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Original Research Paper

Morpho-physiological parameters associated with chlorosis resistance to iron deficiency and their effect on yield and related attributes in potato (*Solanum tuberosum* L.)

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ABSTRACT

The aim of the study was to assess genotypical differences over different stages for morpho-physiological parameters associated with iron (Fe) deficiency and their effect on yield. The factorial pot experiment was comprised of two major factors, i) soil-Fe status of natural vertisol [Fe-sufficient and Fe-deficient soils], and ii) genotypes [CP-3443, CP- 4105, CP-3486 and CP-4069] with differential iron-induced deficiency chlorosis (IDC) response. Data were recorded and associations between different traits were estimated. Under Fe-deficient soil, tolerant genotype (CP-3443) recorded significantly higher chlorophyll content, peroxidase activity in leaves and better yield compared to susceptible genotypes which verified usefulness as IDC tolerant potato genotypes characteristics.

Key words: Correlation, Iron-deficiency chlorosis, Morpho-physiological parameters; Potato and Yield

INTRODUCTION

Iron (Fe), one of the essential micronutrients for the growth and development of all living organisms, its deficiency is a serious problem in both agriculture and human nutrition. Iron is considerably less soluble in soils with a pH value of 8 than Zn or Mn; thus, inorganic Fe makes relatively little contribution to the Fe nutrition of plants in calcareous soils (Singh *et al.*, 2004). Fe level varies with the type and depth of the soil, ranging from 0.2% to 55% (20,000 to 550,000 mg kg⁻¹), and the highest concentration is found at 2–15 cm (Mahender *et al.*, 2019). For instance, the total Fe content in Indian soil ranges from 0.4% to 27.3% (40,000 to 273, 000 mg kg⁻¹), but that accessible to the plant is extremely variable, from 0.36 to 174 mg, which depends on the soil, plant, and environmental factors (Mahender *et al.*, 2019). Calcareous soils may contain high total Fe levels but are not accessible to the plants, because iron forms water-insoluble hydroxides and oxides, and/or Fe carbonates-bicarbonates. Thus, high bicarbonate ion concentration leads to iron-induced deficiency chlorosis (IDC) by suppressing iron uptake and/or translocation in plants (Li-Xuan *et al.*, 2005).

Potato (*Solanum tuberosum* L.) is a multipurpose crop and is the 4th most important crop contributing to the world's food requirement next to rice, wheat and maize with a global production of 388 MMT during 2017 (FAOSTAT, 2019). Potato crop is sensitive to non-optimal iron content in the substrate and marked decreases in tuber quality and yield were reported with reduced biomass, chlorophyll concentrations and alterations in enzyme activities (peroxidase, catalase and acid phosphatase) under Fe-deficient stress (Chatterjee *et al.*, 2006). Susceptibility to Fe chlorosis is based on a plant's response to Fe deficiency stress and this is genetically regulated (Simko *et al.*, 2008). IDC response is usually assessed by chlorophyll content in potato (Chatterjee *et al.*, 2006) and also other legumes like soybean, dry bean etc (Samdur *et al.*, 1999; Samdur *et al.*, 2000). Higher chlorophyll content indicates a lower occurrence of chlorosis in leaves. Iron deficiency was also found to decrease the production of oxidative stress-related enzymes such as peroxidase in several plant species and is due to less Fe concentrations in Fe-deficient leaves (M'sehli *et al.*, 2014).



Developing micronutrient-efficient genotypes can be a quick, fast and successful way to resolve soil micronutrient disorders and improve human health. In previous studies, we have screened genotypes at seedling stage with the intent to identify IDC tolerant potato genotypes. IDC tolerant genotypes had registered a significantly lower reduction in plant height, SPAD value, chlorophyll content, and peroxidase level under Fe-deficient condition. However, the association of morpho-physiological parameters under iron deficiency and their effect on yield in potato genotypes has not been examined. Given that genetic variability for IDC responses exist among potato genotypes. The objective of the study was to access genotypic differences for different morpho-physiological and biochemical parameters associated with IDC tolerance across growth stages and their yield under natural vertisol (normal and calcareous) soils using pot studies.

MATERIALS AND METHODS

Based on our earlier study (Challam *et al.*, 2021), 15 potato genotypes were evaluated for responses to Fe deficiency stress under controlled aeroponic condition. Potato genotypes with differential IDC response (tolerant: CP-3443; moderately tolerant: CP- 4105; moderately susceptible: CP-3486 and susceptible: CP-4069) were further selected for the pot experiment under net house condition at ICAR-Central Potato Research Institute, Regional Station, Shillong. Two soil type viz. Fe- sufficient and Fe-deficient were collected from two different sites Upper Shillong (25.54° N, 91.85° E) and Nongrah (25.57° N, 91.93° E), respectively. Soil samples were collected from identified sites by using hand auger at the soil depths of 0 - 30 cm by adopting random sampling procedure as described by Kalra, 1988. *In vitro* propagated potato plantlets of the genotypes were planted in twenty-five-centimetre plastic pots were filled with vertisol soils (pH: 4.3) having available Fe 7.25 mg/ Kg (DTPA extractable) for Fe sufficient plants whereas Fe deficient plants were filled with calcareous vertisol soil (pH: 8.6) having available Fe 4.53 mg/ Kg (DTPA extractable) (Table 1). All the major nutrients (N, P, K) were supplied in the form of urea, diammonium phosphate, and muriate of potash fertilizers according to the prescribed

dosage. Micronutrients such as Zn, Mn and Mg were added in the form of sulphates to avoid the difficulty of concurrent symptoms of Fe deficiency. One-month old *in vitro* plantlets were transplanted into pots and were irrigated with de-ionized water as and when required. The experimental design was conducted with completely randomized design with four replication per treatment. Throughout the duration of the experiment, all the necessary management practices including pest and diseases control were carried out to ensure good growth and development. Plant growth parameters associated with Fe deficiency were assessed as described below. All the parameters were recorded at different intervals 30, 60, 90 and 120 days (harvest) after planting. The morpho-physiological and biochemical parameters such as plant height, number of main stems, number of leaves, chlorophyll content and peroxidase were recorded across the growth stages. Number and weight of tubers along with root: shoot ratio and were estimated after harvest stage.

All the parameters were recorded at different intervals 30, 60, 90 and 120 days after planting. The growth parameters such as plant height (round level to the tip of the last opened leaf), total number of stem and number of leaves were recorded. Physio-biochemical parameters viz. SPAD values (Boodi *et al.*, 2016), chlorophyll and peroxidase (Chatterjee and Chatterjee, 2003) were measured across the growth stages. To obtain tuber yield plants were maintained till maturity in each treatment. Number and weight of tubers along with root: shoot ratio were estimated after harvest stage.

Table 1: Properties of the initial soil used for pot experiment

Soil properties	Fe-sufficient soil	Fe-deficient soil
Soil pH	5.73	8.13
Electrical conductivity (dS m-1)	0.31	0.23
Organic carbon (%)	1.26	1.67
Free CaCO ₃ (%)	5.70	10.6
DTPA extractable-Fe (mg kg-1)	17.5	4.53

Mean squares for IDC tolerant associated traits, yield and it related traits were estimated with the aid of AGRES statistical package. Comparison between the treatments was made by using common least significant difference at ($P = 0.05$). Pearson's correlation coefficient between IDC tolerance associated traits across four stages, yield and it related traits were estimated for Fe-deficient soils and significance was tested ($P = 0.05$ and 0.01).

RESULTS AND DISCUSSION

Morpho-physiological parameters

Growth and development in plants are a consequence of excellent coordination of several processes operating at different growing phases of plant. Plant height, total number of stem and number of leaves are important morphological character representing vigour of the plant during the growth and development. These growth indicators were measured periodically during the crop period. Being an essential micronutrient, Fe promoted the growth of all potato genotypes when grown at Fe-sufficient soil. Significant difference was observed for plant height, total main stem and number of leaves between the genotypes under different soil condition at all the phenological stages viz., 30, 60, 90 and at harvest stage. The comparison between Fe-sufficient and Fe-deficient soil showed significant reduction in plant height and number of stems under Fe-deficient soil (Table 2). This reduction may be attributed to reduced cell division, meristematic activity in apical tissue, expansion of cell and formation of new cell wall under Fe starvations (Boodi *et al.*, 2016). The reduction of plant height during stress can serve an advantageous purpose for plants to reduce transportation distances, which can help to efficiently distribute water, nutrients and assimilates in plants (Aliche *et al.*, 2020). On the contrary, number of leaves was found to increase as many side shoots were observed as a result of Fe-deficiency in Fe-deficient soil. Tolerant genotype CP-3443 showed least reduction in number of leaves i.e., a greater number of leaves compared to other genotypes although lesser in plant height. Boamponsem *et al.*, (2017) made a similar observation under Fe-deficient conditions, Fe-efficient potatoes have a greater number of leaves

due to shorter internodal distance and stem height. This may be influenced by genetic characteristics of genotype related to its ability to grow better and give the higher yield.

Chlorophylls, the plant pigments responsible for harvesting solar energy and converting into required chemical energy, exhibit a differential pattern in their accumulation in crop plants. Gradual increase in chlorophyll was observed from 30 to 90 days, but decline there onwards until harvest was evident for all the genotypes in Fe-deficient soils. The higher chlorophyll content in plants under Fe-deficient soil for initial growth periods could be attributed to the possible acquisition of appreciable Fe stores during prior growth of *in vitro* plants on sufficient Fe medium and available Fe in soil. Hence, plants were capable of sustaining chlorophyll biosynthesis during early stages on exposure to low Fe supplies. After 90 days, however, the plants could not acquire sufficient Fe from deficient soil led to inhibition of chlorophyll synthesis. The interaction effect was found significant across the growth stage in chlorophyll (a, b and total) (Table 2). Chlorophyll (a, b, and total) were significantly higher among tolerant followed by moderately tolerant genotype, but susceptible ones showed significantly lesser content during all the four crop growth stages under Fe-deficient soils (Table 2). The ability to absorb Fe from the soil under Fe-deficient soil as evident in tolerant and moderately tolerant genotypes helps to produce more chlorophyll pigments, since most of the leaf iron is found in chloroplast, primarily in photosynthetic electron transport chain complexes that comprise about 60 % of the total content of the leaf iron (Terry and Abadia, 1986).

Peroxidase (POD) content underwent a significant increase from 30, 60, 90 days but decline there onwards until 120 days was evident for all the genotypes under both soil conditions. However, Fe-deficient induced a noticeable decrease in the values of peroxidase in comparison to the Fe-sufficient condition (Table 2). The POD is a heme-containing enzyme and therefore its activity and/or synthesis are probably affected by iron deficiency. In the current study, decreased POD activity under limited Fe supply

Table 2 : Mean performance of potato genotypes for morpho-physiological parameters in Fe-sufficient and Fe-deficient soils across growth stages

Time interval		30 days		60 days		90 days		120 days (Harvest stage)		Mean		Change (%)
Trait	Genotype	C	T	C	T	C	T	C	T	C	T	
Plant height (cm)	CP-3486	26.3	21.8	35.6	29.3	48.9	45.9	58.8	50.9	42.4	36.97	13.4
	CP-4069	30.3	28.3	44.6	41.2	55.8	51.8	63.9	58.8	48.65	45.02	8.0
	CP-3443	28.5	23.3	40.9	38.9	52.9	48.4	60.6	54.6	45.72	41.30	9.9
	CP-4105	25.5	20.4	33.4	28.4	50.4	45.2	56.5	48.7	41.45	35.67	13.8
Mean		25.55		36.53		49.91		56.6				
LSD [#]		0.53**	0.37**	0.94**	0.66**	1.15**	0.81**	1.49**	1.06**			
C × T ^S		0.748*		NS		NS		NS				
Total main stem	CP-3486	1.3	1.1	2.2	2.0	2.9	2.2	3.3	2.5	2.42	1.95	24.2
	CP-4069	1.0	1.0	2.0	2.0	2.5	2.4	3.0	2.4	2.12	1.95	20.0
	CP-3443	1.2	1.0	2.3	2.0	2.4	2.3	2.4	2.3	2.07	1.90	4.2
	CP-4105	1.2	1.1	2.1	1.8	2.8	2.3	3.2	2.2	2.32	1.85	31.3
Mean		1.11		2.05		2.47		2.66				
LSD [#]		0.02**	0.01**	0.04**	0.03**	0.05**	0.03**	0.04**	0.04**			
C × T		0.022**		0.058**		0.068**		0.074**				
Number of leaves	CP-3486	15.2	15.0	30.4	36.4	39.2	50.2	56.0	67.9	35.2	42.37	21.3
	CP-4069	15.7	15.9	25.7	29.3	36.1	45.8	51.7	59.7	32.3	37.67	15.5
	CP-3443	15.0	14.8	24.5	28.5	33.1	47.1	50.6	63.0	30.8	38.35	24.5
	CP-4105	16.1	15.7	32.2	38.6	40.6	57.6	58.7	71.5	36.9	45.85	21.8
Mean		15.42		30.69		43.71		59.88				
LSD [#]		0.31*	NS	0.65**	0.46**	0.88**	0.62**	1.67**	1.61**			
C × T ^S		NS		0.921*		1.691**		NS				
Chl-a (mg/g FW)	CP-3486	0.8	0.7	0.9	0.6	1.0	0.7	0.9	0.6	0.9	0.65	33.3
	CP-4069	0.9	0.6	1.0	0.6	0.9	0.8	0.7	0.5	0.87	0.62	28.6
	CP-3443	0.9	0.8	1.1	1.0	1.2	1.0	1.1	1.0	1.07	0.95	9.10
	CP-4105	0.9	0.8	1.0	0.8	1.1	0.8	1.0	0.8	1.0	0.80	20.0
Mean		0.799		0.875		0.937		0.825				
LSD [#]		0.02**	0.01**	0.02**	0.01**	0.02**	0.02**	0.01**	0.01**			
C × T ^S		0.025**		0.024**		0.032**		0.019**				
Chl-b (mg/g FW)	CP-3486	0.4	0.3	0.5	0.4	0.5	0.3	0.4	0.3	0.45	0.32	25.0
	CP-4069	0.3	0.1	0.4	0.2	0.5	0.2	0.4	0.2	0.4	0.17	50.0
	CP-3443	0.4	0.3	0.4	0.3	0.4	0.4	0.4	0.3	0.4	0.32	25.0
	CP-4105	0.4	0.4	0.5	0.4	0.4	0.2	0.4	0.3	0.42	0.32	25.0
Mean		0.325		0.388		0.362		0.337				
LSD [#]		0.006*	0.005*	0.007*	0.005*	0.006*	0.005*	0.007*	0.005*			
C × T ^S		0.009*		0.011*		0.009*		0.011*				
Total Chl (mg/g FW)	CP-3486	1.2	1.0	1.4	1.0	1.5	1.0	1.3	0.9	1.35	0.97	30.8
	CP-4069	1.2	0.7	1.4	0.8	1.4	1.0	1.1	0.7	1.27	0.80	36.4
	CP-3443	1.3	1.1	1.5	1.3	1.6	1.4	1.5	1.3	1.47	1.27	13.3
	CP-4105	1.3	1.2	1.5	1.2	1.5	1.0	1.4	1.1	1.42	1.12	21.4
Mean		1.122		1.263		1.3		1.162				
LSD [#]		0.025*	0.018*	0.019*	0.013*	0.027*	0.019*	0.026*	0.018*			
C × T ^S		0.036*		0.027*		0.038*		0.037*				
POD (OD/min/mg)	CP-3486	1.2	0.8	1.5	1.2	1.8	1.4	1.3	1.0	1.45	1.1	23.1
	CP-4069	1.7	1.0	2.0	1.5	2.3	1.7	1.7	1.5	1.92	1.42	11.8
	CP-3443	1.4	0.9	1.7	1.4	2.0	1.8	1.4	1.3	1.62	1.35	7.10
	CP-4105	1.1	0.7	1.4	1.1	1.7	1.3	1.3	1.1	1.37	1.05	15.4
Mean		1.1		1.475		1.749		1.325				
LSD [#]		0.025*	0.018*	0.031*	0.022*	0.034*	0.024*	0.027*	0.019*			
C × T ^S		0.036*		0.044*		0.048*		0.038*				

- least significant difference (P = 0.05) for Fe-sufficient and Fe-deficient soils individually, S = common least significant difference for both Fe-sufficient and Fe-deficient soils for treatment comparisons, C – Fe-sufficient soil, T – Fe-deficient soil, LSD – Least significant difference (P=0.05), ** - highly significant, * - significant, Change (%) - % change for mean across four stages between Fe-sufficient and Fe-deficient soils.

may have been due to low activation and/or a reduced production of the POD enzyme. In comparison between genotypes, maximum peroxidase activity was noted in CP-3443 followed by CP-4105, CP-3486 and CP-4069 under Fe-deficient soils (Table 2). This implies during redox homeostasis, the antioxidant system in tolerant genotypes maintains a balance between production and scavenging of reactive oxygen species (ROS); such a balance is critical for the protection of the system against oxidative burst (Balakrishnan, 2000).

Yield components

Under Fe-deficient soil, reduced yield was observed in all genotypes compared to plants grown under Fe-sufficient soil. However, IDC tolerant genotype were found to have better yield and its related traits compared to susceptible genotypes. The decrease in yield under Fe-deficient soil being up to 16% in CP-3443, followed by CP-4105 (20%), CP-3486 (27%) and CP-4069 (28%) of its potential yield under Fe-sufficient (Fig. 1a). Similarly, reduced in number of tubers per plant (13-19%) and average tuber weight (3-12%) were recorded in all four genotypes (Fig. 1b & c). Under Fe-deficient soil, tolerant genotypes have better photosynthetic activity as shown by higher number of leaves and chlorophyll content while registered minimum reduction in number of tubers, average tuber weight and overall yield of plants as compared to susceptible genotypes.

Root architecture plays an important role in Fe uptake. Previous studies (Zou *et al.*, 2013; Li *et al.*, 2015) have shown that most species allocate more biomass to roots at reduced availability of Fe and allocate root biomass in shallow soil horizons, as well as increase root length and grow more and more root hairs and lateral roots; some also produce cluster roots, thereby promoting Fe uptake. Root-shoot ratio is the quantity of plant tissue that supports the quantity of those with growth functions. In case of root to shoot ratio an increase (15%) was observed in tolerant genotypes (CP-3443). A slight reduction of 3% was noted in medium tolerant genotype (CP-4105), whereas a reduction of 8% and 17% was observed in CP-3486 and CP-4069, respectively under Fe-deficient soil (Fig. 1d). Dry matter partitioning to different plant parts was significantly influenced both Fe-sufficient and Fe-

deficient conditions. In the study, tolerant genotypes have more root biomass compared to susceptible genotypes. This may be due to better and deeper root system in the genotypes which help in absorption of nutrient from the deeper and surrounding layer of the soils. Root to shoot ratio clearly exhibit significant difference amongst potato genotypes in both the conditions. Genotypes with a higher root biomass actually compete more effectively for soil nutrients with the ability to produce higher yields, particularly under stress conditions. This is supported by the significant relationship of root to shoot ratio with yield (Table 3).

The available nutrient status of the soil is another important property which support the crop growth while, the crop growth and dry matter accumulation depends upon the ability of the soil to supply nutrients, the nutrients release from the soil in turn depends upon the demand from growing plants. Micronutrients, in particular Fe and Zn, either function as metal components of different enzymes or as functional structural or regulatory cofactors and are therefore connected to photosynthesis and protein synthesis. The concentration of Fe and Zn were estimated in the tubers to know their accumulation. A decrease in concentration of Fe ranges from 7.17 to 12.33% in tubers was observed in all the genotypes under Fe-deficient soil (Fig. 1e). This may be attributed to low expression of genes resulting in low xylem mobility and poor translocation capacity under Fe-deficient soil condition. A similar trend was observed in Zn, a stimulator under Fe-deficient conditions (6.54-10.05%; Fig. 1f)

Associations

A significant positive correlation was observed between morpho-physiological, biochemical and yield parameters in the study (Table 3). Number of leaves showed significant ($P < 0.05$) and positive correlation with parameters like total chlorophyll (0.981) and yield (0.970). While, yield per plant was also highly significant with number of tubers (0.957) and root to shoot ratio (0.973), respectively (Table 3). Under Fe-deficient condition, the genotype CP-3443 was able to produce more leaves which participated directly or indirectly in the manufacturing of more chlorophyll (Boamponsem *et al.*, 2017). Further, their leaves function as efficient photosynthesis structures and

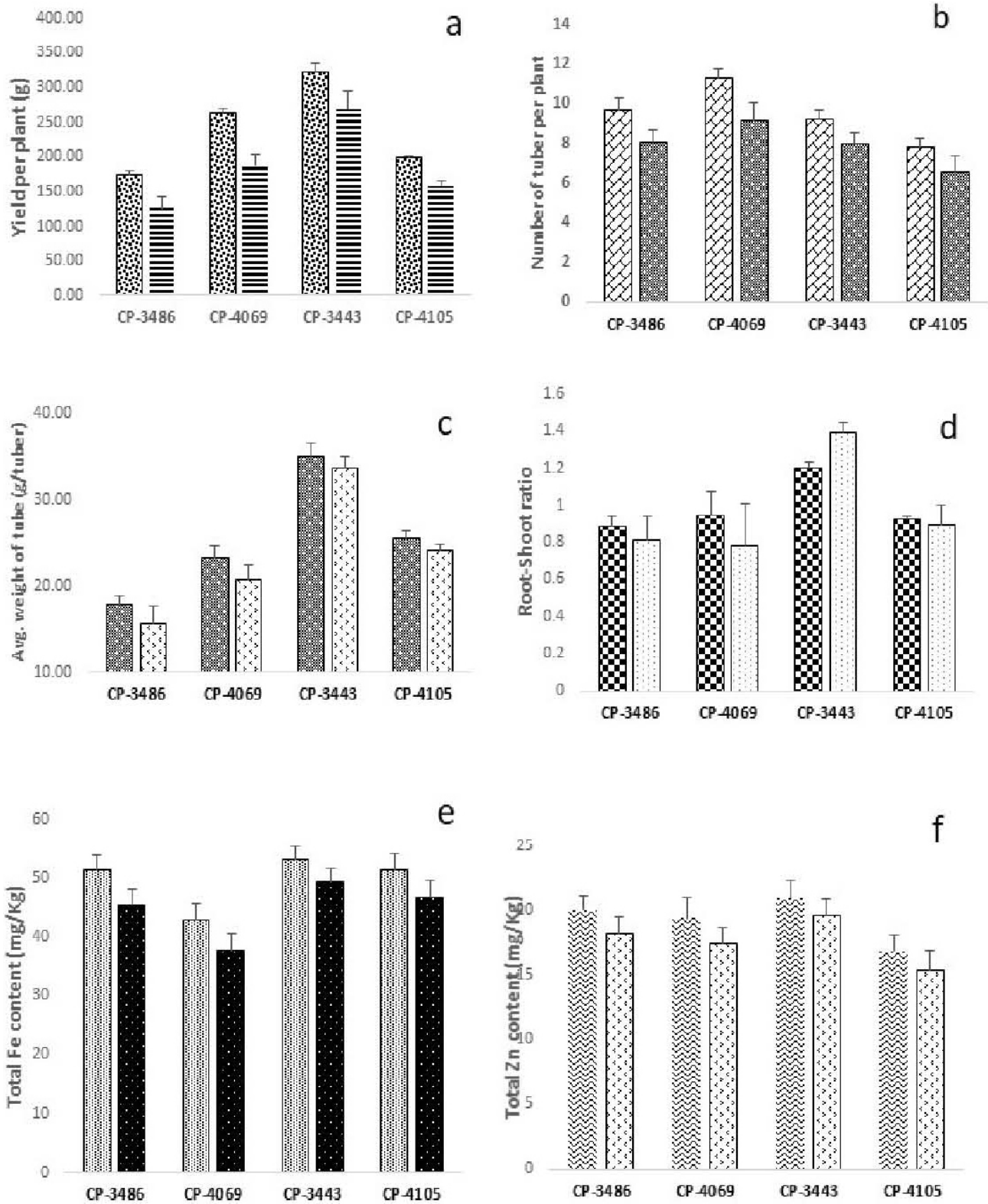


Fig. 1: Difference among IDC resistant and susceptible potato genotypes under normal and calcareous Fe soils for yield and its component traits a) Yield, b) Number of tubers, c) Avg. weight of tuber, d) Root-Shoot ratio, e) Total Fe content, f) Total Zn content. Mean of tolerant and susceptible genotypes considered for comparison; Standard error bar is common for both resistant and susceptible genotypes.

Table 3: Association between mean of morpho-physiological and biochemical parameters across all four stages and yield –related traits

	PH	TMS	NL	TC	POD	Yield	No.Tub	Wt.Tub	R:S
PH	1								
TMS	0.913	1							
NL	-0.542	-0.156	1						
TC	-0.461	-0.059	.981*	1					
POD	-0.027	0.366	0.765	0.867	1				
Yield	-0.352	0.06	.970*	.991**	0.895	1			
No.Tub	-0.358	0.039	0.9	.967*	0.943	.957*	1		
Wt.Tub	-0.914	-0.807	0.497	0.494	0.208	0.376	0.494	1	
R:S	-0.136	0.28	0.888	0.942	.962*	.973*	0.947	0.21	1

PH-plant height, TMS-total main stems, NL-number of leaves, TC-total chlorophyll, POD-peroxidase, No.Tub-number of tubers, Wt.Tub-weight of tubers, R:S-root-to-shoot ratio

* Correlation is significant at P value=0.05 ** Correlation is significant at P value=0.01

produced higher amount of carbohydrates in the plant system registering minimum reduction in number of tubers and overall yield of plants compared to susceptible genotypes (Braun *et al.*, 2016). While the root-shoot ratio indicates that the tolerant genotypes may have efficient root to shoot transport and redistribution of Fe in the plant (Xu *et al.*, 2017). This positive correlation further confirms the involvement of Fe in increasing the photosynthetic apparatus and maintaining the minimal stress in tolerant genotypes to enhance the physiological process under Fe-deficient soils.

CONCLUSION

Under Fe-deficient soil conditions, IDC tolerant genotypes recorded a significantly higher chlorophyll, and peroxidase activity in leaves across all four crop growth stages compared to susceptible genotypes confirming their utility as traits for identification and development of IDC tolerant potato genotypes. Towards developing high-yielding, IDC tolerant potato cultivars for Fe-deficient environments, selection may be practiced for higher tuber weight and root to shoot ratio.

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