Received: 19 July 2021 | Accepted: 4 October 2021 | Published online: 5 January 2022

DOI: 10.1002/csc2.20661

Crop Science

REVIEW AND INTERPRETATION PAPERS

Iron deficiency in soybean

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Assigned to Associate Editor Babu Valliyodan.

Funding information
United Soybean Board

Abstract

Iron deficiency is an important soybean [*Glycine max* (L.) Merr.] nutrient deficiency that is easily identified by interveinal chlorosis of the leaves and reduced plant growth, both of which lead to yield reductions. Research in soybean iron deficiency is often segmented into studies on soil characteristics, microbe interactions, specific phenotypes, or genetics of iron efficiency. Joining these areas of research into a comprehensive literature review will advance our understanding of iron deficiency physiology and help to bridge known iron deficiency chlorosis (IDC) resistance loci with plant responses to iron stress. This review investigates what has been accomplished in the areas of phenotyping and genetics of iron deficiency. Furthermore, this work traces iron deficiency physiology research through the plant, beginning with the role of soil, the transport of iron into and through plant tissues, and the eventual deposition in the seed. While IDC is the most phenotyped and genetically mapped trait relating to iron deficiency in soybean, the whole plant is truly affected by and involved in recovery to the stress. While often neglected in iron deficiency research, the soybean–rhizobia relationship is discussed as an area of opportunity for future advancements. Citrate and nicotianamine were identified as important compounds for iron efficiency in several studies and warrant more in-depth investigation. The aim of this review is to analyze research in soybean iron deficiency phenotyping, genetics, and physiology to reveal connections between these areas and facilitate further discoveries.

1 | INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is a highly valued oil crop worldwide as well as an important source of protein in both livestock feed and human diets (Masuda & Goldsmith, 2009). Consumed directly, soybean is an important dietary source of both protein and iron in developing countries (Messina, 1999). In 2020, soybean was planted on 33.6 million ha in the United States, from which 113 Mt of seed were harvested, second only to maize (USDA, 2021). Although domesticated in China in the 11th century B.C. (Guo et al., 2010), soybean has only relatively recently been introduced to the United States, becoming more common in the early 1900s (Hymowitz, 1970). The genetic diversity of soybean in North America is limited because of several historical factors: the original process of domestication in Asia, a small number of genotypes founding the U.S. germplasm, and subsequent intense breeding for a specific suite of traits (Gizlice et al., 1994). Nevertheless, U.S. soybean yields have been steadily increasing at a rate of 1.5% per year over the last century

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Abbreviations: bHLH, basic helix-loop-helix; BNF, biological nitrogen fixation; IDC, iron deficiency chlorosis; QTL, quantitative trait loci; RH, relative humidity; SM, symbiosome membrane; UAS, unmanned aircraft system.

because of a combination of advances in management and cultivar development (Egli, 2008).

Iron deficiency chlorosis (IDC) is a common symptom of iron deficiency in soybean grown in the upper Midwest of the United States (Hansen et al., 2003). IDC is characterized by interveinal chlorosis of the leaves, stunted plant growth, and often results in substantial yield loss. It has been estimated that yield losses attributed to IDC can cost growers a total of $260 million annually in the U.S. (Peiffer et al., 2012). While it is important to quantify losses resulting from IDC, it should be noted that this figure can vary drastically with the number of soybean acres planted, value of the crop, and IDC symptom severity in any given year.

Inconsistent language describing symptom severity of iron deficiency between studies was noted when compiling this review. While seemingly mundane, the language used to classify soybean genotypes could have important implications on how iron homeostasis is achieved in iron-limiting conditions. Studies often use the terms ‘tolerant’ and ‘susceptible’ to describe a genotype’s relative expression of IDC symptoms in iron-deficient environments (e.g., Assefa et al., 2020; Charlson et al., 2005; Goos & Johnson, 2001; Merry et al., 2019), while others favor ‘efficient’ and ‘inefficient’ (O’Rourke et al., 2007; Rogers et al., 2009). Connotation issues exist with the use of these terms. ‘Tolerance’ to iron deficiency implies that the plant endures the stress with a lack of response or that a plant tolerates IDC without an effect on yield or plant health, both of which are contrary to evidence in this review. ‘Susceptible’ may also be misleading, as this term is commonly used when referring to interactions with biotic diseases. ‘Sensitivity’ to IDC is used on occasion (e.g., Waters et al., 2018) but is better suited for nutrients or compounds that cause toxicity in excess, for example aluminum or salt in soybean (e.g., Patil et al., 2016). Describing a soybean genotype’s ‘resistance’ to IDC (e.g., Charlson et al., 2005) is sensible, as it implies that the plant is actively responding to iron stress and suggests the symptom occurs in varying degrees of severity. For example, if a soybean genotype is described as having high resistance to IDC, it is implied that another genotype may have low resistance to IDC but that complete resistance is not achieved. As this description most accurately reflects IDC as found in this review, soybean genotypes are defined as having high resistance or low resistance to IDC when describing the symptom of IDC per se.

While IDC is the most common symptom of iron deficiency in soybean, it is important to differentiate between IDC and specific genotypic differences in iron physiology (e.g., iron uptake, transport, and utilization). Resistance to IDC is apt for describing the symptom per se, but it does not accurately describe iron physiology of a soybean genotype. Describing iron physiology as ‘iron efficiency’ or ‘iron inefficiency’ has multiple connotations with important implications to physiology and study design. In controlled environment studies where the total amount of iron is reduced compared with a control, efficiency implies that the genotype is better able to maintain homeostasis with reduced total iron. While this may be physiologically possible, iron content within the plant is rarely measured in iron deficiency studies (some exceptions include King et al., 2013; Moosavi & Ronaghi, 2011; Roriz et al., 2014; Wiersma, 2005). ‘Efficiency’ can also refer to the efficiency of a genotype in acquiring available iron from the environment, the efficiency at which it converts unavailable iron to an available form, or the efficiency at which iron is moved throughout the plant. As the terms ‘efficient’ and ‘inefficient’ cover a broad range of iron physiology contexts, they will be used in this review when referring to soybean genotypes in respect to iron physiology and not IDC per se. The terms ‘iron sufficient’ and ‘iron deficient’ will be used when referring to iron availability in growing environments.

Core Ideas

- Iron deficiency in soybean is a pervasive problem for growers and breeders.
- Several environmental factors are responsible for iron deficiency in soybean.
- A gap in understanding exists between IDC QTL and iron deficiency physiology.
- Iron physiology in soybean is complex and involves the whole plant.
- Combining knowledge of iron physiology and QTL will lead to improvements of resistance to IDC.

Iron deficiency is managed through agronomic prevention as well as breeding of iron-efficient cultivars. Agronomic practices to prevent or reduce iron deficiency in soybean include the application of iron chelates (Gamble et al., 2014; Goos & Johnson, 2000; Helms et al., 2010; Kaiser et al., 2014; Wiersma, 2007), reducing excess soil nitrates from previous season carry over (Naeve & Rehm, 2006), nitrate reduction by companion crop uptake (Dragicevic et al., 2015), and increasing soybean seeding rate (Naeve, 2006). Foliar applications of iron can be applied before or after the onset of symptoms (Chatterjee et al., 2017; Franzen et al., 2003; Goos & Johnson, 2000; Lingenfelser et al., 2005). The use of cultivars selected for resistance to IDC is also prominent. In a comparison of agronomic prevention methods and selection of IDC resistant cultivars, it was concluded that cultivar selection is the best strategy to combat iron deficiency in the field (Goos & Johnson, 2000; Kaiser et al., 2014).

From the genetic perspective, many studies have revealed quantitative trait loci (QTL) that confer resistance to IDC (King et al., 2013; Mamidi et al., 2014; Peiffer et al., 2012) (Table 1). Furthermore, the release of

TABLE 1 Soybean iron deficiency chlorosis resistance mapping studies

Study	Method	Chromosomes associated with resistance
Diers et al., 1992	Biparental	None confirmed
Lin et al., 1997	Biparental	Gm03, Gm05, Gm12, Gm14, Gm18, Gm20
Lin et al., 2000b	Biparental	Gm03, Gm12, Gm14, Gm20
Lin et al., 2000a	Biparental	Gm03, Gm11, Gm14, Gm18, Gm19, Gm20
Charlson et al., 2003	Biparental	Gm03, Gm05, Gm12, Gm19, Gm20
Charlson et al., 2005	Biparental	Gm05, Gm19, Gm20
Wang et al., 2008	Association	Gm13, Gm14, Gm20
Mamidi et al., 2011	Association	Gm02, Gm03, Gm06, Gm07, Gm13, Gm19
Pieffer et al., 2012	Biparental	Gm03
King et al., 2013	Biparental	Gm20
Mamidi et al., 2014	Association	Gm02, Gm03, Gm05, Gm07, Gm11, Gm12, Gm15, Gm16, Gm17, Gm18, Gm19
Zhang et al., 2017	Association	Gm02, Gm03, Gm07, Gm16, Gm18, Gm20
Merry et al., 2019	Biparental, Association	Gm03, Gm05, Gm06
Assefa et al., 2020	Association	Gm01, Gm02, Gm03, Gm05, Gm06, Gm07, Gm08, Gm09, Gm10, Gm11, Gm12, Gm13, Gm14, Gm15, Gm16, Gm17, Gm19, Gm19, Gm20

Note. The use of biparental and association mapping approaches to discover loci is indicated in the Method column. Chromosomes on which the loci were discovered are also indicated.

soybean genome sequence assemblies (Liu et al., 2020; Schmutz et al., 2010; Song et al., 2016; Valliyodan et al., 2019) and the development of high-throughput molecular markers (Song et al., 2015) has enabled such studies to be performed at higher resolution and with greater genomic information (e.g., Assefa et al., 2020; Merry et al., 2019).

With the discovery of many IDC resistance QTL, it is timely to better understand the physiology of iron deficiency to aid in identifying the physiological mechanisms and genes underlying IDC resistance and iron efficiency. The aim of this review is to provide a comprehensive analysis of iron deficiency in soybean: (a) iron limitations in the soil, interactions of iron with microbes, and management; (b) IDC phenotyping and genetics; (c) uptake of iron into the root, iron transport, use in the nodule, movement of iron in the stem, iron stress in leaves, and deposition of iron within the seed. By thoroughly reviewing the literature of iron deficiency in soybean, this work seeks to assist researchers in bridging the gap in knowledge between the physiological mechanisms that govern iron deficiency responses and the QTL that confer resistance to IDC.

2 | PART I: ENVIRONMENTAL FACTORS AND MANAGEMENT OF IRON DEFICIENCY

2.1 | pH

While iron is abundant in soils, it is the form of iron and soil chemistry that determines availability for plant uptake (Inkseep & Bloom, 1987; Marschner & Römheld, 1994). As a Strategy I plant, soybean transports ferrous iron (Fe^{2+}) into the root, compared with Strategy II plants, which transport iron into the root as an iron–siderophore complex (Römheld, 1987). In high-pH soils, iron is oxidized to the ferric state (Fe^{3+}), which binds tightly to anions, covalent metals, phosphorus, and soil particles and is unavailable for uptake and transport into the root in Strategy I plants (Marschner & Römheld, 1994). The relative contributions of physical and chemical soil characteristics to soybean iron deficiency have been well characterized during the past two decades (Hansen et al., 2003; Liesch et al., 2012; Robin et al., 2008). A survey of soybean fields exhibiting areas of both iron deficiency symptoms and healthy plants found that pH did not differ significantly between these areas within fields (Hansen et al., 2003), indicating that pH alone does not induce iron deficiency in soybean. Rather, a multitude of soil factors including pH, carbonates, moisture, and salts (measured as electrical conductivity) interact and result in iron deficiency.

2.2 | Soil-moisture-driven carbonate release

Calcareous soils are characterized by a calcium carbonate parent material. Calcareous soils in the upper Midwest commonly have a pH ranging from 7.0 to 8.5, which is highly conducive to iron deficiency in soybean (Hansen et al., 2003). While calcareous soils are conducive to iron deficiency, the severity of the conditions depends, in part, upon soil moisture. Under high moisture, the parent calcium carbonate is dissolved into solution, resulting in Ca^{2+} and carbonate ($CO_3{}^{-2}$) (Hansen et al., 2003) (Figure 1a). Low areas in fields are more

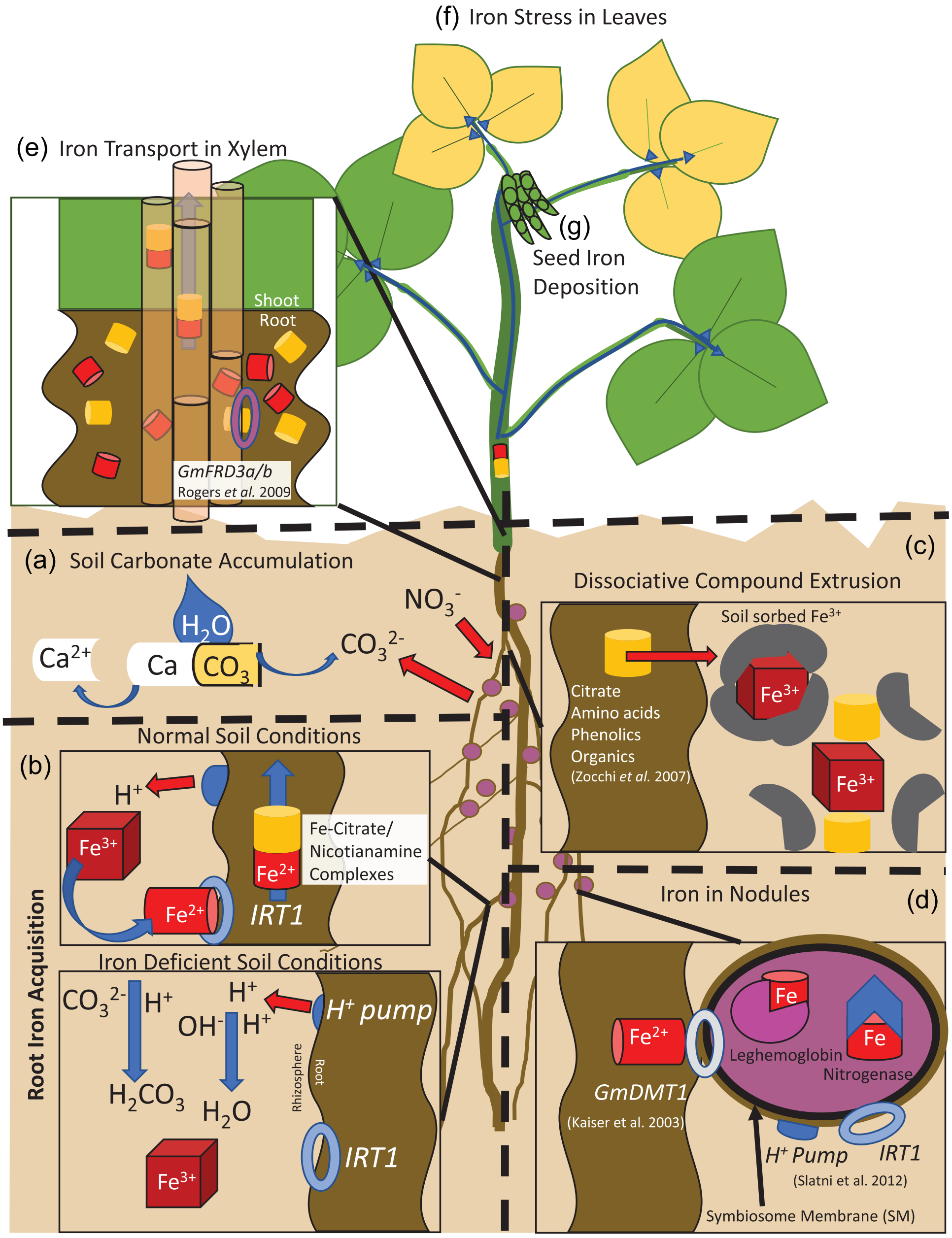

FIGURE 1 The physiology of iron deficiency, movement, and utilization in soybean. Iron deficiency in soybean is controlled by many factors in both soil and the plant. (a) Carbonates are generated in soil through the breakdown of calcium carbonates and through extrusion by the plant when excess nitrates are available. Upon dissolution in water, calcium carbonate is dissolved into Ca^{2+} and CO_3^{2-} (carbonate). Wet field conditions lead to increased carbonate generation and exacerbated iron deficiency symptoms. When excess nitrates are available in the soil, soybean preferentially scavenges the nutrient and extrudes carbonates to balance charge. (b) Under normal soil conditions, Fe^{3+} is reduced to Fe^{2+} via proton extrusion from the root and is then transported into the root by an iron transporter (likely IRT1). In iron-deficient soil conditions (lower panel b), a buffering effect occurs when carbonates accumulate in the soil coupled with high soil pH. Soil buffering prohibits protons extruded from the root to reduce Fe^{3+} to the available Fe^{2+} for uptake. (c) Iron readily precipitates when soil pH exceeds 6.5. To overcome precipitated iron sorbed to soil particles, soybean extrudes citrates, amino acids, phenolic compounds, and organic carbon compounds to increase solubility of Fe^{3+}. Freed Fe^{3+} can then be reduced to Fe^{2+} for plant uptake. (d) Nitrogen fixation in the nodules requires large quantities of iron as constituents of leghemoglobin and nitrogenase. Nodules in common bean accumulate high levels of proton pumps and IRT1 proteins on the surface to support this iron requirement. The soybean iron transport protein GmDmt1 is also crucial for proper nodule function. (e) Once iron has entered the root, it is transported throughout the plant as iron-citrate and iron-nicotianamine complexes in the xylem. In some iron-efficient soybean genotypes, increased expression of gene *GmFRD3b* leads to higher levels of citrate in the xylem sap. Reduced citrate and nicotianamine results in iron accumulation in the root. (f) Iron deficiency in soybean is easily characterized by interveinal chlorosis. Iron deficiency chlorosis most often occurs in newly developing trifoliates, as iron is immobile in the plant. Chlorophyll production in iron-deficient leaves is reduced, resulting in yield loss. (g) Iron deposition in seeds occurs through phloem and xylem loading, transported by citrate and nicotianamine. As the plant senesces, iron pools from other tissues such as leaves, roots, and nodules, are transported to the seed. Increased iron accumulation within the seed may provide escape from early season iron deficiency for the next generation

prone to iron deficiency because of increased moisture accumulation and dissolving of the parent material (Robin et al., 2008). Once in soil solution, carbonate acts as a strong base, raising the pH and oxidizing Fe^{2+} to Fe^{3+}. Carbonate also acts as a buffer in the soil, inhibiting the conversion of Fe^{3+} to Fe^{2+} (Hansen et al., 2003) (Figure 1b). The effect of the buffering capacity of carbonate will be discussed in greater detail in a forthcoming section of this review, as it intimately relates to root acquisition of iron.

2.3 | Soil nitrates

Soil nitrates have been shown to increase the severity of soybean iron deficiency in high pH soils (Wiersma, 2010). When available in soil, soybean will preferentially scavenge nitrogen (Bloom et al., 2011) likely to reduce inputs required for biological nitrogen fixation (BNF). To balance intracellular charge and pH, the uptake of nitrate is coupled with carbonate extrusion into the soil, which results in further pH increases and buffering of the soil in the rhizosphere, exacerbating IDC symptoms (Bloom et al., 2011; Rogovska et al., 2007; Wiersma, 2010) (Figure 1a). For this reason, fields in which soybeans use BNF, as opposed to scavenging for soil nitrates, would be expected to display reduced IDC symptoms.

2.4 | Compounded nutrient stress

Several other nutrients become limited in high-pH soils in a similar matter as iron. Phosphorus, potassium, manganese, boron, copper, and zinc become less available for plant uptake at pH >5 (Truog, 1946), suggesting iron-deficiency-similar responses may be employed to acquire these nutrients as well. Multiple nutrient stresses synergistically increase iron deficiency responses to overcome iron stress (Romera et al., 2003). Under simultaneous copper and iron stress, soybeans increase soil acidification and metabolite release, enhancing iron reduction and acquisition (Romera et al., 2003). Soybean cultivars that absorb phosphorus with high efficiency (i.e., phosphorus-efficient soybean) have also been found to have improved iron uptake in iron-limiting conditions (Romera et al., 2003). Because multiple nutrient stresses occur at high pH, it is logical that plants would develop complementary mechanisms to acquire unavailable nutrients. However, additional research in this area is needed to understand the impact of multiple nutrient stresses on soybean more thoroughly. In high-pH soils, iron deficiency is commonly identified because of the striking interveinal chlorosis. However, iron may simply be the most limiting nutrient and the plant may be under additional nutrient stresses. Recent advances in ionomics (Mosa et al., 2017) may make it possible to better investigate the correlations between the acquisition of different nutrients in high-pH soils by soybean plants.

2.5 | Microbial interactions in the soil

Microbial communities in the rhizosphere and their influence on plant health has been well documented in the last decade (comprehensively reviewed in Finkel et al. [2017]). Microbes face many of the same challenges that plants encounter in iron-limiting conditions including the need to free iron tightly sorbed to the soil and other compounds and reduce Fe^{3+} to Fe^{2+} (reviewed by Marschner, 2011). Bacteria using Fe^{2+} and Fe^{3+} as an electron donor in the denitrifying process (reviewed by Weber et al., 2006) could also compete with soybean for available iron. Competition for Fe^{2+} between soybeans and denitrifying bacteria may also be occurring in high-pH soils and should be investigated as another potential iron sink. While most of the microbial research within soybean systems focus on rhizobium and nodulation, several studies have investigated the importance that microbial communities have on iron acquisition. The diversity of bacterial species present in the soil allows for siderophore production (Finkel et al., 2017) as well as other iron reducing strategies allowing plants to scavenge a portion of iron reduced in the rhizosphere by bacteria (Khandelwal et al., 2002; Zhang et al., 2009). While microbial effects on plants in general have been well characterized, relatively few studies have been conducted specifically on the effects of microbial communities on soybean in iron-deficient soils.

Fungal associations allow plants access to nutrients beyond the reach of the rhizosphere. Fungi also release siderophores, which allow them to acquire Fe^{3+} in high pH soils (Reviewed by Crowley et al. [1991]), which may lead to transport of iron into the soybean via mycorrhizal relationships. In noncalcareous soil, mycorrhizal relationships were found to increase manganese, iron, and phosphorus in soybean (Ibiang et al., 2017; Karaca et al., 2013). With the growing popularity of biological seed treatments for commercial soybean production, there is a great opportunity to further research this topic and identify fungal species that can alleviate iron deficiency in calcareous soils.

2.6 | Management of iron deficiency in soybean

2.6.1 | Iron chelates

The use of iron chelates [such as diethylenetriamine pentaacetate (DTPA), ethylenediaminetetraacetic acid (EDTA), and ethylenediamine di(o-hydroxyphenyl acetic) acid (EDDHA)] as soil amendments to alleviate iron deficiency in soybean is common in high-pH soils (Gamble et al., 2014; Goos & Johnson, 2001; Helms et al., 2010; Kaiser et al., 2014; Wiersma, 2007). Iron chelates have a high affinity for Fe^{3+}, increasing the solubility of iron in the soil. Roots are better able to reduce iron to Fe^{2+} when Fe^{3+} is freed from bonds with other anions and soil surfaces and instead form complexes with chelates. Once reduced, the iron is released from the chelate and transported into the root (Wiersma, 2007). Chelates can be applied as a seed treatment or in furrow at planting (Kaiser et al., 2014). The success of chelate application relies heavily on the buffering capacity and pH of the soil. Suboptimal application rates are unable to overcome high pH and buffering and results in only short-term improvements, which are followed by a return to chlorotic symptoms (Wiersma, 2007). The economics of applying iron chelates must also be considered when managing iron deficiency. For best results, chelates must be applied before symptoms occur (Gamble et al., 2014). In years with minor iron deficiency, such as dryer than normal years, the application of chelates would be an added cost with little to no benefit to the grower. While chelates can improve iron deficiency symptoms, it is recommended to plant an IDC-tolerant cultivar as a first line of defense (Kaiser et al., 2014; Liesch et al., 2011).

2.6.2 | Managing nitrates

Several management strategies preventing excess soil nitrates have proven beneficial in reducing iron deficiency severity (Dragicevic et al., 2015; Naeve & Rehm, 2006). Corn (*Zea mays* L.) and soybean rotations, in which the quantity of nitrogen is carefully applied so that excess nitrogen does not carry over to the following soybean crop, was found to increase not only iron availability but also the availability of other nutrients that are often unavailable at high pH (Naeve & Rehm, 2006). Using a companion crop was also found to alleviate IDC symptoms (Dragicevic et al., 2015). When oat (*Avena sativa* L.) was used as a companion crop with soybean, oat was able scavenge soil nitrogen more efficiently, forcing soybean to rely on BNF and thereby reducing the extrusion of carbonates into the rhizosphere (Dragicevic et al., 2015).

2.6.3 | Foliar iron applications

Foliar applications of iron compounds have been investigated for alleviating iron deficiency symptoms (Hecht-Bucholz & Ortmann, 1982; Moosavi & Ronaghi, 2011; Rodriguez-Lucena et al., 2010), including the effect of iron foliar application on yield in iron-deficient soils (Chatterjee et al., 2017; Goos &Johnson, 2000; Franzen et al., 2004; Lingenfelser et al., 2005). In controlled environments, foliar applications of iron increases iron supply of the leaf (Moosavi & Ronaghi, 2011), improving chlorophyll development and reducing chlorotic symptoms (Hecht-Bucholz & Ortmann, 1982; Rodriguez-Lucena et al., 2010). Foliar treatment, however, has not been effective in symptom alleviation when applied to soybean with severe IDC symptoms (Hecht-Bucholz & Ortmann, 1982) especially in field conditions (Goos & Johnson, 2000; Franzen et al., 2004; Lingenfelser et al., 2005). The primary challenge with foliar applications is a physical one. Leaf area of soybean prior to the development of symptoms is very small and soybean plants that are stressed by IDC are unable to efficiently take up the iron required for recovery. It is important to differentiate between regreening of soybean (symptom alleviation) and improvements in yield because of foliar iron application. While leaves may appear less chlorotic (although this result is not consistent across locations or studies), studies have found that soybean yield was not increased with foliar applications of iron (Chatterjee et al., 2017; Franzen et al., 2004; Goos & Johnson, 2000; Lingenfelser et al., 2005). Rodriguez-Lucena et al. (2010) screened 15 different synthetic and natural iron sources for efficacy through foliar application, finding that only iron-lignosulfate was translocated from leaves to roots after application. This suggests that while the leaves recover, soybean nodules could still be iron limited after foliar iron applications, and thus reduced nitrogen fixation in the nodules may limit protein production resulting in decreased plant yield later in the season as reported in several studies (Franzen et al., 2004; Goos & Johnson, 2000; Lingenfelser et al., 2005).

3 | PART II: IRON DEFICIENCY PHENOTYPING AND GENETICS

3.1 | Phenotyping iron deficiency in soybean

Iron deficiency chlorosis, the most visual symptom of iron deficiency in soybean, is often used synonymously with iron deficiency in the literature and is the most commonly measured symptom of iron deficiency. Traditionally, IDC severity has been measured using a visual rating on a 1-to-5 scale, with a score of 1 indicating no symptoms and a score of 5 indicating severe symptoms (Figure 2) of either single leaf trifoliates (e.g., Cianzio et al., 1979) or on a plot basis (e.g., Mamidi et al., 2011). Ratings based on a single leaf only capture a specific moment in plant development and may not represent overall plant health. Visual rating on a plot basis is more informative, as symptom severity can be assessed on how much of the canopy appears chlorotic. For example, a score of 1 would indicate no symptoms within the plot, 2 would indicate some chlorosis in the canopy, 3 would indicate chlorosis throughout the canopy, 4 would indicate chlorosis throughout canopy combined with some necrosis, and 5 would indicate severe chlorosis and major necrosis or plant death (Figure 2). As can be inferred by the previous statements, the visual rating system is extremely subjective and prone to

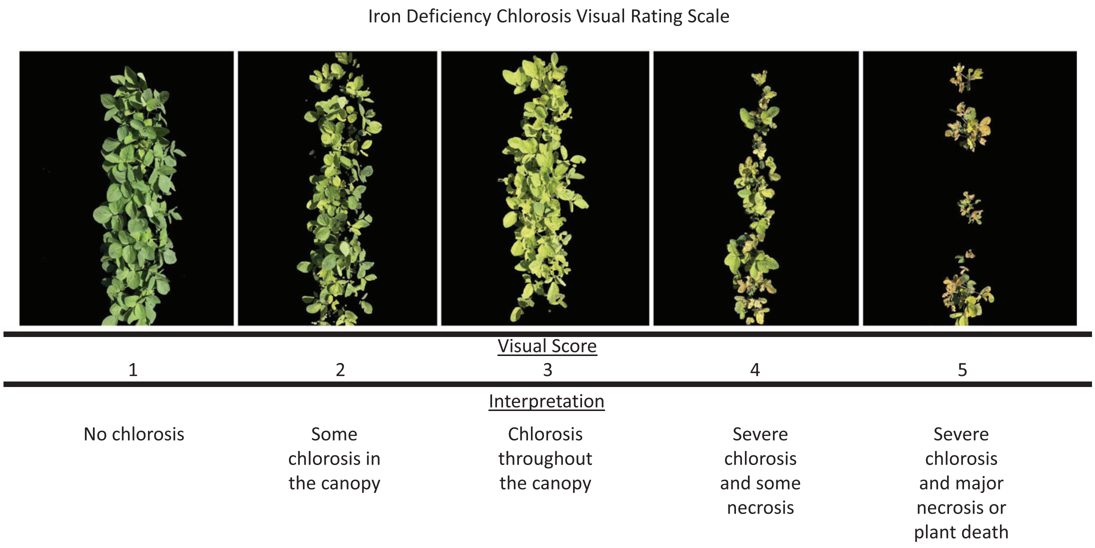

FIGURE 2 One-to-five visual rating scale of iron deficiency chlorosis. Soybean rating of iron deficiency chlorosis (IDC) is often conducted on a visual scale. The 1-to-5 visual scale is the most widely used and can be conducted on individual trifoliates or on a plot basis. When conducted on a plot basis, a score of 1 indicates no chlorosis within the plot. A score of 2 indicates some chlorosis, most likely in the upper part of the canopy where iron chlorosis initiates. A score of 3 indicates chlorosis throughout the canopy. A score of 4 indicates severe chlorosis and the onset of some plant necrosis resulting from iron deficiency. A score of 5 indicates severe necrosis and plant death as a result of iron deficiency

variability between raters. One benefit and challenge with whole-plant or whole-plot scoring is that these naturally integrate estimates of biomass as well as the chlorosis of the existing biomass.

Recently, researchers have developed image-based IDC rating systems (Naik et al., 2017; Zhang et al., 2017). In this approach, pictures of plots are taken with digital cameras, and computer imaging software is then used to extract three features - green pixel count, yellow pixel count, and brown pixel count. Using machine-learning algorithms, these features are related to the IDC severity scores taken in the field. Approaches such as this have offered great success in hill plots, achieving a mean per-class accuracy (the accuracy of a plot being correctly rated on the 1-to-5 scale) of ∼96% (Naik et al., 2017).

Four-row field trial IDC plots have also been imaged to measure IDC severity (Bai et al., 2018). A high-throughput phenotyping cart was used to capture red-green-blue (RGB) images over the center two rows of each plot and an overall classification accuracy of 81% was achieved (Bai et al., 2018). Throughput can be further increased using unmanned aircraft systems (UASs), in which classification accuracies up to 77% were reported by Dobbels and Lorenz (2019). Moreover, greater precision was achieved via the UAS analysis compared with human visual ratings (Dobbels & Lorenz, 2019). The level of throughput afforded by UAS image collection and analysis is necessary for a substantive impact on efficiency of breeding programs, which may screen up to tens of thousands of plots in IDC nurseries. More work, however, is needed to optimize models and streamline the image analysis and prediction pipeline to enhance user efficiency and justify the automated phenotyping approach. Without such advances, time in the field collecting visual ratings is only replaced by time at the computer spent on image analysis.

While categorical classification of IDC symptoms may be adequate for characterizing IDC resistance among varieties and genetic mapping of QTL, alternative phenotyping procedures would benefit a deeper physiological understanding of iron stress at the plant level. Relative leaf chlorophyll concentrations can be measured by destructively sampling soybean leaves and extracting the chlorophyll in acetone solutions (Lin et al., 1997). Other researchers have measured chlorophyll concentration using a SPAD meter (Minolta) (Bloom et al., 2011; Helms et al., 2010; Vasconcelos & Grusak, 2014). While this approach is more time and labor intensive, it can provide an objective measurement of IDC symptom severity provided variation within plots and plant canopy are accounted for.

The lack of standard phenotyping conditions can be a challenge for researchers investigating physiological differences between iron efficient and inefficient soybean genotypes. Using soil from IDC nurseries in potted greenhouse experiments has been attempted but has not yielded reliable results (Fairbanks et al., 1987) Byron and Lambert (1983). Soil collection, transport, and storage for experiments also brings into question the feasibility of this method.

Many controlled IDC studies have been performed using hydroponic methods. Most often in these experiments, iron concentrations are limited in the nutrient solution and sometimes pH or carbonate levels are adjusted to induce IDC

(Dragonuk et al., 1989; Lin et al., 1998; O'Rourke et al., 2007; Peiffer et al., 2012). The use of carbonates to limit iron and induce IDC symptoms in hydroponics has been conducted using solid $NaHCO_3$ (Coulombe et al., 1984), which can be slow to dissolve and is chemically different than the $CaCO_3$ found in most IDC-prone soils. The use of a predissolved liquid source of $CaCO_3$ has recently been studied in a potted controlled environment study along with pH adjustments and inoculation with *Bradyrhizobium japonicum* to induce nodulation (Merry et al., unpublished data, 2021). Merry et al. (unpublished data, 2021) found that calcium carbonates affected iron deficiency more than pH per se, and nodulation improved the iron efficiency of both iron efficient and deficient genotypes. The effect of nodulation on iron deficiency in soybean in controlled environments is rarely studied, although previous research has indicated that soybean nodules may help initiate an iron deficiency response (Soerensen et al., 1988, 1989).

3.2 | Genetics of soybean iron deficiency

Early screening of the soybean germplasm collection failed to find an accession that was completely resistant to IDC (Fehr, 1982). Complete resistance to IDC is most likely unattainable for reasons similar to complete drought resistance in soybean. Just as some amount of water is required for survival, plants must be able to acquire iron to reach maturity and reproduce. However, genetic variation for resistance to IDC is abundant in soybean germplasm, making it possible to develop cultivars with improved resistance. Planting of such cultivars remains the preferred management tool for avoiding or minimizing yield losses (Goos & Johnson, 2000; Kaiser et al., 2014). Initial genetic studies of IDC resistance erroneously suggested the trait was controlled by a single recessive gene (Cianzo et al., 1979; Weiss, 1943). It is now understood that the inheritance of IDC resistance is quantitative, as first reported by Cianzio and Fehr (1982) and since verified in many genetic studies (see Table 1).

A major IDC resistance QTL on Gm03 was first identified by Lin et al. (1997), and further characterized by Peiffer et al. (2012). Based on mapping and genomic analysis, the candidate gene in the Gm03 locus is a basic helix-loop-helix (bHLH) transcription factor, with a mutation in a protein dimerization region hypothesized to confer low resistance to IDC. It is thought that this gene activates the iron deficiency response in the plant under iron stress (Peiffer et al., 2012), although what that response specifically entails remains elusive. Isolines Clark (PI 548533) and IsoClark (PI 547430) (which differ in allelic state at the Gm03 locus), show exceptional differences in gene transcription throughout the genome under iron stress (O'Rourke et al., 2007, 2009). Part of the response appears to involve transport of citrate-iron compounds in the stem (Rogers et al., 2009), discussed later in detail. Discovery of the Gm03 bHLH transcription factor has led to the discovery of additional soybean bHLH transcription factors involved in regulating the response to iron deficiency as well (Li et al., 2018).

Although many IDC resistance QTL have been mapped (Table 1, Figure 3), the physiological bases of iron efficiency associated with these loci are rarely identified in soybean. An exception to this is the characterization of an iron efficiency locus identified on chromosome 20 (Gm20), first identified by Lin et al. (1997). This locus was later found to be associated with seed-iron accumulation, indicating a physiological basis for resistance to IDC (King et al., 2013). While King et al. (2013) found that genotypes carrying the resistance allele at the Gm20 locus had higher average seed-iron content under iron-sufficient soil conditions, the way in which increased iron is deposited within the seed has not been investigated. It may be possible to speculate on the mechanism of iron efficiency in cases such as this using sequence information and gene annotations. The improved understanding of how iron efficiency is achieved may greatly enhance the ability to detect gene candidates within the Gm20 locus. Colocalizing other iron efficiency traits with previously discovered IDC resistance loci may be advantageous in better solidifying our understanding of iron efficiency in soybean and the development of IDC-resistant cultivars. To this end, an in-depth discussion of iron physiology in soybean is warranted in this review.

4 | PART III: PHYSIOLOGY OF IRON DEFICIENCY IN SOYBEAN

4.1 | Root acquisition of iron

Previous sections have addressed the importance of soil characteristics and microbial communities in iron acquisition, while this section addresses the importance of root function for the transport of ferrous iron into the plant. While Strategy II plants (graminaceous monocots) release siderophores into the rhizosphere, which can bind Fe^{3+} and transport the entire complex into the root, soybean must ultimately rely on reduction of Fe^{3+} to Fe^{2+} (Marschner & Römheld, 1994).

Proton extrusion has been well characterized as a mechanism to acidify the rhizosphere and increase iron uptake (Marschner & Römheld, 1994). The effectiveness of this mechanism is greatly reduced in calcareous soils with the high buffering capacity of carbonates, resulting in little to no pH change (Figure 1b). In controlled conditions, Zocchi et al. (2007) found that soybean had little ability to lower the pH of the rhizosphere but instead increased extrusion of citrates, amino acids, phenolic compounds, and organic carbon. These compounds can act to disassociate Fe^{3+} from bonds with other compounds in the soil to improve iron uptake (Figure 1c).

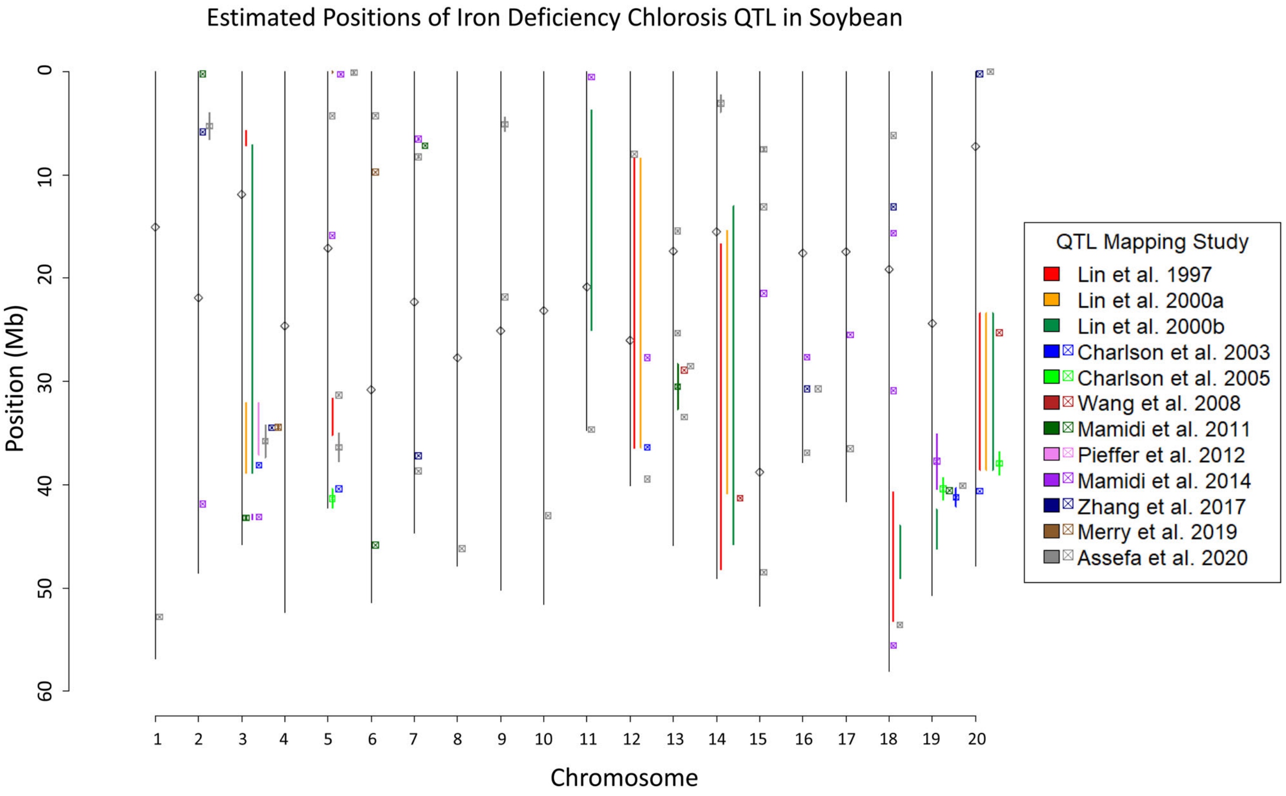

FIGURE 3 Estimated quantitative trait loci (QTL) intervals of iron deficiency chlorosis (IDC) mapping studies. Interval (indicated with a colored line) or significant single nucleotide polymorphism (SNP) (indicated with a boxed "x") positions from previous IDC QTL mapping studies were estimated using marker information and the Wm82.a2.v1 soybean reference genome assembly. For Lin et al. (1997, 2000a, 2000b), Charlson et al. (2005), and Pieffer et al. (2012), QTL interval positions were taken from soybase.org iron efficiency QTL data estimates. The estimated regions or SNPs were then projected onto the 20 chromosomes of soybean located on the *x* axis. The *y* axis indicates genomic position in megabases (Mb). Several loci appear to overlap from multiple studies, such as on Gm03 (loci containing the major effect QTL), Gm05, Gm12, Gm13, Gm14, Gm19, and Gm20

4.1.1 | Flavonoids

The importance of the flavonoid class of phenolic compounds to iron acquisition in plants was reviewed extensively by Cesco et al. (2010). In many species, flavonoids are highly responsive to iron deficiency conditions in the soil and act to reduce iron, thus increasing mobility. In soybean, several flavonoids have been identified as root exudates during soybean growth (d'Arcy-Lamenta, 1986; Graham, 1991; Römheld & Marschner, 1986; Zocchi et al., 2007) and in nodule formation (Liu & Murray, 2016; Skorupska et al., 2017; Subramanian et al., 2007); however, there is a lack of research on the role of flavonoids in soybean IDC. More research on the role of flavonoids in improving IDC should be conducted for two reasons. First, flavonoids are widely accepted as important root exudates in iron deficiency responses in a range of species (Cesco et al., 2010). Second, plants use flavonoids as key signaling molecules to bacteria (Abdel-Lateif et al., 2012; Liu & Murray, 2016; Römheld & Marschner, 1986). The signaling of flavonoids to potential iron-reducing bacteria or fungi could have profound effects on iron acquisition in soybean.

4.1.2 | Iron reductase

Iron reductase enzymes are crucial for the uptake of Fe^{2+}. Once Fe^{3+} is released from iron oxides or other chemical bonds, it can be reduced at the root surface by iron reductase and transported into the root. A hydroponics-based experiment in common bean (*Phaseolus vulgaris* L.) has shown variation for iron reductase activity under iron stress (Blair et al., 2010). A similar study conducted in soybean could be beneficial for identifying QTL or genes to alleviate IDC symptoms. Transgenic overexpression of a ferric reductase gene (*AtFRO2*) in soybean resulted in upregulation of iron transporting citrates and malates (Vasconcelos et al., 2014). Iron levels were also increased within roots, pod walls, leaves, and seeds of the transgenic plants compared with wild-type plants (Vasconcelos et al., 2014). While promising, this study was conducted in hydroponic conditions where total iron amount (but not availability) was reduced. Further experiments would be needed to identify whether this approach would be feasible in high-pH calcareous soils.

4.2 | Iron in the nodule

As a legume species, soybean engages in a symbiotic relationship with rhizobia in which atmospheric nitrogen is fixed by the rhizobia in exchange for nutrients and safety provided by the soybean plant. Nitrogenase, the enzyme that fixes atmospheric nitrogen, is highly sensitive to oxygen (Gallon, 1981). However, oxygen is also important for respiration in the rhizobia, which supplies the massive amount of energy needed to fix nitrogen. To circumvent this, leghemoglobin acts as an oxygen transporter to reduce the concentration of oxygen near nitrogenase while still allowing it to be available for respiration (Appleby, 1984). Both leghemoglobin and nitrogenase require iron as a constituent (Figure 1d). Iron is used for binding and transporting oxygen in leghemoglobin and as an electron transporter in nitrogenase (Guerinot, 1991). Therefore, nodules rely on a large supply of iron from the roots for effective nitrogen fixation. In fact, when the iron transporter gene *fegA* is mutated in rhizobia, nitrogen fixation does not occur normally (Benson et al., 2005). This is most likely because nitrogen fixation cannot occur without iron, and the soybean plant responds by minimizing inputs to the nodule.

For iron to enter the rhizobia, it must first cross the symbiosome membrane (SM), which physically separates the plant and bacterium (Guerinot, 1991) (comprehensively reviewed by Moreau et al. (1998)). While iron is transported through the SM as both Fe^{2+} and Fe^{3+}, the rate of transport for Fe^{2+} is much faster (Clarke et al., 2014), indicating the possible importance of reducing iron near the SM. In common bean, nodules were found to have an abundance of proton pumps and the iron transporter protein IRT1 (IRON-REGULATED TRANSPORTER 1) is located on the surface and within the nodule (Slatni et al., 2012) (Figure 1d). This is highly beneficial for accumulating iron where it will be used and for reducing iron that reverted to Fe^{3+} after entering the root or nodule. Proton pumps and IRT1 are also highly upregulated in the nodule under iron stress (Slatni et al., 2012). These physiological mechanisms help to ensure a steady iron supply for nitrogen fixation. The ferrous iron transporter Glycine max divalent metal transporter 1 (GmDmt1) was discovered as a major iron transporter across the SM (Kaiser et al., 2003) (Figure 1d). GmDmt1 was found to be most highly expressed in developing nodules. As iron is not depleted within rhizobia during nitrogen fixation, it is possible that iron is accumulated early in nitrogen fixation and then maintained within the bacteroid throughout the life of the soybean plant. An experiment could be designed to track the cycling and retention time of iron in the nodule using Fe-57, a stable iron isotope that can be used to trace iron transport in soybean (Oliveira et al., 2014). Another recently discovered transporter of iron across the SM, GmVTL1, was found to be expressed in mature nodules (Brear et al., 2020). The use of multiple genes for iron transport most likely reflects the tight regulation and evolutionary importance of iron homeostasis in both the plant and rhizobia bacteria.

It is unclear from the literature whether nitrogen fixation or photosynthesis is first effected by iron stress. It is logical that nodulation may receive a greater portion of acquired iron during iron stress, as nodules would be one of the first tissues to come into contact with mineral nutrients after transport into the roots, whereas iron must be transported into stems and then leaves for photosynthesis. This hypothesis could be tested with a simple physiological experiment looking at iron partitioning in the nodules and leaves during iron stress. Nitrogen fixation levels, as well as chlorophyll content (and resulting photosynthetic levels), could also be monitored to better assess the physiological response to low iron in both systems on a temporal level. An experiment such as this could also characterize a possible negative feedback loop in which reduced chlorophyll production because of low iron results in less photosynthate produced for nitrogen fixation, resulting in a subsequent reduction in nitrogen fixation.

4.3 | Iron transport and partitioning in the stem

Strategy II plants uptake iron in the form of complexes with siderophore compounds that can be directly transported into the stem. As a Strategy I plant, soybean primarily transports iron in the stem in complexes with nicotianamine or citrate (Figure 1d). In a comparison of six species, soybean was found to have the highest levels of both nicotianamine and citrate in xylem sap (Ariga et al., 2014), suggesting that these compounds may be important for iron transport.

Constitutive expression of the barley nicotianamine NA synthase 1 (*HvNAS1*) in transgenic soybeans greatly increased nicotianamine production, nearly doubled seed-iron concentration, and provided increased resistance to IDC on calcareous soil in a controlled environment (Nozoye et al., 2014). While the transgenic plants appeared to grow normally (Nozoye et al., 2014), soybean yield was not measured. Increasing transport of iron away from the roots at a faster rate may stimulate an iron deficiency response in roots. It is also possible this result is caused by a reduction in iron transport to the nodules because of increased movement of iron into the stem. In this study, iron accumulation in the seed was increased with increased nicotianamine production (Nozoye et al., 2014), suggesting a connection may exist between nicotianamine and the increased seed-iron QTL on Gm20

(King et al., 2013). Transgenic approaches may be successful in improving resistance to IDC in soybean; however, the costs associated with deregulating such transgenic varieties may deter their use.

The transporter proteins of citrate have been found to be important in iron efficiency in soybean (Rogers et al., 2009). The Multidrug and Toxin Efflux (MATE) protein ferric reductase defective 3 (FRD3) has been identified in *Arabidopsis thaliana* (L.) Heynh. as a transporter of citrate into the xylem, with defective mutants accumulating Fe-citrate compounds within the root (Durrett et al., 2007). Two FRD3 homologs identified in soybean showed high expression in an iron-efficient line (Clark) and low expression in an iron-inefficient near-isogenic line (IsoClark) (Figure 1e). Clark had much higher levels of citrate in the xylem than IsoClark, most likely attributable to the increased expression of the citrate transporter (Rogers et al., 2009). Other MATE genes have been identified as important for iron homeostasis in *Arabidopsis* as well. A MATE protein localized to the golgi apparatus, Bush and Chlorotic Dwarf 1 (BCD1), hyperaccumulated iron within the golgi when overexpressed. This hyperaccumulation resulted in leaf chlorosis and senescence throughout the plant (Seo et al., 2012). A similar result occurred with the overexpression of the MATE gene *Early Leaf Senescence-1*(ELS1) in *Arabidopsis* (Wang et al., 2016). Interestingly, both of these genes appear to have little homology with FRD3.

Lack of movement of iron from the roots and into the shoots and leaves has been identified as a characteristic of iron-inefficient soybeans (Santos et al., 2015). Defective nicotianamine and citrate transporters in the shoot may be prevalent in soybean germplasm. An intriguing question is why these inefficient genotypes can transport iron from their roots into their shoots and leaves when grown in soils and conditions that do not induce IDC. Gene expression studies using Clark and IsoClark have indicated that IsoClark fails to respond to iron stress (O'Rourke et al., 2007, 2009). As a result, cell repair genes were activated to regulate the iron stress (Santos et al., 2015). Iron-inefficient genotypes with this stress response could be sequestering iron in their roots to maintain homeostasis. Such a response would be beneficial if iron became available to the plant later in the season, keeping cells in the roots healthy enough to transport iron into the plant when conditions improve.

4.4 | Iron deficiency in leaves

Iron deficiency is visually characterized by interveinal chlorosis of the leaves (Figure 1f). Under iron-deficient conditions, chlorophyll content and carotenoids in leaves are decreased, although only minor decreases are seen for carotenoids (Abadia et al., 1999). A reduction in photochemical efficiency was also found (Abadia et al., 1999), as is expected with reduced chlorophyll content. Iron is necessary for chlorophyll synthesis, most likely as a precursor (Pushnik et al., 1984). Iron also plays an important role in the photosynthetic electron transport chain. With reduced iron available to carry out electron transport, excess light energy intercepted could generate reactive oxygen species and damage the leaf (Asada, 2006; Zhao, 2018).

A metabolomics study has been conducted on the leaves of soybean cultivars that were iron efficient or inefficient (Lima et al., 2014). A key finding in this study was a reduction in citrate levels in leaves of iron efficient and inefficient genotypes, again illustrating the importance of this compound in iron transport. Multiple amino acids (alanine, asparagine, threonine, and valine) were found at higher levels in iron-deficient leaves, as well as increased compounds involved in oxidative stress protection (Lima et al., 2014). Iron-deficient leaves may be damaged by intense levels of photosynthetically active radiation because of reduced chlorophyll content.

While it is often assumed that transpiration in plants is necessary to transport minerals from roots to leaves, mineral transport has been shown to occur regardless of transpiration, most likely through some form of active transport (Tanner & Beevers, 2001). In fact, iron has been shown to accumulate in leaves more efficiently under high relative humidity (RH) (and thus lower transpiration) in an iron-efficient soybean genotype, while no differences were seen in an iron-inefficient genotype (Roriz et al., 2014). While the physiological basis of this response has not been elucidated, one possibility is that increased photosynthetic activity, resulting from reduced stomatal closure in high RH, provides additional energy for the iron-efficient genotype to overcome iron stress. During high RH, water losses in the leaf would be reduced, allowing for longer opening of the stomata and increased CO_2 uptake (Mortenson & Gislerod, 1990) and thus increased photosynthetic activity. While it is impossible to control humidity at the field level for management of IDC in soybean, it is important to consider RH when conducting studies in controlled environments and the effects it may have on IDC symptoms.

4.5 | Iron in the seed

Developing soybean seedlings use nutrients stored in the seed before roots and photosynthetic capacities are established. Sufficient iron is usually stored within the cotyledons to supply the emerging soybean plant until the third vegetative (V3) growth stage, corresponding to the full emergence of the third soybean trifoliate. For this reason, iron-deficiency symptoms are often not visualized until the V3 growth stage or later. Iron-efficient varieties have been found to have higher seed-iron content than inefficient varieties when grown in either iron-sufficient or iron-deficient conditions (Wiersma, 2005, 2012). Larger stores of initial iron within the seed may allow for increased initial plant growth before the onset of iron deficiency (Figure 1g).

Iron is loaded into the seed via both xylem and phloem (Grillet et al., 2014). During seed fill, iron acquired from the roots is transported directly to the seed via the xylem (Blair et al., 2010). It can therefore be assumed that nicotianamine and citrate play an important role in this iron loading into the seed, as evidenced by Nozoye et al. (2014). Sinclair and de Wit (1976) classified soybean as a self-destructive plant, meaning that nearly all the nutrients stored within root, stem, and leaf tissues are remobilized and loaded into seeds, causing plant senescence. An iron isotope study has indicated that perhaps nearly half of iron loaded into seeds is remobilized from the nodules, while the remaining iron is transferred from the leaves and stem as well as directly loaded from the soil (Burton et al., 1998).

The use of iron seed content as a proxy of IDC resistance has been suggested (Blair et al., 2010; Wiersma, 2005, 2007, 2012) and may be of use in breeding programs. One major downfall of these studies is that they sampled a small number of soybean cultivars, and proper analyses on the response to indirect selection have not been conducted. To better establish a relationship between high seed-iron levels and resistance to IDC, a large-scale study should be conducted to analyze the seed-iron content of large populations of breeding lines representing the germplasm basis of a typical cultivar development program across environments with ranges in IDC stress.

5 | CONCLUDING REMARKS

As a globally valuable crop for both its oil and protein, the need for highly productive soybean under many different abiotic stresses is vital. High-pH soil conditions around the globe can result in severe yield losses because of iron deficiency, although the extent of the severity is dependent on transient factors such as soil moisture and nitrate content. The aim of this review was to analyze research in soybean iron deficiency phenotyping, genetics, and physiology to reveal connections between these areas and facilitate further discoveries.

Iron deficiency in soybean results in physiological changes in all tissues; however, nearly all phenotyping of this trait, to date, has focused on leaf chlorosis. This narrow view may explain the popularity of foliar iron applications, as these may elicit some recovery of the chlorosis, while being largely ineffective for yield recovery (Chatterjee et al., 2017; Franzen et al., 2004; Goos & Johnson, 2000; Lingenfelser et al., 2005). As most foliar-applied iron sources are not transported from the leaves (Rodriguez-Lucena et al., 2010), other plant tissues could remain under iron stress. The high iron required for proper nitrogen fixation in the nodules makes this tissue especially vulnerable if foliar applied iron is sequestered in the leaves and not transported in the roots. Because soybean seeds are ∼40% protein, persistence of iron deficiency in the nodules would logically result in yield loss regardless of leaf recovery.

Advances in high-throughput phenotyping allow for screening of more plots, which helps to overcome issues of field heterogeneity by enabling increased replication. The ability to phenotype IDC nurseries at higher frequency could reveal physiology related to iron deficiency recovery that cannot be observed when only scoring plots once or twice a year. Moving beyond an ordinal rating system and developing a more quantitative score using pixel ratios or another metric may also allow for the discovery of smaller-effect QTL in the future.

Breeding for IDC resistance is still the most practical approach in combating iron deficiency in soybean. While many IDC resistance QTL have been identified in soybean (Table 1, Figure 3), only a handful of these loci have been studied in detail, with the exceptions of the major-effect QTL on Gm03 (Pieffer et al., 2012) and the QTL on Gm20 that colocalized with seed-iron content (Lin et al., 1997; King et al., 2013). The Gm03 QTL appears to regulate the iron deficiency response in soybean, and differences in the transcriptomes of near-isogenic lines for the locus have been conducted (Pieffer et al., 2012). It may be possible to connect differentially expressed genes with physiological responses outlined in this review as was done with the MATE citrate transporters by Rogers et al. (2009). Such a connection may be made with the Gm20 QTL as well. The literature reviewed consistently pointed toward a correspondence between increased nicotianamine production and increased deposition of iron within the seed. It therefore seems logical that nicotianamine production or transport may underlie the physiological efficiency of the Gm20 QTL. Screening diverse soybean genotypes for increased nicotianamine production could also provide new breeding material for iron-efficient cultivar development.

Nicotianamine and citrate were identified in many studies as important to iron efficiency. In fact, all soybean genes directly identified in iron efficiency had some relationship to these two iron-binding compounds. Overexpression of a barley nicotianamine synthase resulted in reduced chlorosis in controlled conditions and increased deposition of iron in the seed (Nozoye et al., 2014). Constitutive expression of *AtFRO2*, a ferric reductase, resulted in increased levels of citrate production (Vasconcelos et al., 2014). Expression differences potentially caused by the Gm03 bHLH transcription factor affected citrate transport in near-isogenic lines (Rogers et al., 2009). It may be fruitful in future gene cloning efforts to consider the nicotianamine and citrate transport systems when searching for candidate genes.

While formidable, further investigation into all areas of soybean iron deficiency will provide a more complete insight into this nutrient stress. This review identified certain areas of soybean iron deficiency research as underdeveloped, such as understanding the role of root exudates in the iron deficiency response and the role of microbes in iron efficiency. Advancements in controlled-environment experimentation for iron deficiency will perhaps alleviate research difficulties in these areas. Identifying mycorrhizal fungi that can acquire iron through siderophores and then transport that iron into soybean could be a breakthrough in the era of biologicals research. With millions of acres of soybean production annually stressed by IDC, solutions in these areas of soybean iron deficiency research will be celebrated.

ACKNOWLEDGMENTS

This work was supported by the United Soybean Board project #2120-172-0145. Dr. Ryan Merry was supported by a United Soybean Board Graduate Fellowship (project #1620-732-7228).

AUTHOR CONTRIBUTIONS

Ryan Merry: Conceptualization; Writing-original draft. Austin Dobbels: Writing-original draft. Walid Sadok: Writing-review & editing. Seth Naeve: Writing-review & editing. Robert Stupar: Conceptualization; Writing-review & editing. Aaron J. Lorenz: Conceptualization; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Merry, R., Dobbels, A. A., Sadok, W., Naeve, S., Stupar, R. M., & Lorenz, A. J. (2022). Iron deficiency in soybean. *Crop Science*, *62*, 36–52. https://doi.org/10.1002/csc2.20661