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Proceeding Paper

Effects of Alkalinity-Induced Iron Deficiency on Physiological and Growth Variables of some Upland Rice Cultivars under Laboratory Condition †

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Abstract: Prevalence of iron deficiency in upland rice under alkalinity stress is capable of constraining its production. This investigation aimed to explicate the physiological basis of iron deficiency tolerance in some upland rice genotypes. Eighty upland rice genotypes were characterized for iron deficiency tolerance at seedling growth stage in a sand-culture hydroponics with varying $NaHCO_3$ concentrations (0, 15 and 25 mM). The treatments were arranged in completely randomised design with three replicates. Significant decrease was observed on leaf iron concentration, SPAD meter readings, leaf photosynthetic efficiency, quantum yield and growth variables with increasing concentration of $NaHCO_3$. Iron tolerance index was further estimated based on these parameters and used for ranking the genotypes. Based on iron tolerance index, genotypes were divided into 6 groups with Caipo and NERICA 7 identified as the most and least tolerant to iron deficiency respectively. The basis of iron deficiency tolerance is discussed on relation to the stability of photosynthetic apparatus and plan growth under alkalinity stress.

Keywords: Quantum yield; Photosynthetic apparatus; NERICA; Sand-culture hydroponics; iron tolerance index

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1. Introduction

The competing need for freshwater in the cultivation of lowland rice production system necessitated the need to explore other ecologies. Upland rice cultivation is another option in rice production. In the tropics, it accounts for 8% of cultivated land while lowland rice was reported to be 92% [1]. According to [2], the cultivation pattern of upland rice in the tropics is associated with high rainfall, reduced sufficiency for rice and Gross National Income level; however, this had been a subject of debate by other stakeholders.

Iron deficiency is one of the production constraints in the establishment of upland rice in the tropics. It is closely associated with the bicarbonate content in the soil with increased soil and plant alkalinity [3]. The increasing soil water content could lead to availability of excess bicarbonates in the soil as CO_2 content increases with reduced soil porosity. Furthermore, increased soil pH had been reported to induce iron deficiency chlorosis and reduce the solubility of iron in the soil. Iron is a component of the electron transport chain in mitochondria and chloroplast. Thus, the disruption of electron transfer would elicit reactive oxygen radicals, causing cellular oxidative damage and reduced growth [4]. As a component of cellular anti-oxidant systems [5], deficiency of iron will also affect redox homeostasis in the cell. Increased soil alkalinity resulting from high bicarbonate concentrations may negatively affect mineral composition, especially micronutrients such as Zn, Mn and Mg [6].

Genotypic variation in response to iron availability in the soil has been reported in the literature [4,7–8] and may provide a basis for breeding-based crop improvement strategies. However, understanding the physiological basis of crop performance may complement crop improvement efforts for developing iron deficiency tolerant rice. The present work is therefore aimed at investigating the physiological characteristics of some selected upland rice genotypes under varying concentration of bicarbonate in the growth medium.

2. Materials and Methods

2.1. Plant Materials and Experimental Setup

Eighty upland rice genotypes of a broad range of morphological diversity and origins were used in the screen house experiment. The experiment was conducted at the International Institute of Tropical Agriculture, Ibadan sub-Station, Nigeria, with day/night temperature range of 30/22 °C and relative humidity of at least 50% during the day. The screen house was disease-free and lit by natural lighting. The treatments consisted of eighty genotypes and concentrations of $NaHCO_3$ (0 mM, 15 mM and 25 mM). The genotypes were planted in a completely randomised design with three replications.

River sand was collected and washed thoroughly under water, air dried, autoclaved at 70 °C for 72 h and sieved at 25 mm particle size. The experimental plants were raised in 3-litre dark-coloured pots to minimise light penetration into the culture solution thereby, reducing algal growth. The surfaces of the 3-litre pots were wiped with 70% ethanol and washed thrice with 5% HNO_3 to remove any contamination. Each plant pot was labelled and filled with an equal amount of sand to hold the plants in place during growth. A modified basal nutrient solution suggested by [9] for normal growth of seedlings was utilised. Rice seeds were directly sown into the pots and irrigated with the optimum nutrient solution till 15 Days After Sowing (DAS).

2.1. Sampling and Data Collection

Four rice seedlings were maintained per pot. SPAD meter readings of seedlings were measured using the SPAD-502 meter (Minolta Ltd., Tokyo, Japan) and leaf colours were visually scored thrice during the screening period i.e., 15, 25 and 35 DAS (5 = normal growth, no chlorosis; 4 = slight yellowing of upper leaves and the leaf veins; 3 = interveinal chlorosis in the upper leaves; 2 = interveinal chlorosis of the upper leaves along with some apparent stunting of growth; 1 = severe chlorosis, stunted growth and necrosis in the youngest leaves [10].

The pH of the nutrient solution in all pots was monitored with a pH meter (Hanna Instruments, Italy). Plant height (PHT) was measured with a metre rule at 15, 25 and 35 DAS. At the 35 DAS, biomass index was estimated by weighing oven-dried shoots and roots of two seedlings per pot. Leaf Fe concentrations were also determined and calculated following the [11] protocol. At 35 DAS at 9.00 a.m quantum yield (Fv/Fm) was measured with a pocket PEA Chlorophyll fluorimeter (Hansatec Instruments Ltd., Norfolk, UK). Photosynthetic rates were measured using the LI-6400XT portable photosynthesis system (LI-COR Biosciences, Lincoln, USA).

2.3. Data Analysis

Fe tolerance indexes for each parameter and the average Fe tolerance indexes per genotype were calculated as described by [12,13]. The genotypes were grouped into clusters based on average Fe tolerance indexes using the agglomerative clustering method. All data collected except Fe tolerance indexes were subjected to Analysis of Variance (ANOVA) using R statistical package. Means were separated using LSD at 5%, 1% and 0.1% probability level.

3. Results

The average Fe tolerance indexes of the 80 upland genotypes ranged from 34.3 to 109.0. They were categorised into 6 clusters with CAIPO and NERICA 7 as first and last genotype on the table as shown in Table 1. At 35 DAS, there was a significant reduction in the SPAD meter readings in all the genotypes with increasing concentrations of $NaHCO_3$ (Table 2). A similar pattern was observed on the leaf iron concentration, leaf photosynthetic rate, quantum yield, plant height, shoot dry weight and root dry weight in all the upland rice genotypes (Table 2). Similar to all other parameters, there was a significant reduction in visual chlorosis scoring with increasing concentration of $NaHCO_3$ with the exception of Caipo that maintained stable visual chlorosis score at 15 and 25 mM $NaHCO_3$.

Table 1. Characteristics of clusters obtained from average iron tolerance indexes based on physiological and growth parameters of upland rice genotypes.

Cluster	Membership	Size	Min	Max	Mean	StdDev
1	Caipo, OFADA3, LAC23	3	91.2	109	97.5	9.98
2	FARO65	1	85.1	85.11	85.11	
3	PCT11-1-3-1, NERICA3, DURADO, NERICA15, NERICA16, CIRAD409, IGBEMORED, Azucena, IAC120, CIRAD403, Palapo, NERICA4, IRAT13, EbonyiLocalBest, Wayrem, OS4, NERICA18	17	61.4	80.36	67.33	5.53
4	NERICA13, NERICA11, NERICA17, ARICA5, NERICA14, CIRAD358, IRAT170, NERICA8, NERICA5, CT13582-15-M, IRAT226, IRAT2	12	49.7	59.17	54.76	3.13
5	CIRAD394, WAB56-50, ITA301, FARO63, IRAT216, NERICA12, Vandana, IRAT212, IRAT257, ARICA4, IAC47, ChinaBest, IRAT364, OS6	14	37.9	53.42	47.4	5.33
6	WAB181-18, WABC165, ITA128, IR7267-12-2-3, WAB56-104, MOROBEREKAN, NERICA6, Pamira, NERICA9, IRAT133, WAB99-16, APO, NERICA10, IRAT144, CIRAD488, NERICA1, OFADA4, Palawan, IGUAPECATETO, IRAT112, IRAT109, ART-27-58-7-1-2, OFADA1, IRAT362, ART27-190-1-3-3, FARO64, Curinga, SabonDaga, NERICA2, Primavera, OFADA2, WAB638-1, NERICA7	33	34.3	51.99	43.89	4.77

Table 2. Effect of $NaHCO_3$ concentrations on some physiological and growth parameters in upland rice genotypes using a representative of the 80 upland rice genotypes.

[A]												
Genotype	SPAD reading			Leaf Fe concentration (mg/kg)			Photosynthetic rate (μmol(CO_2) $m^{-2}s^{-1}$)			Quantum yield (F_v/F_m)		
	0mM	15mM	25mM	0mM	15mM	25mM	0mM	15mM	25mM	0mM	150Mm	25mM
Most tolerant												
Caipo	40.54	29.61	27.38	86.64	61.32	49.57	27.44	20.33	8.28	0.787	0.736	0.731
OFADA3	33.03	24.84	21.95	85.34	62.51	50.76	28.66	21.54	9.49	0.783	0.734	0.727
LAC23	35.19	25.97	23.26	89.54	62.21	49.96	27.21	20.09	8.05	0.787	0.738	0.731
FARO65	37.42	27.88	14.09	86.24	38.61	15.05	26.77	11.37	6.49	0.786	0.698	0.599
PCT111	35.44	23.33	20.33	85.15	55.89	37.14	27.98	20.44	7.98	0.781	0.724	0.698
Least tolerant												
NERICA2	37.91	12.06	9.79	88.54	36.72	13.08	27.50	12.08	6.45	0.787	0.702	0.601
PRIMAVER	38.44	23.01	8.77	91.94	40.92	16.48	27.87	12.45	6.80	0.785	0.697	0.594

OFADA2	32.36	12.59	9.31	86.94	35.18	11.48	27.86	12.44	6.81	0.786	0.699	0.600
WAB638-1	31.26	17.56	7.52	89.84	38.82	14.38	26.65	11.23	5.58	0.780	0.690	0.590
NERICA7	41.21	10.59	8.47	88.34	36.58	12.88	26.86	11.44	5.81	0.784	0.694	0.594
Mean	36.28	19.37	16.47	87.85	46.87	27.08	27.483	15.343	7.177	0.785	0.712	0.646
F values (ANOVA)												
G		462.81***			1389.8***			451.52***		2880.9***		
T		7851.76***			48719.0***			37613.52***		57428.9***		
G x T		209.84***			453.6***			183.45***		1351.2***		

[B]

Genotype	Colour visual score			Plant Height (cm)			STDRYWEIGHT (g)			RTDRYWEIGHT (g)		
	0mM	**15mM**	**25mM**	**0mM**	**15mM**	**25mM**	**0mM**	**15mM**	**25Mm**	**0mM**	**15mM**	**25mM**
					Most tolerant							
Caipo	5.0	4.0	4.0	43.71	36.47	32.27	0.949	0.706	0.629	0.694	0.554	0.489
OFADA3	5.0	4.0	3.0	42.21	32.84	19.44	0.713	0.423	0.397	0.402	0.311	0.219
LAC23	5.0	4.0	3.0	41.21	30.52	29.20	0.902	0.607	0.403	0.408	0.314	0.289
FARO65	5.0	4.0	2.0	58.93	43.02	33.73	1.029	0.576	0.199	0.750	0.282	0.094
PCT111	5.0	3.5	3.0	44.11	34.69	29.85	0.746	0.346	0.149	0.252	0.174	0.089
					Least tolerant							
NERICA2	5.0	2.0	1.5	53.54	20.86	10.88	0.814	0.402	0.017	0.409	0.084	0.011
PRIMAVERA	5.0	2.5	1.0	54.51	33.72	21.16	0.837	0.449	0.092	0.457	0.189	0.002
OFADA2	5.0	2.0	1.0	56.94	20.18	19.43	1.013	0.795	0.022	0.352	0.036	0.008
WAB638-1	5.0	2.0	1.0	57.52	23.48	16.24	0.669	0.201	0.043	0.208	0.054	0.005
NERICA7	5.0	1.5	1.0	65.32	33.29	24.17	0.813	0.402	0.022	0.409	0.084	0.011
Mean	5.00	2.95	2.05	51.79	30.90	25.12	0.849	0.491	0.197	0.429	0.208	0.122
F values (ANOVA)												
G		41.00***			75.55***			371.04***		1618.75***		
T		627.25***			2162.414***			6920.06***		6706.19***		
G x T		19.75***			65.73***			111.69***		234.94***		

G, Genotypes; T, Treatments (0Mm, 15Mm and 25Mm of $NaHCO_3$); G x T, Genotype by Treatment interactions. *, **, *** indicate probability of significance at 0.05, 0.01 and 0.001, respectively.

4. Discussion

Nutrient solutions containing bicarbonate or complete absence of iron have been used extensively in the literature in the investigation of iron deficiency chlorosis in laboratory conditions [14,15]. Literature has also proven a significant correlation between the results in laboratory conditions and that of field trials as recorded in research conducted by [16,17]. Chlorosis is a common physiological marker used to express bicarbonate stress, thus it informs the selection of tolerant or susceptible genotypes.

The selected upland rice genotypes were phenotyped to determine their variations to alkalinity stress in the Laboratory. The reduced SPAD meter reading as a result of increasing $NaHCO_3$ concentration in the growth medium was corroborated visually with increasing chlorosis. This could be linked to the fact that iron constituted 80% of the chloroplast [18]. Furthermore, 60% of iron was involved in the electron transport chain [19]. This disruption in the functional integrity of the chloroplast could have explained observed reduction in the quantum yield and subsequently leaf photosynthesis with increasing concentration of the $NaHCO_3$. A similar position was also reported by [20] when maize was subjected to iron deficiency in the growth medium. The reduced availability of assimilates from the reduction in leaf photosynthesis under iron deficiency could have explained the reduced growth under this condition among all the upland rice genotypes investigated.

An alternative physiological mechanism linking iron deficiency with leaf photosynthesis and concentration of iron in different organs of the plant was proposed by [21]. In their explanation, it was proposed that iron deficiency linked with a reduction in leaf photosynthesis could result in the disruption of sucrose transportation in the phloem. It could also lead to the differential distribution of iron in the plant shoot and root, with more of iron found in the plant shoot than in the plant root [21]. It was posited that this observed response pattern would have elicited a signal from the plant shoot to the roots leading to changes in the reactive oxygen system and consequently resulting in oxidative damage of organs. However, this distribution of iron was observed in matured shoot and roots, unlike what was obtained in our investigation, where the leaf iron concentration was sampled form the flag leaf.

In context of iron tolerance stress index, it was revealed that the upland rice genotypes were categorized into 6 groups with Caipo and NERICA 7 being the most and least ranked respectively. This could be attributed to their pattern of growth and chlorosis response with increasing bicarbonate concentration in the medium. While the former maintained a steady decline in its growth variables with decreasing iron concentration, the later had a twofold reduction in growth, especially at bicarbonate concentration of 25 mM.

5. Conclusions

These evidences, taken together indicated that physiological responses to iron deficiency, especially under alkalinity stress, is expressed in the reduction of leaf iron concentration. This may perhaps be linked with the observed decreased in the SPAD-meter reading, quantum yield and leaf photosynthetic rate. The reduced availability of assimilates would negatively affect carbon budgeted for growth as portrayed in reduced plant height, shoot and root dry weight at 35 DAS. In addition, it was found that within this germplasm evaluated, Caipo and NERICA 7, were regarded as the most and least tolerant to iron deficiency.

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