CORN HYBRIDS DIFFER IN THEIR 24-HOUR PATTERNS OF PHYTOSIDEROPHORE RELEASE

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ABSTRACT

Iron efficient gramineous species respond to Fe-deficiency stress by releasing phytosiderophores; thus, increasing Fe solubility and uptake of Fe^{3+}-phytosiderophore. In small grains, phytosiderophore release peaks in a 4 to 6 h period beginning 2 h after the initiation of light, but in maize (*Zea mays* L.) phytosiderophore release is reported to be continuous during light and dark photoperiods. However, our prior work with 11 hybrids suggests possible differences in patterns of phytosiderophore release among hybrids. Our objectives were to examine six hybrids varying in Fe efficiency for differences in 24-h phytosiderophore release patterns and to verify if these patterns differ when phytosiderophore is collected under light and dark photoperiods. Individual Fe-stressed maize plants were grown in a collection solution for six consecutive 4-h periods or alternatively in collection solutions with a different plant transferred for each of six consecutive 4-h periods. This 24-h measurement cycle was repeated up to fourteen times. Root exudates were analyzed for phytosiderophore release using an indirect Fe-binding assay. Iron-efficient hybrids sustained moderate (P3279, DK655) to high (N7070) levels of phytosiderophore release for the first 12 to 16 h after collection began, but release declined during the final 8 to 12 h of collection (usually in the dark period). Iron-inefficient hybrids released either high (DK566) or low (N4640Bt, P3489) levels of phytosiderophore during the first 4 h of collection, and then all three hybrids released low and declining amounts for the next 20 h. The consistent decline in later collection periods for all hybrids was observed with plants collected for 24 h whether collection was initiated just before darkness or just after light initiation, or whether micropur was used or was not used during collection. This consistent decline in phytosiderophore release is attributed to deactivation of the Fe-stress response mechanism from dissolution of apoplastic Fe during the 24-h collection period (40% dissolved in 4 h and 58% in 24 h). The only factor which eliminated the decline was measuring phytosiderophore on new plants every four hours. Although the majority of data suggests that phytosiderophore is released by maize continuously in light and dark photoperiods, at least one hybrid produced significantly more phytosiderophore during the light than the dark photoperiod. This points out the importance of using multiple cultivars or varieties when studying or establishing physiological plant parameters.

INTRODUCTION

•Fe-deficiency chlorosis can devastate yields. Susceptible crops typically develop Fe-deficiency chlorosis on high pH, calcareous soils where the forms of Fe taken up by plants ($Fe^{2+}$and Fe^{3+}) are sparingly soluble.

•Phytosiderophore release in wheat and oat predicts Fe-efficiency or -inefficiency (Hansen and Jolley, 1995; Hansen et al., 1996), but this correlation has not been established in maize (Bernards et al. 2002).

•Yehuda et al. (1996) and Ueno et al. (2009) confirmed that phytosiderophore release in maize continues in both light and dark periods. However, their measurements did not distinguish between primary and nodal roots (Bernards et al., 2002) and their experiments focused on but one cultivar.

OBJECTIVES

The two objectives of our series of experiments were (a) to determine if the continuous release pattern of phytosiderophore extends into the dark period when measured on nodal roots and (b) if patterns of release differ among various Fe-efficient and Fe-inefficient maize hybrids.

MATERIALS & METHODS

All Experiments

•All maize seeds used were germinated in darkness and grown in growth chambers using elongation, pretreatment, and treatment nutrient (modified Steinberg) solutions.
•Phytosiderophore was measured using an indirect Fe-binding assay (Bernards et al., 2002)

Experiment 1

•Two maize hybrids, Fe-efficient P3279 and Fe-inefficient P3489, were grown at two solution Fe levels (1.5 and 4.5 mg L^{-1}).

•Phytosiderophore was collected (beginning Day 6 after initiation of treatments) from the roots of an individual plant taken from each treatment and placed in a new collection solution every 4 h for 24 h (six measurements in 24 h). This cycle was repeated over four 24-h periods.

•The collections for each 24-h period began at 0900 and final collections began at 0500.

Experiment 2

•Four hybrids (Fe-efficient N7070 and DK655 and Fe-inefficient N4640Bt and DK566) were selected according to differences in phytosiderophore release based on a previous study (Bernards et al., 2000).

•Phytosiderophore was collected from a new plant of each hybrid placed in a new collection solution every 4 h for 24 h. This cycle was repeated 14 times, resulting in phytosiderophore measurements every 4 h for fourteen 24-h periods.

•For harvests 1, 2, 3, 5, 7, 9, 11, and 13, collection began at 0500 and for harvests 4, 6, 8, 10, and 12, phytosiderophore collection began at 1700 h.

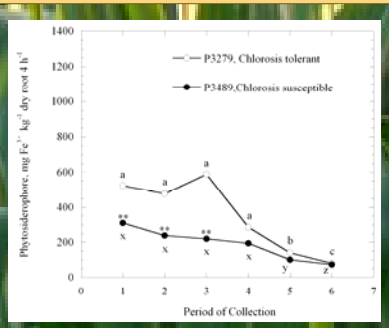

Figure 1. Phytosiderophore exudation of two maize hybrids, P3279 (chlorosis-tolerant) and P3489 (chlorosis-susceptible), across six periods of collection. Collection periods 1-6 represent phytosiderophore measurements at 1300, 1700, 2100, 0100, 0500, and 0900, respectively. For comparing phytosiderophore release between the two cultivars, ** represents a significant difference at the 0.01 level of probability. For comparing phytosiderophore release across periods of collection for each hybrid, points with the same letter are not significantly different at the 0.05 level of probability, Duncan-Waller K Ratio Test.

RESULTS & DISCUSSION

Experiment 1

•Iron-efficient maize hybrid P3270 secreted more phytosiderophore in the first three collection periods than Fe-efficient hybrid P3489 (Fig. 1). Release appeared continuous for both hybrids, although phytosiderophore release was significantly lower in the final two collection periods (0500 and 0900) than in earlier collections (Fig. 1).

Experiment 2

•There was a unique pattern of phytosiderophore exudation for each hybrid examined (Fig. 2). Yet, phytosiderophore release was generally highest over time with the two Fe-efficient hybrids and lowest with the two Fe-inefficient hybrids.

•With three of the four hybrids (DK566, N4640Bt, and N7070), phytosiderophore release was statistically similar at every collection period regardless of whether collection took place during the light or dark periods (Fig. 3). With the fourth hybrid (DK655), phytosiderophore collection for half of the collection periods was the same regardless of the collection time, but for the other three periods phytosiderophore release differed statistically (Fig. 3).

CONCLUSIONS

•The three Fe-efficient cultivars reported herein can be distinguished from the Fe-inefficient hybrids based on quantity of phytosiderophore release--especially if multiple measurements within the same day are used.

•Phytosiderophore exudation was continuous and independent of light or dark photoperiods (collection time) for three cultivars, but less phytosiderophore was released during the dark photoperiod with the fourth hybrid.

•The best explanation for the decline in phytosiderophore observed in later collection periods when the same plant was used for collection for 24 h is the mobilization of root apoplast Fe and concomitant deactivation of phytosiderophore production and release.

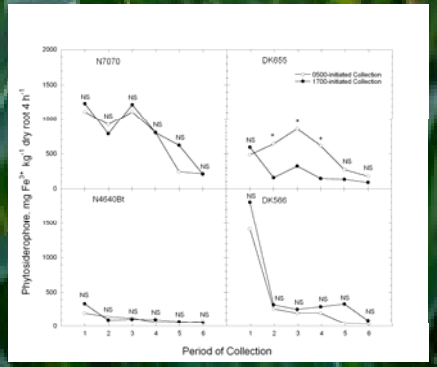

Figure 3. Phytosiderophore exudation of four maize hybrids (N7070, DK655, DK566, and N4640) across six periods of collection. Collection periods 1-6 (0500-initiated collection) represent measurement times of 0900, 1300, 1700, 2100, 0100, and 0500, respectively. Collection periods 1-6 (1700-initiated collection) represent measurement times of 2100, 0100, 0500, 0900, 1300, and 1700, respectively. For comparing phytosiderophore release between 0500- and 1700-initiated collections, ** represents a significant difference at the 0.01 level of probability and NS indicated that the difference was not significant at 0.05 level.

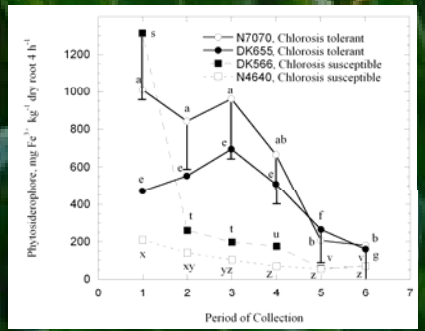

Figure 2. Phytosiderophore exudation of four maize hybrids (N7070, DK655, DK566, and N4640) across six periods of collection. Collection periods 1-6 represents phytosiderophore measurements at 0900, 1300, 1700, 2100, 0100, and 0500, respectively. Error bars originating on data points associated with the highest data point for a given collection period represent the Least Significant Difference ($LSD_{.05}$) to use for comparing hybrids. For comparing phytosiderophore release across periods of collection for each hybrid, points with the same letter are not significantly different at the 0.05 level of probability, Duncan-Waller K Ratio Test.

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