PHYTOSIDEROPHORE EXUDATION FROM THE ROOTS OF IRON STRESSED KENTUCKY BLUEGRASS

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ABSTRACT

Some Kentucky bluegrass (*Poa pratensis* L., KBG) cultivars are susceptible to iron (Fe) deficiency chlorosis when grown on calcareous soils and are routinely treated with Fe fertilizers. Aesthetics could be improved and this costly practice could potentially be eliminated with the use of cultivars resistant to Fe deficiency. Grasses are known to release phytosiderophore into the rhizosphere to dissolve Fe for plant use, and this characteristic has been used to screen for resistant cultivars with other species. A chelator-buffer hydroponic study was conducted to stress Baron, Award, Limousine, and Rugby II cultivars at 1 and 10 µM Fe in complete nutrient solution buffered at pH 7.4. Shoot yields were significantly greater at 10 than 1 µM Fe for all cultivars. The cultivars other than Baron had similar and consistent increases in chlorosis at 1 µM Fe and nearly no chlorosis at 10 µM Fe. Baron was significantly different than the other cultivars in that it developed chlorosis earlier and more severely at both levels of Fe, although chlorosis at 10 µM Fe was not as severe as 1 µM Fe for this cultivar. In addition, Baron exhibited re-greening at the 10 µM Fe. Phytosiderophore exudation was significantly greater for all cultivars at 1 than at 10 µM Fe. Surprisingly, the apparently Fe chlorosis susceptible Baron cultivar not only released phytosiderophore, but released it at a significantly higher level than the other cultivars. These results imply that Fe deficiency susceptibility in Kentucky bluegrass may be related to inefficient uptake mechanisms rather than production and release of phytosiderophore.

INTRODUCTION

Fe is an essential nutrient in plants because it is directly involved in chloroplast development and the important reactions of photosynthesis. Although it is the fourth most abundant element in the earth's crust (Tisdale et al., 1993), its bioavailability in calcareous soils is very low as Fe is rapidly oxidized and immobilized.

Despite iron's low availability in calcareous soils, many plants have been able to adapt and grow well there. Grasses, including KBG, are known to produce phytosiderophores (organic chelates) as a way of taking up Fe from the soil for plant use and these compounds have been identified and characterized (Cesco et al., 2006; Ueno et al., 2007). Quantifying phytosiderophore exudation over time while Fe-deficiency stressed has been used to identify Fe-deficiency resistant genotypes in other monocots such as corn, oat, and wheat (Lytle et al., 1990; Hansen and Jolley, 1995; Hansen et al., 1996) and could, potentially, be used as a screening technique for turf.

OBJECTIVES

A hydroponic study was conducted using chelator-buffered techniques (Hopkins et al., 1998; Yang et al., 1994) with the objectives of (a) confirming release or lack of release of phytosiderophore from an Fe-deficiency susceptible cultivar, and (b) comparing phytosiderophore release of four cultivars differing in susceptibility to Fe-deficiency chlorosis. The techniques allow the maintenance of micronutrients in solution with equal molar levels of chelates and micronutrients plus a slight excess of Trisodium N-(2-hydroxyethyl)ethylenediamine-N,N’,N’-triacetate hydrate (NaHEDTA) to sequester contaminant metals.

METHODS

ABS DWV Fittings, assembled with one layer of cheesecloth and one layer of plastic mesh between the fitting and screw-on top, were used to germinate the KBG seeds. Once germinated (15 days), intact plants were transferred with roots and placed in a complete-nutrient pretreatment solution (Steinberg, 1953). The solutions were changed and the grass in the fittings clipped to 5.2 cm (2 in) every three to four days for the duration of the pretreatment (21 days). Selecting fittings containing healthy plants, a hydroponic experiment was conducted by growing four cultivars of Kentucky blue grass, ’Award’, ‘Baron’, ‘Limousine’, and ‘Rugby II’ at 1 and 10 µM Fe . Treatment solutions were made with optimum levels of essential nutrients except for the Fe variable. Solution pH was buffered to 7.4 with MES and KOH. The experiment consisted of eights treatments (four cultivars and two Fe levels) of eight plants each with three replications in a complete random block design. After imposition of treatments, chlorosis scores were made daily using a scale of 0 (no chlorosis) to 5 (severe chlorosis and necrosis). On days 5-13, root exudates (phytosiderophore) were collected for 4 h, beginning 2 h after light initiation. An indirect Fe-binding assay was used to determine the concentration of phytosiderophore in the root exudates (Hansen and Jolley, 1995). One plant from each treatment was used for phytosiderophore collection each day (Fig. 4), followed by harvest of shoots and roots separately. Shoots and roots were oven dried, weighed, ground, digested in nitric-perchloric acid, and analyzed by inductively coupled plasma (ICP).

RESULTS AND DISCUSSION

All cultivars showed greater chlorosis at 1 than at 10 µM Fe. However, at 10 µM Fe, Baron developed chlorosis earlier and more severely than the other three cultivars (Figure 1). In addition, Baron re-greened after Day 9, where chlorosis worsened in all others as the treatment progressed. In cultivars grown at 1 µM solution Fe, all cultivars responded similarly in that their chlorosis worsened as the treatment progressed (Figure 1). Surprisingly, Baron produced more total phytosiderophore than Award, Limousine, or Rugby II (Table 1) despite developing more severe chlorosis,. All cultivars produced more phytosiderophore at 1 than at 10 µM Fe (Table 2). Ten µM Fe treatments produced greater shoot yield while 1 µM Fe produced greater root yield (Table 2). These results imply that Fe deficiency susceptibility in Kentucky bluegrass may be related to inefficient uptake mechanisms rather than production and release of phytosiderophore.

Figure 1. Chlorosis scores for four KBG cultivars at 1 and 10 µM Fe. Error bars drawn on Baron represent $LSD_{0.05}$ to compare the four cultivars.

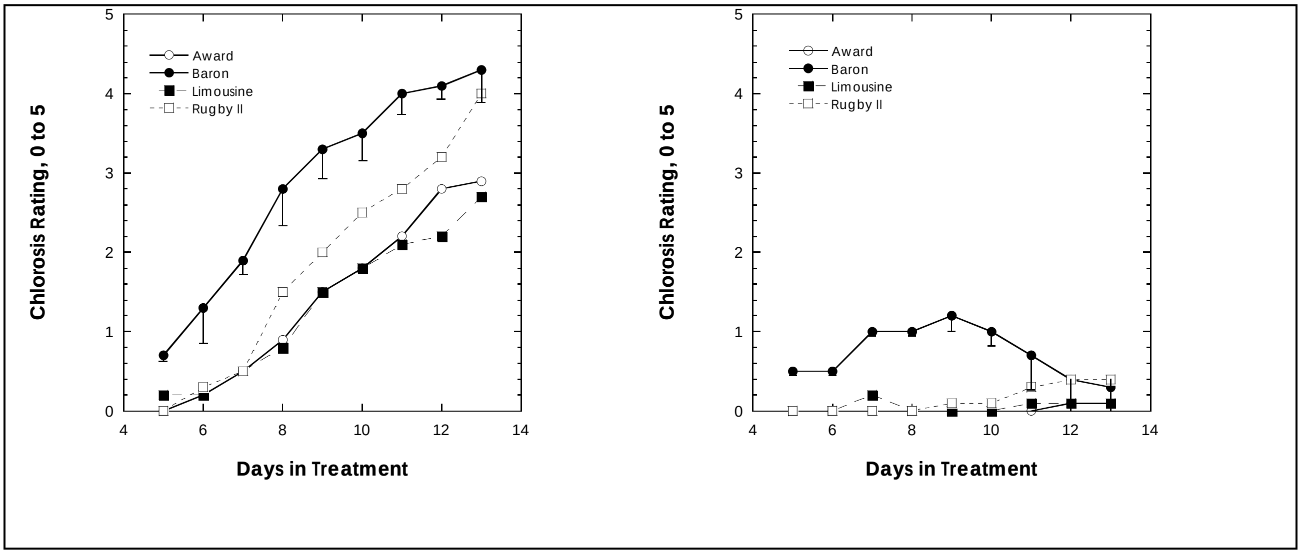

Table 1. Total yield and phytosiderophore production for four cultivars of Fe-stressed KBG. Differing letters next to values represent significant differences.

Cultivar	Total Yield (g)	Phytosiderophore ($\mu g\ ml^{-1}$)
Award	0.76 b	3.22 b
Baron	0.78 b	3.55 a
Limousine	0.82 a	3.07 b
Rugby II	0.77 b	3.19 b

Table 2. Shoot yield, root yield, and phytosiderophore production at 1 and 10 µM Fe of KBG (average of four cultivats).

Fe Level (µM)	Shoot Yield (g)	Root Yield (g)	Phytosiderophore ($\mu g\ ml^{-1}$)
1	0.761	0.117	3.54
10	0.780	0.109	2.98
Significance	0.005	0.014	<0.001

REFERENCES

Cesco, S., A.D. Rombolà, M. Tagliavini, Z. Varanini, and R. Pinton. 2006. Phytosiderophores released by graminaceous species promote ^{59}Fe-uptake in citrus. *Plant and Soil* 287:223-233.

Hansen, N.C. and V.D. Jolley. 1995. Phytosiderophore release as a criterion for genotypic evaluation of iron efficiency in oat. *Journal of Plant Nutrition* 18:455-465.

Hansen, N.C., V.D. Jolley, W.A. Berg, M.E. Hodges, and E.G. Krenzer. 1996. Phytosiderophore release related to susceptibility of wheat to iron deficiency. *Crop Science* 36:1473-1476.

Hopkins, B.D., D.A. Whitney, R.E. Lamond, and V.D. Jolley. 1998. Phytosiderophore release by sorghum, wheat, and corn under zinc deficiency. *Journal of Plant Nutrition* 21: 2623-2637.

Lytle, C.M., V.D. Jolley, and J.C. Brown. 1990. Iron-efficient and iron-inefficient oats and corn respond differently to iron-deficiency stress. *Plant and Soil* 130:165-172.

Steinberg, R.A. 1953. Symptoms of molybdenum deficiency in tobacco. *Plant Physiology* 28:319-322.

Tisdale, S.L., W.L. Nelson, J.D. Beaton, and J.L. Havlin. 1993. *Soil Fertility and Fertilizers*, 5th ed. Macmillan Publishing Co., New York, NY, USA.

Ueno, D., A.D. Rombolà, T. Iwashita, K. Nomoto, and J.F. Ma. 2007. Identification of two novel phytosiderophores secreted by perennial grasses. *New Phytologist* 173: 304-310.

Yang, X., V. Romheld, H. Marschner, and R.L. Chaney. 1994. Application of the chelator-buffered nutrient solution technique in studies on zinc nutrition in rice. *Plant and Soil* 163:85-94.

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