.12 g	1.23 fg	0.29 g	0.29 g
PS-12	16.96 de	20.90 b	1.33 e	1.75 b	0.33 e	0.43 b
PS-32	15.30 fg	17.66 d	1.27 e-g	1.53 c	0.31 f	0.37 c
PS-01	18.90 c	23.83 a	1.43 d	1.99 a	0.35 d	0.49 a
PS-41	16.16 ef	18.56 c	1.29 ef	0.44 b	0.32 ef	0.44 b

Means sharing the same letter(s) do not differ significantly at $p \leq 0.05$

The data (Table 3) regarding yield per plant of mash bean revealed that PSB strain (PS-01) without PrM increased the yield per plant up to 54.2 % as compared to un-inoculated control without PrM amendment. The maximum increase in yield was observed by inoculation of PSB strains in combination with PrM than sole inoculation of PS-01 and un-inoculated control without PrM. The data regarding P contents in roots and shoots (Table 3) of mash bean revealed that inoculation of PSB strains in combination with PrM caused increase in P contents of roots and shoots. Application of PrM significantly increased the P contents in plant root and shoot up to 60 and 25%, respectively, as compared to control. Inoculation of PSB strain (PS-01) without PrM increased P contents of root and shoot up to 123.25 and 120%, respectively, as compared to un-inoculated control without PrM. Combined application of PSB strain (PS-01) and PrM significantly increased the P contents of root and shoot up to 4.28 and 1.96 folds, respectively, as compared to un-inoculated control without PrM.

Table 3. Effect of PSB inoculation and PrM on grain yield and root and shoot P contents of mash bean.

Treatments	Grain yield plant^{-1} (g)		P in root (%)		P in shoot (%)	
	Without PrM	With PrM	Without PrM	With PrM	Without PrM	With PrM
Control	1.40 g	1.75 f	0.29 e	0.45 de	0.27 h	0.33 g
PS-12	1.92 ef	2.62 b	0.59 cd	1.18 b	0.47 f	0.70 b
PS-32	1.91 ef	1.97 de	0.48 de	0.70 cd	0.38 g	0.56 de
PS-01	2.16 cd	2.97 a	0.64 cd	1.52 a	0.59 cd	0.78 a
PS-41	1.93 ef	2.26 c	0.50 c-e	0.80 c	0.51 ef	0.63 c

Means sharing the same letter(s) do not differ significantly at $p \leq 0.05$

DISCUSSION

Phosphate solubilizing bacteria (PSB) with multiple plant growth promoting activities could be one of the viable supplement to chemical fertilizers and play an important role for enhancing availability and uptake of plant nutrients and have significant role in the bio-fertilization of field crops (Sharma *et al.* 2013). These bacteria have ability to convert unavailable form of nutrients to plant available form through biological process (Vessey, 2003). Results of our study showed that PSB strains significantly increased the mash bean growth and yield. Phosphate solubilizing bacterial strains increased the shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, yield per plant and P contents in roots and shoots of mash bean plants as compared to un-inoculated control. This increase in growth and yield attributes of mash bean might be due to involvement of PSB strains for increasing the availability of nutrients, biosynthesis of plant growth regulators *i.e.* auxins, phosphatase activity and/or ACC-deaminase activity (Hussain *et al.* 2013a, 2013b). Phosphate solubilizing bacterial strains increased the mash bean growth and yield which might be due to enhanced P accumulation and biomass production in plants (Barea *et al.* 2005). Phosphate solubilizing bacteria enhanced plant growth by solubilization of insoluble P in soil and increased its acquisition by increasing root growth of plant (Malboobi 2009). Increase in plant growth and yield of mash bean might also be attributed to biosynthesis of siderophores which provides iron to plants, biosynthesis of different phytohormones like cytokinin and auxins, solubilization of mineral P through different mechanisms and/or biosynthesis of various enzymes (Gyaneshwar *et al.* 2002). Moreover, PSB could also increase the biological nitrogen fixation efficiency of rhizobium-legume symbiosis, therefore increase in growth and yield of mash bean might be attributed to more nitrogen fixation (Son *et al.* 2006; Mohammadi, 2011). Increase in P contents of root and shoot of mash bean by inoculation of PSB strains might be due to solubilization of inorganic phosphate through production of organic acids and/or mineralization of organic P by phosphatase enzymes (Hussain *et al.* 2013a; Sharma *et al.* 2013). As, PSB solubilize the low soluble calcium phosphate compounds through the production of organic acids and make P available to plants (Walpola and Yoon, 2013a). Ahemad and Kibret (2014) stated that mobilization of mineral nutrients like P and Fe in soil by bacteria could be the main mechanism for increased growth and development of plants which makes these nutrients in more readily plant available forms. Improvement in shoot length, root length, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight, yield per plant and P contents of root and shoot by inoculation of PSB strains in combination with organic amendment (PrM) was more prominent as compared to sole inoculation of PSB strains without PrM. This increase in growth and yield attributes of mash bean might be due to increase in soil organic matter status of soil. As PrM is rich source of organic matter that increases the activity of PSB bacteria, improves their efficiency for releasing organic acids and phosphatases enzymes that might be involve in enhancing P availability and other nutrients to plants (Winarso *et al.* 2011; Ahemad and Kibert, 2014). Moreover, PSB containing phosphatase enzymes liberate P through mineralization of organic matter which promote the growth and yield of plants (Trolove *et al.* 2003; Walpola and Yoon, 2013b). Data regarding increase in plant growth, yield and P contents of root and shoot by the application of organic amendment *i.e.* PrM might be due to supply of more nutrients to plants and improved soil physico-chemical properties (Cellier *et al.* 2014).

Conclusion: From present research it might be concluded that if PSB strains containing phosphatase activity are applied in combination with organic amendments *i.e.* PrM, both organic and inorganic pool of soil P may be mobilized more efficiently and can be translated in to enhanced growth and yield of mash bean. Among the PSB strains, PS-01 was most effective and improved yield up to 38 and 69% as compared to sole use of PS-01 and PrM, respectively. This approach could be very effective in legumes because enhanced availability of P could also assist the nitrogen fixation process in legumes.

Acknowledgements: Facilities for this study were provided by the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad.

REFERENCES

Abbasi, M. K., N. Musa and M. Manzoor (2015). Phosphorus release capacity of soluble P fertilizers and insoluble rock phosphate in response to phosphate solubilizing bacteria and poultry manure and their effect on plant growth promotion and P utilization efficiency of chilli (*Capsicum annuum* L.). Biogeosci. Discuss.12:1839–1873.

Ahemad, M. and M. Kiber (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. J. King Saud Uni. Sci. 26: 1-20.

Balemi, T. and Negisho, K. (2012). Management of soil phosphorus and plant adaptation mechanisms to phosphorus stress for sustainable crop production: A review. J. Soil Sci. Plant Nutr. 12 (3): 547-561.

Barea, J.M., R. Azco'n, and C.A. Aguilar (2005). Interactions between mycorrhizal fungi and bacteria to improve plant nutrient cycling and soil structure. Buscot F. and Varma S. (eds). Microorganisms in soils: Roles in genesis and functions. pp. 195-212.

Cellier, A., T. Gauquelin, V. Baldy, and C. Ballini (2014). Effect of organic amendment on soil fertility and plant nutrients in a post-fire Mediterranean ecosystem. Plant Soil. 376: 211–228

Datta, M. and R.K. Gupta (1983). Utilization of pressmud as amendment to acid soil in Nagaland. J. Indian. Soc. Soil Sci. 31:511-516.

Duncan, D. B. (1955). Multiple range and multiple F-test. Biometrics. 11: 1-42.

Ghimire, R., J.B. Norton, P.D. Stahl, and U. Norton (2014). Soil microbial substrate properties and microbial community responses under irrigated organic and reduced-tillage crop and forage production systems. PLoS One. 9(8): e103901. doi:10.1371/journal.pone.0103901.

Gyaneshwar, P., G.N. Kumar, L.J. Parekh, and P.S. Poole (2002). Role of soil microorganisms in improving P nutrition of plants. Plant Soil. 245: 83-93.

He, Z.Q., T.S. Griffin, and C.W. Honeycutt (2004). Enzymatic hydrolysis of organic phosphorus in swine manure and soil. J. Environ. Qual. 33: 367–372.

Hussain, M.I., H.N. Asghar, M. Arshad, and M. Shahbaz (2013b). Screening of multi-traits rhizobacteria to improve maize growth under axenic conditions. J. Anim. Plant Sci. 23(2): 514-520.

Hussain, M.I., H.N. Asghar, M.J. Akhtar, and M. Arshad (2013a). Impact of phosphate solubilizing bacteria on growth and yield of maize. Soil Environ. 32(1): 71-78.

Khan, M. S., A. Zaidi, and P. A. Wani (2006). Role of phosphate solubilizing microorganisms in sustainable agriculture-A review. Agron. Sustainable Dev. 26: 1-15.

Malboobi, M.A., M. Behbahani, H. Madani, P. Owlia, A. Deljou, B. Yakhchali, M. Moradi, and H. Hassanabadi (2009). Performance evaluation of potent phosphate solubilizing bacteria in potato rhizosphere. World J. Microbiol. Biotecnol. 25: 1479–1484.

Mohammadi, K. (2011). Soil, plant and microbe interaction. Lambert Academic Publication. 120 pp.

Muhammad. D. and R.A. Khattak (2009). Growth and nutrient concentrations of maize in pressmud treated saline-sodic soils. Soil Environ. 28(2): 145-155.

Niaz, A., A.M. Ranjha, Rahmatullah, A. Hannan, and M. Waqas (2007). Boron status of soils as affected by different soil characteristics-pH, $CaCO_3$, organic matter and clay contents. Pak. J. Agric. Sci. 44(3): 428-433.

Olsen, S. R. and L. E. Sommers (1982). Phosphorus. pp 403–430 In: Methods of Soil Analysis Part 2 Chemical and Microbiological Properties, edited, by Page, A. L., Miller, R. H., and Keeney, D. R., SSSA Madison, WI.

Sarker, T.C., M.A. Mannan, P.C. Mondol, A.H. Kabir, S.M. Parvez, and M.F. Alam (2013). Physico-chemical profile and microbial diversity during bioconversion of sugarcane press mud using bacterial suspension. Not. Sci. Biol. 5(3): 346-353.

Saxena, J., G. Rana, and M. Pandey (2013). Impact of addition of biochar along with *Bacillus* sp. on growth and yield of french beans. Sci. Hortic. 162: 351-356.

Shaharoona, B., M. Arshad and Z.A. Zahir (2006). Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.) Lett. Appl. Microbiol. 42: 155–159.

Sharma, S.B., R.Z. Sayyed, M.H. Trivedi, and T.A. Gobi (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus. 2: 587.

Son, T.T.N., C.N. Diep, and T.T.M. Giang (2006). Effect of bradyrhizobia and phosphate solubilizing bacteria application on Soybean in rotational system in the Mekong delta. Omonrice. 14: 48-57.

Trolove, S.N., M.J. Hedley, G.J.D. Kirk, N.S. Bolan, and P. Loganathan (2003). Progress in selected areas of rhizosphere research on P acquisition. Aust. J. Soil Res. 41: 471–499.

Vessey, J.K. (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant Soil. 255: 571–586.

Walpola, B.C. and M. Yoon (2013a). Isolation and characterization of phosphate solubilizing bacteria and their co-inoculation efficiency on tomato plant growth and phosphorous uptake. Afr. J. Microbiol. Res. 7: 266-275.

Walpola, B.C. and M. Yoon (2013b). Phosphate solubilizing bacteria: Assessment of their effect on growth promotion and phosphorous uptake of mung bean *(Vigna radiate* [L.] R. Wilczek). Chil. J. Agric. Res. 73: 275-281.

Winarso, S., D. Sulistyanto, and Handayanto (2011). Effects of humic compounds and bacteria on phosphorous availability in an acid soil. J. Ecol. Nat. Environ. 3:232-240.

Wolf, B. (1982). A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. Commun. Soil Sci. Plant Anal. 13:1035-1059.