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Increase in root branching enhanced ferric-chelate reductase activity under iron stress in potato (*Solanum tuberosum*)

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ABSTRACT

In response to Fe-deficiency, various dicots increase their root branching to improve ferric-chelate reductase activity. It still remains unclear, whether the response caused by Fe-deficiency ultimately improves the plant's ability to withstand Fe-deficiency. In this experiment conducted at ICAR-Central Potato Research Institute, Regional Station, Shillong during 2020, we demonstrated a substantial increase in the growth of the lateral root of potato genotype (CP 3443), when grown in the iron-stress, in relation to control plants, and the total lateral root number is well linked to ferric-chelate reductase (FCR) activity. These findings showed that FCR is involved in root Fe uptake in potato (*Solanum tuberosum* L.) and they suggest a role in Fe distribution throughout the plant. In view of these findings, the Fe-deficiency induced increases in the lateral roots suggested that these play a significant role in Fe-deficiency tolerance in potato, which can serve as useful trait for the identification of chlorosis tolerance and/or nutrient-deficiency stress.

Keywords: Fe-deficiency, Ferric-chelate reductase, Lateral root, Potato

Iron (Fe) is a crucial micronutrient for plants as it is a key cofactor for various enzymatic reactions. However, Fe solubility is low in aerobic and neutral pH environments, leading to limited bioavailability and consequent Fe deficiency. Under low Fe conditions, rather than forming ectopic root hairs, plant roots increase their absorptive surface by inducing root-hair branching (Vissenberg *et al*. 2020). Generally, the increased root branching is proposed to facilitate the uptake of nutrients (Boamponsem *et al*. 2017a). The enlarging root surface area of *Arabidopsis* increased the root hair count and volume. The increased root area facilitates iron reduction and transport of iron. Therefore, whether the increases of root branching are also important for tolerating Fe deficiency in plants still remains unknown.

Strategy-I plants include non-graminaceous monocots and dicots exposed to iron-deficient conditions have been shown to first acidify the soil in order to increase its iron soluble content followed by a reduction of Fe^{3+} to the more soluble Fe^{2+} by a ferric chelate reductase (FCR) and subsequent uptake of Fe^{2+} by a $Fe^{(II)}$ transporter in plant root cell (Boamponsem *et al*. 2017a). FCR is proposed to play a vital role in Strategy-I response because the *Arabidopsis FRO2* loss-of-function mutant, *frd1*, developed severe chlorosis when grown on iron-deficient medium. In addition, generally the Fe-deficiency induced FCR activity was confined to the young lateral root apex (Dasgan *et al*. 2002), subapical root regions or young lateral roots (Boamponsem *et al*. 2017a). In potato (*Solanum tuberosum* L.), formation of lateral roots and root hairs was enhanced in Fe-efficient plant lines compared to inefficient and control plants (Boamponsem *et al*. 2017a) and enhanced ferric-reduction capacity was noted in response to a deficit in Fe supply (Boamponsem *et al*. 2017b). Therefore, the increases in lateral root density are expected to provide more sites for FCR inducing in a Fe-deficient plant, and hence increase the total FCR activity. This is the first study to demonstrate the relationship between Fe-deficiency induced FCR activity and lateral-root density in potato.

MATERIALS AND METHODS

Based on the result of differential iron-deficiency chlorosis (IDC) response of 15 genotypes under aeroponic culture (Challam *et al*. 2021), CP 3443 exhibited tolerance to IDC hence the genotype was considered for the study at ICAR-Central Potato Research Institute, Regional Station, Shillong during 2020. One month old *in-vitro* plantlets were used for the root morphological changes and $Fe^{(III)}$ chelate reductase (FCR) activity induction during Fe-

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deficiency using hydroponic culture. Two different nutrient compositions were followed for FCR activity in potato, viz. Fe-sufficient media (Fe-EDTA 50 µM/L) and Fe-deficient media (Fe-EDTA 0 µM/L). The basic nutrient solution with minor modification was followed as described by Buckseth *et al*. (2016). The *p*H (5.7±2) and EC (1.8-2.0) of solutions were monitored daily. All the nutrient solutions were renewed once in two days. FCR activity in freshly weighed (0.5g) root samples was determined following Boamponsem *et al*. (2017b), whereas number of lateral roots, lateral-root density and root biomass was calculated as described by Jin *et al*. (2008). The data were recorded from 10 plants per conditions.

RESULTS AND DISCUSSION

The roots of dicotyledonous plants cause a series of reactions in response to Fe deficiency, allowing Fe to be absorbed more efficiently by rhizosphere. These adaptive reactions were found to occur on the root surface of potato and include an increased FCR activity, acidification of the rhizosphere to increase Fe mobility and root morphology changes. These responses became apparent 10 days after

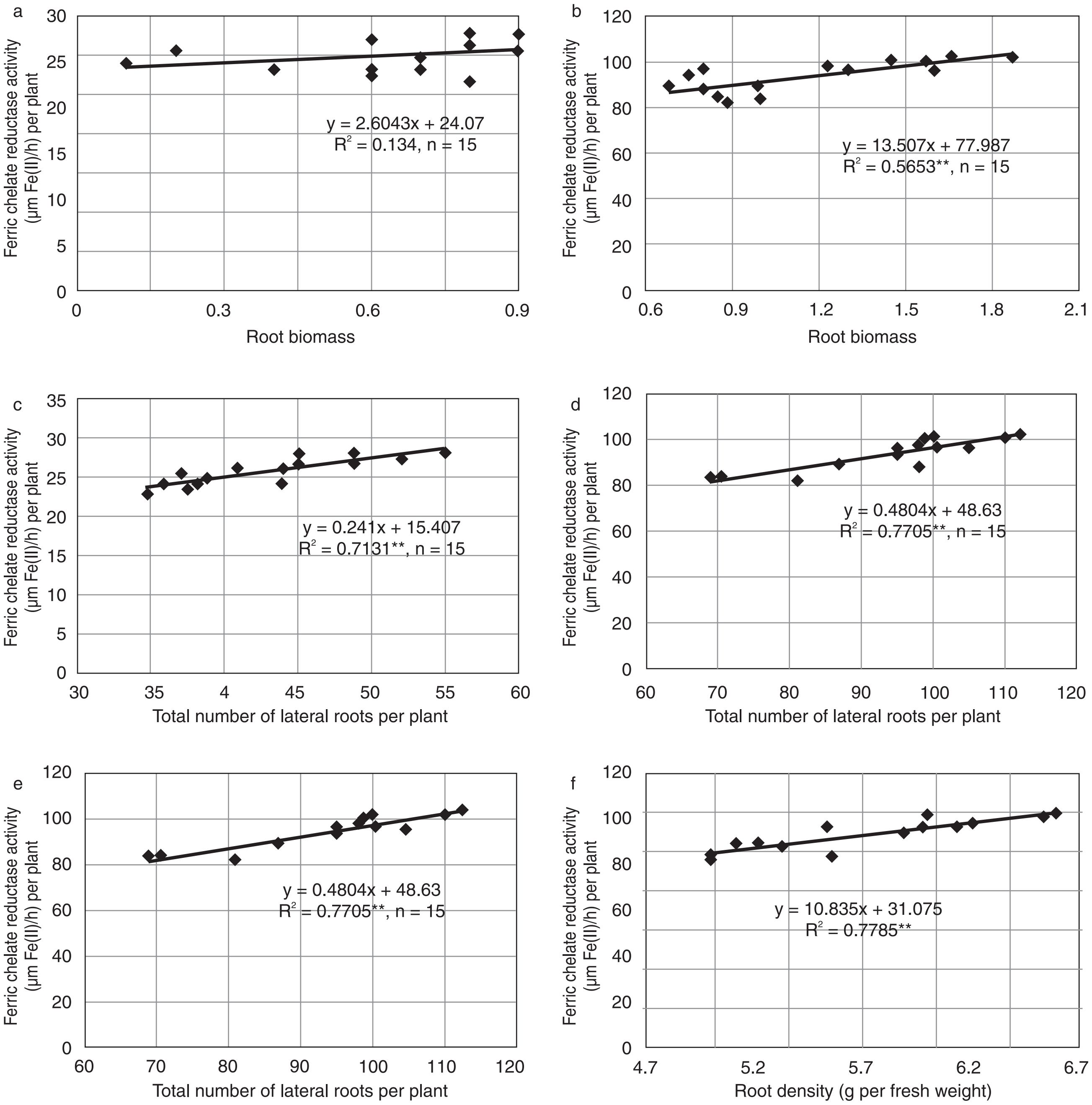

Fig 1 The relationship between ferric-chelate reductase (FCR) activity and root biomass or lateral-root development. (a & b) The relationship between FCR activity and root biomass at 10 and 20 days; (c & d) The relationship between FCR activity and lateral roots number at 10 and 20 days; (e & f) The relationship between FCR activity and lateral roots density of root at 10 and 20 days.

the onset of Fe-deficient conditions. On the 10th day following treatment with Fe-deficit, the total number of lateral roots in Fe-deficient plants increased by about 38 % and the lateral-root density, expressed as the lateral root number per g of fresh weight, increased by approximately 50%. On the 20th day, the growth in lateral roots was even greater with a total increase of 46% and an increase in density of 66%. In determining Fe-acquisition efficient for potato seedlings, Boamponsem *et al*. (2017a) showed that lateral rooting is critically important. Larger root system gives the soil nutrients an improved adsorption surface which is especially significant in terms of soil Fe as a less mobile ion. This morphological change could increase root reduction in Fe-limited conditions. Studies using different genotypes of beans and wheat show, for example, that P-efficient genotypes have a vigorous, highly branched root system with a larger number of apices (Shen *et al*. 2018). In addition, the development of root hair was increased under Fe-deficiency medium which are densely covered, while in control conditions (Fe-sufficient) there was little root hair. In low iron conditions the root is shorter, more lateral roots are formed, and root hairs grow much longer and denser, facilitating increased nutrient uptake. Consequently, dynamic root hair morphogenesis is a key trait for improving the acquisition of essential nutrients in heterogeneous soils. Therefore, a better understanding of the root hair developmental pathway is crucial. Various studies, mostly based on the model organism *Arabidopsis*, have led to a better understanding of the genetic and molecular cascade that lies at the base of root-hair development.

Another major response of the Strategy-I plants to Fe deficiency is the induction of FCR activity for the reduction and uptake of Fe (Legay *et al*. 2012). FCR activity was evaluated under Fe deficiency conditions to determine whether FCR was induced by Fe deficiency as suggested by other scientists (Dasgan *et al*. 2002, Boamponsem *et al*. 2017b), also whether increased root branching enhanced FCR activity in potato. In the study, a concomitant increase in root FCR activity was observed similar to values typically found in roots of Fe-deficient tomato plants (Dasgan *et al*. 2002). On the 10th day of Fe-deficiency treatment, the root FCR activity was poorly associated with root biomass ($r = 0.134$) (Fig 1a) in each plant. However, the total lateral root number ($r = 0.713^{**}$) (Fig 1c) correlated well. In addition, it had a much better comparison to lateral-root density ($r = 0.917^{**}$, $P < 0.05$) (Fig 1e). Almost the entire analysed root area shows high FCR activity on the 20th day of Fe-deficient treatment. The radical FCR behavior of individual plants therefore was very much correlated with the root biomass ($r = 0.565^{**}$, $P < 0.05$) (Fig 1b), with total lateral root number or lateral-root density (Fig 1d,f). The increase in lateral roots therefore tends to lead to the change in Fe-induced activity of FCR. Nonetheless, because FCR is caused only by Fe deficiency, the function of increasing root branching is not determinative. The role of FCR activity in a Fe-deficient plant should be supported by the increased sites for FCR activity of the entire root system. All plants, except grasses, use a reduction strategy to facilitate Fe uptake, and the FCR has proven to be the rate-limiting step for Fe uptake in Fe-limiting conditions. A number of ferric reductase oxidase (*FRO*s) genes were detected, identified and reported on iron uptake and homeostasis for a variety of plant species such as *Arabidopsis* (Mai *et al*. 2016); potato (Legay *et al*. 2012 and Boamponsem *et al*. 2017b). Tiwari *et al*. (2020) also reported the up-regulation of *FRO* under nitrogen stress, indicating the diverse roles for *FROs* in both iron and nitrogen metabolism Therefore, enhancing FCR activities through increasing lateral-root development is expected to improve the ability to resist Fe-deficiency induced chlorosis.

Many of the morphological changes in Fe deficient plants observed in this work can be associated with a change in the balance of phytohormones. Phytohormones such as auxin, cytokinin, ethylene, and gibberellins are the major regulatory agencies for the specification, proliferation and expansion in the roots and extensive crosstalks between them can ensure quick response to external and internal cues. The signal pathways that lead to the enhanced development of the lateral root by Fe deficiency still remain obscure. Auxin, ethylene, and nitric oxide have been implicated as positive regulators of the *FIT, FRO2,* and *IRT1* genes in an Fe-deficient environment to promote Fe acquisition (García *et al*. 2021). *FIT* and *FRO2* expression was found to be enhanced in an auxin over-producing mutant, *yucca*, compared to that wild type (Chen *et al*. 2010). Auxin plays a role in both physiological and root morphological responses to Fe deficiency (Chen *et al*. 2010, García *et al*. 2021). Auxin may therefore be the right chemical signal leading to the Fe-deficiency induced increases of the lateral root. This hypothesis will be investigated in our future research as to whether auxin might be involved in the shoot to root signaling network regulating Fe-deficiency root responses in potato.

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