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SCREENING FOR IRON DEFICIENT CHLOROSIS (IDC) TOLERANT GENOTYPES IN POTATO (*SOLANUM TUBEROSUM*, L.) UNDER AEROPONIC SYSTEM

CLARISSA CHALLAM[1,2], SOM DUTT[1*], JAGDEV SHARMA[2], TUSAR KANTI BAG[1], MUTHURAJAN RAVEENDRAN[2] AND DURAIALAGARAJA SUDHAKAR[2]

[1]ICAR – Central Potato Research Institute, Shimla 171 001, Himachal Pradesh, India
[2]Department of Plant Biotechnology, Centre for Plant Biotechnology and Molecular Biology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

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Abstract – A study was carried out under controlled aeroponic culture for identifying IDC tolerant potato genotypes. Micro-plantlets of 15 potato genotypes were evaluated for Fe deficiency response. Two different nutrient compositions *viz.*, Fe-sufficient (Fe-EDTA 50 mg/l) and Fe-deficient (Fe-EDTA 0mg/l) media were supplied to the root zone by periodic spraying in fine mist to keep it saturated. Morphoo-physiological and biochemical parameters such as plant height, root length, root-shoot biomass, SPAD, chlorophyll content, peroxidase and Fe content were recorded for each genotype under both conditions. Genotypes were ranked by multivariate cluster analysis. IDC tolerant genotypes, *viz*., CP-1435, CP-3772 and CP-3443 showed less reduction in chlorophyll, SPAD values,peroxidase and leaf Fe content compared to the susceptible genotypes. This work could result in new screening technique which can be utilized in breeding programme for preliminary identification of IDC tolerant potato genotypes.

INTRODUCTION

Despite the fact that iron (Fe) is the fourth most abundant element in the earth's crust, its deciency is by far the most widespread micronutrient deciency and is a serious problem in both human nutrition and in agriculture. Its deficiency affects nearly 2 billion people and the problem is severe enough to cause anemia in over 40% of the world's population (WHO, 2015). Potato is the fourth most important food crop in the world and is considered as the future crop to address global food security and poverty alleviation by FAO, Rome. In today's world, over a billion people consume potato regularly (Barrell *et al.*, 2013). Potato is a good source of vitamin C, vitamin B_6 and a modest contributor of minerals such as magnesium, phosphorus, manganese, zinc and iron in the human diet. The bioavailability of iron in potato can be greater than in cereals and legumes due to the presence of high levels of ascorbic acid up to 46 mg per 100 g tubers fresh weight (Bandana *et al.*, 2015), and low levels of phytic acid, an inhibitor of iron absorption.

Iron is a crucial mineral in the living kingdoms. In plants, it participates in several key chemical processes. For example, Fe is involved in the synthesis of chlorophyll, the vital pigment required for photosynthesis, the most important physiological process in plants. About 80% of Fe in plants is concentrated in chloroplasts (Brumbarova *et al.*, 2015). In addition to its importance to photosynthesis, Fe is involved in cell detoxification. Fe is part of the active centre of the cell defence enzyme system for peroxidase (POD). Iron is sparingly soluble under aerobic conditions, especially under alkaline conditions and calcareous soils, which account to 30% of the world's cultivated soils (Kobayashi *et al.*, 2012) and is a limiting factor for normal plant growth. In order to deal with the limiting amounts of Fe, plants have evolved strategies for Fe acquisition– Strategy-I (reduction-based) and Strategy-II (chelation-based) (Romheld and Marschner, 1986). Strategy-I plants, including the dicots (such as potato) and non-graminaceous monocots, rely on a number of biochemical processes including: (i) acidification of the rhizosphere to solubilise $Fe^{(III)}$ (ii) reduction of the solubilized $Fe^{(III)}$ to $Fe^{(II)}$ by a membrane-bound $Fe^{(III)}$chelate reductase at the root surface, (iii) transport of $Fe^{(II)}$ into the root cells by metal transporters, and for some species possibly (iv) the secretion of flavins to also facilitate the solubilization of ferric iron. Strategy- II plants are unique to graminaceous plants and involve the release of $Fe^{(III)}$-specific phytosiderophores (PS) that can bind $Fe^{(III)}$ with high affinity and subsequent uptake of the $Fe^{(III)}$ phytosiderophore complexes via a specific transport system.

The potato crop is sensitive to both excess and low iron conditions: non-optimal iron content in the soil results in a consequent yield loss and can even deteriorate tuber quality (Chatterjee *et al.,* 2006). Cultivation of iron deficiency chlorosis (IDC) tolerant cultivars is an economically viable option as they perform well under Fe-deficient conditions (Boodi *et al.,* 2016). The identification of IDC tolerant genotypes can lead to the enhancement of crop productivity in calcareous soils. Development of IDC tolerant genotypes/cultivars presents the most important method of controlling Fe chlorosis development in field crops (Vasconcelos *et al.*, 2014). Classical breeding approaches were employed to breed for plants adapted to high lime soils by identification of genotypes tolerant to IDC (Cianzio *et al.*, 2006). However, the major breeding problem is the development of reliable screening procedures that produce reproducible results. In most cases screening has been conducted in field nurseries where environmental factors, including rainfall patterns and temperature, may influence the expression of chlorotic symptoms. Heterogeneity of soil composition and water content contribute to non-genetic variation for expression of chlorotic symptoms within field nurseries and over years. To compensate, breeders must replicate entries within a field nursery and over a period of several years to obtain reliable estimates of the genetic tolerance inherent in a particular genotype. In some years, chlorosis symptoms are so mild in a field nursery that it is not possible to adequately distinguish genotypes from one another for their chlorosis tolerance, resulting in substantial losses of time and effort. To overcome these difficulties, we conducted a nutrient solution-based screening under aeroponic culture as a more reliable indicator of IDC tolerance than field tests or tests in potting soil. Different parameters like leaf color, chlorophyll content, POD, Fe content and root Fe reduction capacity have been employed for identification of IDC tolerant genotypes, either singly or in combination. Higher SPAD values and chlorophyll content indicate a lower incidence of leaf chlorosis. Iron deficiency has also been found to reduce the activity of oxidative stress-related enzyme like POD in several plant species and is attributed to less Fe concentration in Fe-deficient leaves. However, there is no standard method for screening for IDC reported in potato. In this study, several morphological traits, physiological and biochemical parameters were recorded to identify IDC tolerant genotypes in potato under aeroponic cultureandcorrelation among these were worked out. A few IDC tolerant potato genotypes were identified.

MATERIALS AND METHODS

Virus-free *in vitro* plantlets of fifteen potato genotypes including CP-1435, CP-3781, CP-1616, CP-3486, CP-3772, CP-3803, CP-2067, CP-1549, CP-1544, CP-3443, CP-3781, CP-4105, CP-3893, CP-3443 and CP-2138 were obtained from Indian Council of Agricultural Research-Central Potato Research Institute, Shimla, India.

An aeroponics system was established for potato crop growth as described by Bag *et al.* (2015). The experiment was carried out at the aeroponic unitat ICAR- Central Potato Research Institute, Regional Station, Shillong (1800 m above mean sea level, 25.54°N, 91.85°E). *In vitro* plants were grown in aeroponic culture following our standard practices (Bag *et al.*, 2015). Twenty-eight well hardened plantlets per accessions were transplanted on the styrofoam sheet panel under two aeroponic culture. Two different nutrient compositions were followed for screening the IDC tolerance in potato *viz.*, Fe-sufficient media (Fe-EDTA 50 mg/l) and Fe-deficient media (Fe-EDTA 0 mg/l). The basic nutrient solution contained 2.0 mM $Ca(NO_3)_2$, 0.75 mM K_2SO_4, 0.1 mM KCl, 0.25 mM KH_2PO_4, 0.65 mM $MgSO_4$, 1.0 µM $MnSO_4$, 1.0 µM H_3BO_3, 1.0 µM $ZnSO_4$, 0.1 µM $CuSO_4$, 0.005 µM $CoCl_2$ and 0.005 µM $(NH_4)_6Mo_7O_{24}$. Nutrient solutions were supplied to the plant roots for 30 s at an interval of every 180 s by spraying (mist form) through nozzles with an automated timer and motor-pump attached with pipe under the aeroponics system. The nutrient solutions were changed at every 7 days interval. The pH of the solution was adjusted to 5.8 using 1 mol l^{-1} H_2SO_4 or 1 mol l^{-1} NaOH. All the following observations, with the exception of VCR, were recorded on standard leaf (third fully opened leaf from the top on main stem) of five plants per accession in both the treatments to estimate mean. The experiment was carried out for 30 days. Various observations were recorded *viz.,* colour score (Boamponsem *et al.*, 2017), SPAD value, chlorophyll content (Arnon, 1949), peroxidase (Boamponsem *et al.,* 2018), Fe content (Kalra, 1988) and root-shoot ratio (Qi *et al.*, 2019).

The mean values of morpho-physiological and biochemical associated traits were estimated. The obtained means were then converted to relative values, i.e. Fe tolerance indexes. Iron tolerance index is defined as the observations under Fe-deficient divided by the means of the Fe-sufficient. Grouping of genotypes within Fe-deficient and Fe-sufficient conditions was basedon Ward's minimum variance analysis. Pearson's correlation coefficient was used to determine the relationship between variables and related traits significant at 0.05 and 0.01 level.

RESULTS AND DISCUSSION

There was a marked difference in fifteen potato genotypes in response to Fe deficiency when grown under aeroponic culture. Few genotypes were highly tolerant to Fe deficiency while others were moderate and highly sensitive. Height and root length of the plant are important morphological characters representing the vigor of the plant during the growth and development and have positive relationship with yield. An overall decrease in plant height under Fe-deficiency was observed compared to the plant grown under Fe-sufficient conditions. The relative plant height measured as a ratio of mean under Fe-deficient to mean under Fe-sufficient conditions ranged from 0.54 to 0.84 (Table 1). Lowest reduction in plant height was shown by CP-1435 followed by CP-3772, CP-2140 and CP-3443 whereas the highest decrease was recorded in CP-4069 followed by CP-4105 and CP-3803. The increase in relative plant height observed in tolerant genotypes may be attributed to the involvement of Fe in chlorophyll production, which favorcell division, meristematic activity in apical tissues, expansion of cell and formation of new cell wall (Singh *et al.*, 1989). In case of root length, relative values ranged from 0.75 to 1.09 where CP-3772 registered the highest value followed by CP-3803 and CP-3443 whereas lowest value was noticed in CP-3893 followed by CP-4105 and CP-2140 (Table 1). Except for CP-3772 and CP-3803, reduction in root length was observed under Fe-deficient compared to Fe-sufficient condition. In addition, Fe deficiency is known to alter both chloroplast structure and photosynthetic rate in higher plants, but our knowledge of the impact of Fe homeostasis on photosynthesis efficiency, and therefore on biomass production, is still very limited. The study revealed an increase root-shoot biomass of potato genotypes under Fe-deficient conditions. It is indicating that in conditions of limited plant matter accumulation, the photosynthetic products synthesized in shoots were preferentially distributed to the root system to maintain root system growth (Long *et al.*, 2020). The relative mean of root-shoot biomass ranged from 0.48 to 0.72 and the maximum increase was noted in tolerant genotypes (CP-3772, CP-3443 and CP-1435) (Table 1). The increase in the root-shoot biomass was significantly higher in tolerant than in susceptible, indicating that the tolerant genotypes may have efficient root to shoot transport and redistribution of Fe in the plant (Xu *et al.*, 2017).

The selection of IDC tolerant plants solely based on morphology may not be an exact indicator of tolerant genotypes, particularly if not grown under controlled conditions for trait that are influenced by environmental factors.To obtain a deeper knowledge of the tolerance mechanism (s) involved, the physiological and biochemical parameters which make a certain genotype more tolerant are being investigated in the study. VCR, SPAD values and chlorophyll content are directly or indirectly associated with photosynthesis which is considered a physiological indicator or biomarker of plant tolerance to stress since plants reduce their photosynthetic rate as a first response to unfavorable environmental conditions. In the present studies, early signs of Fe deficiency such as retarded plant growth, slow expansion of young leaves and turning to pale green as early as 10 days were observed in the susceptible genotypes.

Low chlorophyll content (chlorosis) of young leaves is the most evident sign of Fe-deficiency as Fe plays a critical role in chlorophyll biosynthesis. The lowest VCR scores were recorded by tolerant genotypes (CP-1435, CP-3772 and CP-3443) compared to susceptible genotypes after 30 days of no Fe supply (Fig. 1). Relative comparison between Fe-sufficient and Fe-deficient conditions indicated that tolerant genotypes did not show big changes in VCR scores, while the susceptible genotypes showed significantly higher VCR under Fe-deficient conditions. The SPAD values ranged from 0.52 to 0.72 and were significantly higher in tolerant genotypes compared to susceptible genotypes of potato under Fe-deficient conditions. Relative mean values compared to Fe-sufficient and Fe-deficient conditions showed higher (CP-1544,CP-4069 and CP-2138) and less (CP-3772, CP-3443, and CP-1435) reduction among the genotypes (Table 1). Under Fe-deficient conditions, highly tolerant genotypes recorded very low VCR score and high SPAD values compared to other genotypes. SPAD values were able to clearly differentiate the severity of chlorosis as compared to VCR scores among genotypes. Being a quantitative measure, SPAD is simple, robust and more reliable in judging IDC tolerant compared to VCR (Boamponsem *et al.,* 2017).

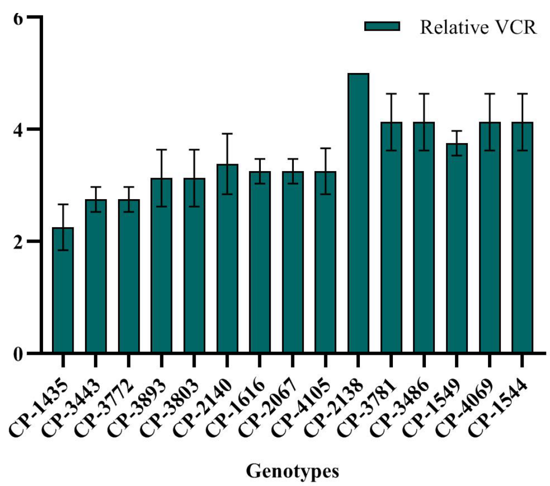

Fig. 1. Relative VCR scores for fifteen potato genotypes

Iron is a component of enzyme complexes in chloroplasts and mitochondria and is known to be required for biosynthesis of chlorophyll and carotenoid (Tewari *et al.,* 2013). Chlorophyll contents were detected to be dependent on the bioavailability of Fe. In the present study, chlorophyll content (a, b and total) decreased significantly under Fe-deficiency. However, the decreases in photosynthetic parameters were significantly lower in tolerant genotypes (CP-1435, CP-3443 and CP-4105) compared to susceptible genotypes under Fe-deficiency conditions (Table 1). The relative mean of total chlorophyll content ranged from 1.04 to 1.82. The total chlorophyll content, which is related to photosynthetic activity, revealed differences in IDC tolerance among potato genotypes both in Fe-deficient and Fe-sufficient condition. The specific differences identified between Fe-deficient and Fe-sufficient genotypes in relation to leaf chlorosis under low Fe supplies could be explained by the decrease in chlorophyll status, predominantly in the sensitive genotypes compared to tolerant genotypes. This can be attributed to the regulatory role of Fe in the formation of aminolevulinic acid and protochlorophyllide, the precursors of chlorophyll biosynthesis as stated by Broadley *et al.* (2012). Increase in chlorophyll concentration and a decrease in chlorosis symptoms appear to be associated with Fe-efficiency. Due to an adequate supply of photosynthetic products, tolerant genotypes (CP-1435, CP-3443 and CP-3772) could effectively control the decline of photosynthetic area and photosynthetic rate under low Fe stress, maintain higher matter accumulation, and coordinate the growth of shoot and root, hence improving its overall adaptability to Fe-deficient condition.

When plants are stressed, they can activate defense mechanisms to adapt to the adverse environment. For example, the enzymes POD can eliminate accumulation of reactive oxygen species in plants, keep free radicals in plant cells at a low level, reduce membrane damage caused by membrane lipid peroxidation, and maintain normal plant life activities. In the current study, the relative mean of POD ranged from 0.66 to 0.80 and the maximum activity was noted in tolerant genotypes (CP-3443, CP-3772, CP-3803 and CP-1435) compared to susceptible genotypes (CP-1544, CP-2138 and CP-4069) under Fe-deficient condition (Table 1). POD activities in potato decreased under low Fe stress. This may have been due to low activation and/or a reduced production of the POD enzyme since it requires Fe for its function. Decreased POD activity was also detected in okra and flax under iron deficiency conditions (Kabir *et al.,* 2015). Transcripts encoding POD enzyme was found to be down-regulated in tomato leaves under low Fe conditions. The results are clearly consistent with the notion that the amount of Fe in the nutrient medium influenced antioxidant enzyme activities in potato leaves. Plant cells exposed to Fe deficiency can be sensitive to oxidative stress due to a low level of POD. POD activity was significantly higher in tolerant genotypes than in susceptible genotypes under control as well as low Fe treatments. The findings reported herein suggests that antioxidant activity levels could be associated with tolerance to Fe-deficiency stress since the POD activity of the sensitive genotypes were more affected by Fe starvation than that of the tolerant genotypes. As

Table 1. Fe tolerance indices of morphological and physiological parameters and ranking of genotypes based on Ward's minimum variance analysis. All the data was presented in relative values (%age) calculated per plant.

Genotypes	Plant Height (cm)	Root Length (cm)	Root-Shoot Biomass (g/plant)	SPAD value	Chl. a (mg/g FW)	Chl. b (mg/g FW)	Total Chl. (mg/g FW)	POD (ΣOD/ min/ mg)	Leaf Fe content (µg/g DW)	Root Fe content (µg/g DW)	Average (%)	Cumulative sum	Genotype Ranking
CP-1435	83.81	95.34	69.36	68.44	83.24	83.89	167.13	76.09	83.69	38.53	75.82	75.49	1
CP-3443	78.17	97.96	71.06	70.18	87.33	79.20	166.53	80.40	80.72	51.32	77.37		1
CP-3772	81.00	109.22	72.58	72.22	73.68	62.20	135.88	76.79	69.23	42.49	73.27		1
CP-3893	72.21	74.73	56.86	59.26	68.65	68.31	136.96	75.02	66.28	87.93	69.92	69.18	2
CP-3803	61.21	104.22	72.76	63.31	63.13	72.83	135.96	76.62	70.27	54.01	70.93		2
CP-2140	78.57	86.21	67.43	58.53	57.84	67.19	125.03	74.18	66.99	52.10	67.67		2
CP-1616	71.69	91.91	69.87	59.31	69.10	54.38	123.48	75.57	63.32	83.31	70.94		2
CP-2067	75.77	89.00	68.51	59.99	67.08	65.98	133.06	75.07	66.93	52.63	69.00		2
CP-4105	57.45	83.52	48.30	57.64	73.42	71.94	145.36	74.53	83.67	49.42	66.65		2
CP-2138	70.67	95.03	67.43	54.69	68.19	69.09	137.28	66.60	60.06	52.33	67.12	66.54	3
CP-3781	69.90	90.44	58.93	55.80	60.80	62.86	123.66	72.50	63.71	42.68	64.18		3
CP-3486	70.03	90.03	62.14	54.10	80.26	62.04	142.30	70.05	71.52	55.27	68.38		3
CP-1549	73.72	91.13	48.90	56.84	66.21	67.22	133.43	72.21	65.06	57.10	66.49		3
CP-4069	53.69	92.83	55.36	54.02	56.89	44.74	101.63	67.54	61.50	41.31	58.65	61.11	4
CP-1544	64.59	97.23	67.08	51.84	56.48	54.70	111.18	65.89	60.18	54.07	63.56		4
Range	54-84	75-109	49-73	52-72	56-87	44-84	101-167	66-80	62-84	39-88	-	-	-
S. ED	0.74	1.38	0.83	0.79	1.12	0.78	1.87	0.90	0.99	0.94	-	-	-
CD (0.05)	1.48	2.78	1.68	1.59	2.26	1.57	3.77	1.81	2.02	1.89	-	-	-
CD (0.01)	1.97	3.71	2.24	2.13	3.01	2.09	5.04	2.42	2.69	2.52	-	-	-
CV(%)	1.47	2.11	1.85	1.87	2.31	1.67	1.97	1.74	2.05	2.44	-	-	-

Chl- Chlorophyll; POD – Peroxidase.Data are presented in relative values (%).

such, susceptible genotypes may be incapable of activating and/or sustaining the biosynthesis of POD in response to Fe shortage.

Total Fe content was recorded from both leaf and root samples. The relative amount of Fe content varied in the leaves (0.60 to 0.84ìg g$^{″1}$) and roots (0.38 to 0.87ìg g$^{″1}$). Total leaf Fe content was found to be highest in CP-1435, CP-4105, CP-3486 and CP-3443 while least recorded in CP-2138, CP-1544 and CP-4069 (Table 1). While in roots, Fe content was found higher in CP-3893, CP-1616, CP-1549 and CP-3486 and lowest in CP-1435, CP-4069 and CP-3772 (Table 1). Generally, plant roots acquired iron to distribute it to other tissues and organs. Iron is transported from the root epidermis to the central vascular cylinder for xylem loading and translocated to the aerial sections of the plant (Bairu *et al.,* 2011). The present study showed that the susceptible genotypes like CP-1544 had a high level of root Fe content while low level in leaves. This susceptible genotype having high amount of iron in the vascular cylinder of the roots but may have difficulty in transporting iron through and out of the xylem into the shoot symplast and the chloroplasts under low Fe supply. In the case of tolerant genotypes, iron has been distributed to various aerial plant parts and movement of iron from older leaves to younger leaves occurs by means of phloem transport. In leaf cells, Fe is distributed to other cellular compartments to function in chlorophyll synthesis and act as a co-factor for enzymes while the excess is stored in *ferritin* for future use.

A multivariate analysis using Fe tolerance index of morphological and biochemical parameters was performed employing Ward's minimum variance analysis, a four-cluster grouping was generated. Based on this analysis, CP-1435, CP-3772 and CP-3443 were ranked first with an averaged Fe tolerance indexes of 75.49 % whereas CP-4069 and CP-1544 were among the susceptible genotypes ranking fourth with averaged Fe tolerance indexes of 61.11 %. Genotypes, CP-2067, CP-2140,CP-1616, CP-3893, CP-4105 and CP-3803 produced average Fe tolerance indexes of 69.18 %, and thus were rated as moderately tolerant to Fe deficiency (Table 1).

Pearson's correlation coefficients between morpho-physiological and biochemical parameters were calculated and the data are presented in Table 2. The results of the correlation analysis showed a significant relationship among different parameters studied at 0.05 and 0.01 level of significance. Plant morphological characters *viz.*, plant height was positively correlated with four (SPAD, Chlorophyll a, b and total) and negatively correlated with VCR out of eleven parameters assessed whereas root length was found significantly correlated with SPAD androot-shoot biomass. At the leaf level, plant physiological characters *viz.* SPAD values, chlorophyll a, chlorophyll b, total chlorophyll, POD and Fe content were significantly positively correlated with each other except being negatively correlated with VCR.

The mean performance of biochemical parameters varied significantly among the fifteen potato genotypes, indicating that a broad genetic base was present among the genotypes. Boodi *et al.* (2016) similarly observed wide variation in chemical properties of soybean among the genotypes. Morpho-physiological traits that are highly correlated with biochemical parameters can influence a plant's ability to survive in iron-limiting soils. The correlation analyses suggests that tolerant genotypes having low VCR in conjunction with higher SPAD value, increased chlorophyll, POD, leaf Fe content and root-shoot biomass may exhibit tolerance to IDC. These parameters could be effectively utilized for identifying IDC tolerant genotype.

CONCLUSION

The usefulness of aeroponic culture as an effective technique for the screening and selection of IDC tolerant genotypes in potato has been demonstrated. IDC tolerant genotypes had registered a significantly lower reduction in plant height, VCR, chlorophyll content, SPAD, POD, leaf Fe content and root-shoot biomass compared to susceptible ones confirming their utility as traits for identification and development of IDC tolerant potato genotypes. VCR and SPAD values are extremely useful for preliminary and large-scale screening of germplasm or breeding material due to their simplicity and robustness. However, compared to other parameters, chlorophyll content, POD, root-shoot biomass and leaf Fe content are more reliable and may serve as predictors for Fe-efficiency trait in potato. The findings of this study

Table 2. Pearson's correlation coefficient along with probability of significance between different parameters

Correlation	Plant Height	Root Length	VCR	SPAD	Chl. a	Chl. b	Total Chl.	POD	Leaf Fe	Root Fe	Root-Shoot Biomass
Plant Height	1										
Root Length	0.124	1									
VCR	-0.456*	-0.094	1								
SPAD	0.586*	0.446*	-0.837**	1							
Chlorophyll a	0.479*	0.184	-0.465*	0.594**	1						
Chlorophyll b	0.477*	0.004	-0.508*	0.551*	0.649**	1					
Total Chlorophyll	0.527*	0.152	-0.511*	0.635**	0.990**	0.735**	1				
Peroxidase	0.426	0.087	-0.852**	0.825**	0.552*	0.555*	0.583*	1			
Leaf Fe content	0.204	0.057	-0.566*	0.505*	0.810**	0.708**	0.830**	0.570*	1		
Root Fe content	-0.392	-0.285	0.223	-0.435	-0.312	-0.512*	-0.382	-0.213	-0.368	1	
Root-Shoot Biomass	0.479	0.623*	-0.247	0.488	0.268	0.182	0.234	0.285	0.068	-0.058	1

* Correlation at 0.05 (Two-tailed) ** Correlation at 0.01 (Two-tailed)

will be helpful in better understanding of some of the mechanisms behind mineral partitioning and resources allocation in potato. Since, the nutrient solution cannot fully simulate agricultural, saline-alkaline or calcareous soil conditions and as such further studies are necessary to confirm IDC tolerance of the tolerant genotypes under alkaline/calcareous soils.

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