Title
The effect of Fe injection on flowering in soybean without Fe-deficiency symptoms

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Introduction

We found that application of Fe fertilizer to soybean (*Glycine max*) plants that did not show Fe-deficiency symptoms brought about an increase in the pod yield. However, the Fe content of soybean plants did not increase, even though the amount of Fe available in the soil increased on application of the Fe fertilizer. Hence, the relationship between the Fe fertilizer and pod yield remains unclear. In order to understand this relationship, we examined the effect of injecting the stems of soybean plants with citrate-Fe solution. The administration of Fe during the vegetative growth period had no apparent effect on the growth of soybean (whole-shoot dry weight, or chlorophyll content), but during the reproductive growth period, it enhanced flowering. In this report, we discuss the effect of Fe on floral meristems.

Materials and methods

Plants were hydroponically grown in an air-conditioned greenhouse (temperature, 25°C; day length, 13 h; photosynthetic photon flux density, approximately 400 μmol s^{-1} m^{-2}). For this experiment, we used the full-strength nutrient solution employed by Tanaka and Tadano, which has the following composition: 1.3 mM NH_4NO_3, 2 mM $Ca(NO_3)_2$, 0.2 mM $NH_4H_2PO_4$, 1 mM K_2SO_4, 2 mM $MgSO_4$, 50 μM Fe-EDTA, 18 μM $MnSO_4$, 46 μM H_3BO_3, 3 μM $ZnSO_4$, 0.16 μM $CuSO_4$, and 0.05 μM $(NH_4)_6Mo_7O_{24}$.

The stem cortex was shaved using a razor, and the vascular bundles were cut just above the primary leaves. The shaved surface was covered with a small ball of quartz wool and sealed with Parafilm. In the morning, 0.5 mL of treatment solution was injected into the ball of quartz wool.

The floral meristems or axillary buds present at the junction of the petioles and stems were harvested and used for reverse transcriptase-polymerase chain reaction (RT-PCR). The following primers were used: GmLEAFY-LikeF, 5′-GAGCAATGCCGTGAGTTCTT-3′; GmLEAFY-LikeR, 5′-TTCTCTCAGCGTGACAAAGC-3′ (Meng et al 2007); ActinF, 5′-GTTCTCTCCTTGTATGCAAGTG-3′; ActinR, 5′-CCAGACTCATCATATTCACCTTTAG-3′ (Aeschbacher et al 1999); LjAPETALA1F, 5′-TGAAGAGGATAGAGAACAAGATC-3′; and LjAPETALA1R, 5′-AGAGCAGTATCTAACTGCTGCTCCA-3′ (derived from AY770395).

Results and discussion

First, we determined the concentration of Fe to be injected. In the mornings, the Fe concentration in the xylem sap was approximately 10 μM. We tested the effect of administering citrate-Fe or EDTA-Fe, and 50 μM or 100 μM Fe during the vegetative growth period. Injection of 50 μM citrate-Fe resulted in an increase in the Fe content, particularly in the upper leaves, and an increase in the number of axillary buds, but it did not influence the whole-shoot dry weight. Moreover, the soybean plants did not present with any symptoms of Fe excess. Therefore, in our

experiments, we used the 50-μM citrate-Fe solution for injecting soybean plants.

At 4 weeks after germination, i.e., before flowering, we started injecting the plants with Fe. We injected 0.5 mL of treatment solution everyday for 1 week. In this week, both the control plants and the Fe-injected plants started flowering; however, the Fe-injected plants flowered earlier (Fig. 1). The total number of flowers though remained unchanged.

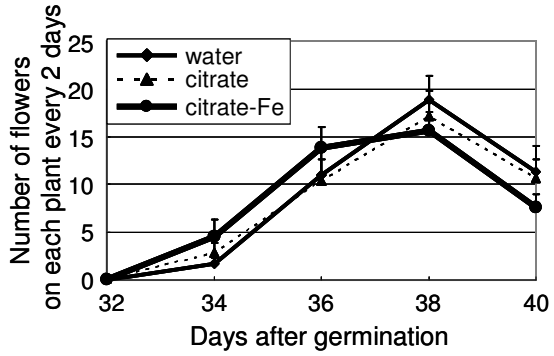

Fig. 1 Time course of flowering.
Soybean plants were supplied daily with water, 50 μM citrate, or 50 μM citrate-Fe for 24–30 days after germination (n = 8).

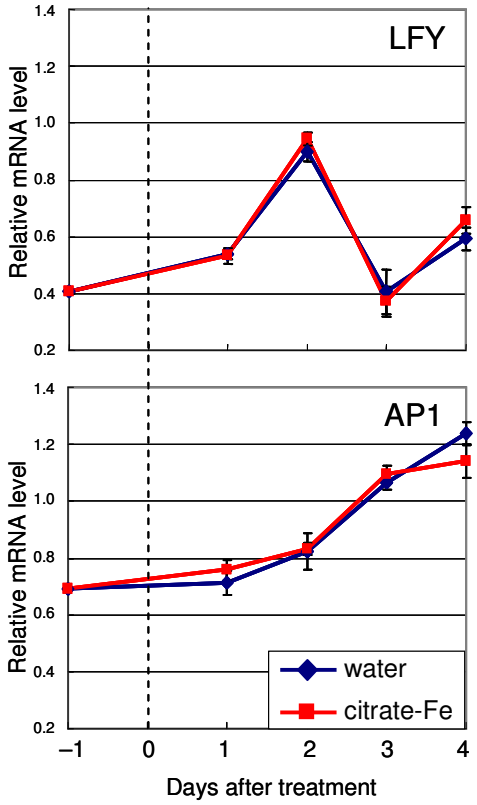

Fig. 2 The expression pattern of *LFY*-like and *AP1* genes in axillary meristems.
Soybean plants were supplied daily with water or 50 μM citrate-Fe for 29–33 days after germination. Treatments were started at day 0. The relative mRNA levels were normalized with actin mRNA (n = 4).

The flowers of the soybean plant are very small; hence, we could not determine whether Fe enhanced the differentiation of floral primordia or whether it facilitated flowering by visual observation. Therefore, using RT-PCR, we analyzed the expression patterns of the *LEAFY* gene (*LFY*) and *APETALA1* gene (*AP1*), which is an intermediate target of the *LFY* gene (Wagner et al. 1999). These genes are essential and are sufficient for inducing the transition to the reproductive phase. We detected an increase in the mRNA accumulation of the *LFY* gene and the *AP1* gene in that order; moreover, the expression patterns of both these genes were not altered on injecting the plants with Fe (Fig. 2). These results suggested that application of Fe facilitates flowering and not differentiation of floral primordia.

It has been reported that the seed-setting rates during the late flowering period are lower than those during the early flowering period (Kuroda et al. 1992, in Japanese). Our results suggest that the Fe supply facilitated the flowering of soybean, and that early flowering increased the seed-setting rates even though the soybean did not show Fe deficiency. We think that the foliar application of Fe before flowering may lead to an increase in the soybean yield.

References

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