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Morphological and Physiological Responses of Weedy Red Rice (*Oryza sativa* L.) and Cultivated Rice (*O. sativa*) to N Supply

Marites A. Sales[1], Nilda R. Burgos[2], Vinod K. Shivrain[2], Brad Murphy[4], Edward E. Gbur, Jr.[5]

[1]Department of Plant Pathology, University of Arkansas, Fayetteville, USA; [2]Department of Crop, Soil and Environmental Sciences, University of Arkansas, Fayetteville, USA; [3]Syngenta Crop Protection, Vero Beach, FL, USA; [4]Department of Horticulture, University of Arkansas, Fayetteville, USA; [5]Agricultural Statistics, University of Arkansas, Fayetteville, USA.

Email: nburgos@uark.edu

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ABSTRACT

Red rice (*Oryza sativa L.*), *a noxious weed in rice production*, *competes with cultivated rice for nutrients*. *Accumulation of more N in red rice than in cultivated rice may be due to a mechanism different from that of cultivated rice*. *To test this assumption*, *red rice and cultivated rice were grown in nutrient solution to compare their growth and physiological responses to N supply*. *Experimental design was a split-plot*, *where main plot factor was rice type* (*Stf*-3, '*Wells*'); *split-plot factor was N treatment* [T_1 (*complete nutrient solution*); T_2 (*–*NH_4NO_3); T_3 (*+*NH_4NO_3 *for* 24-*h post-N deficiency*); *and* T_4 (*+*NH_4NO_3 *for* 48-*h post-N deficiency*)]. *Nitrogen deficiency was defined as N sufficiency index* (*NSI*) < 95%. *Height*, *tiller number*, *biomass*, *and root morphology were monitored to determine morphological responses*. *Stf*-3 *red rice had significantly greater growth measurements than Wells in terms of shoot and root characteristics*. *At* T_4, *Stf*-3 *showed higher increment in root length and surface area than Wells. Shoot tissue concentrations of N and total sugars were measured to determine physiological response in N-deficient and N-supplemented plants*. *Stf*-3 *had greater N and sucrose tissue concentrations at N-deficient conditions compared with Wells*, *implying a stress-adaptive molecular mechanism regulated by N and sucrose availability*.

Keywords: *Hydroponics*, *Nitrogen Concentration*, *N Uptake*, *Rice* (*Oryza Sativa L.*), *Root Morphology*, *Sucrose Concentration*, *Sugars*

1. Introduction

Rice is a staple food for more than half of the world's population. The United States produces less than 2% of the volume of world rice production, but is a major rice exporter, providing 12% - 14% of the annual volume of the global rice trade [1]. Arkansas is the largest rice-growing state, containing over 45% of U.S. rice acreage, according to the USDA National Agricultural Statistics Service. A major challenge facing rice producers in the southern U.S. is weed competition. Red rice, a weedy rice relative belonging to the same genus and species as the cultivated rice (*Oryza sativa*) is one of the most difficult weed species to control because of its similarity to the crop [2]. About 60% of the rice fields in Arkansas are infested by red rice [3]. Red rice has a competitive advantage over cultivated rice because it grows taller and faster, and tillers profusely, thus depriving cultivated rice of necessary nutrients, light and space owing to its height and massive root system. Under non-competitive conditions, red rice produces almost double the grain yield of commercial cultivars [4]. When competing with cultivated rice, one red rice plant·m^{-2} reduced yield of 'Newbonnet' rice, a tall cultivar by 219 kg·ha^{-1} [5]. Red rice caused an estimated loss of $ 275 ha^{-1} in 2006 alone [3]. These economic losses include damaging effects of plant lodging and price docking of rice grains contaminated with red rice kernels. In addition, red rice uptake of even half of the optimum fertilizer N requirement for rice cultivars, estimated at 200 kg·N·ha^{-1} in the southern U.S. [6], is enough to drastically reduce rice yields and the economic benefits of N fertilization. Red rice accumulated more fertilizer N and produces more biomass than 'Drew' rice under field conditions, suggesting that it could have higher yields even in low N supply [7]. The

implied tolerance to N-deficient conditions in weedy red rice is a trait that would be of agronomic importance in cultivated rice.

Nitrogen is the most important inorganic macronutrient and is a limiting factor in crop productivity. It is a major constituent of proteins, cofactors, and secondary metabolites [8], and thus affects all levels of plant function [8-10]. Plants contain 1% - 6% N by weight and absorb N as both nitrate (NO_3^-) and ammonium (NH_4^+), depending on plant age and type, environment, and other factors [11]. Before NO_3^- can be used in the plant, it must be reduced to NH_4^+ or ammonia (NH_3). The NH_3 produced is assimilated into amino acids that are subsequently combined into proteins and nucleic acids. Nitrogen is also an integral part of chlorophyll needed for photosynthesis [12], so high photosynthetic activity, vigorous vegetative growth, and a dark green color are indicators of adequate N supply. Plants regulate photosynthesis to balance the flow of C through an optimized distribution of its N resources [13]. The profound effect of N supply on overall plant growth and development is modulated by C status [14], and most likely, cross-talk with other factors, such as hormones, cytokinins and abscissic acid [15]. Nitrogen deficiency, therefore, affects other metabolic pathways.

In recent years, elucidating plant response to stress has been facilitated by investigations at the cellular level. One morphological adaptation to nutrient deficiency is alteration of root architecture, such as increased number and length of root hairs to reach a wider area of the environment and, consequently, increase nutrient acquisition [8]. Molecular analyses have also revealed other phenotypic expressions of nutrient stress adaptation, such as increased densities of transport molecules to enhance nutrient utilization [16], release of plant compounds to increase bioavailability of soil nutrients [17,18], and enhanced nutrient uptake capacities regulated at the level of membrane transport [19-21]. General response systems to nutrient stress involve use of stored polysaccharides or recycling of cellular components to prevent severe deficiencies in respiratory substrates and maintain important biochemical pathways [22-24]. A degradative process known as vacuolar autophagy was induced by starch starvation in maize [25,26] and rice [27] and would be a likely process in any stress response pathway which uses starch as a precursor. Alteration in carbohydrate metabolism in response to N also indicates changes in the flux of soluble sugars in the plant.

Removing weeds from the paddy field increases the amount of N in the rice plant [28]. Plant density is an important factor in competition because it is inversely related to resources available to the plant [29]. When cultivated rice was planted with red rice at varying densities, only rice cultivars with comparatively high tillering capacity, leaf area and dry stem weight could compete very well with red rice [30,31]. Since red rice has morphological and physiological features that suggest competitive advantage over cultivated rice in adapting to N-poor conditions, it is expected to accumulate more N and produce more biomass compared with cultivated rice at low N conditions. Comparing morphological and physicological responses of weedy and cultivated rice types under N stress conditions is the first step towards elucidating adaptive mechanisms in red rice that are either absent or less efficient in the cultivated rice, hence, this study.

2. Materials and Methods

2.1. Plant Material

Rice types compared were the tall, awnless, medium-grain red rice accession Stf-3 and 'Wells'. Accession Stf-3 is a strawhull red rice collected from St. Francis County, Arkansas, USA. A strawhull red rice was selected because it is the most prevalent red rice type based on hull color, and is most similar to Wells rice in height at maturity [32]. Wells is a long-grain rice cultivar, which matures approximately 124 d after planting. Because of its high milled rice yield, stable head rice yield, and tolerance to rice blast and sheath blight [33], Wells was planted in 31% of rice production areas in Arkansas by 2006, making it the rice cultivar of choice [34]. The second most popular cultivar was planted in only 13% of rice area.

Seeds were surface-sterilized with 10% H_2O_2 for 10 min followed by 70% ethanol for 5 min, then washed thoroughly in sterile deionized water and germinated at 30˚C for 48 h in Petri dishes lined with moist filter paper. Uniformly germinated seeds were transferred into 6 cm diameter wells in black plastic trays (27 cm × 53 cm) (Pro-Tray, Hummert, MO, USA) fitted into 35-L plastic tubs (36 cm × 62 cm × 31 cm) (Multi-Reservoir, American Agritech, AZ 85283, USA) containing aerated, deionized water until a week after germination when it was replaced with half-strength nutrient solution [35].

2.2. Nutrient Solution

The nutrient solution was composed of NH_4NO_3 (40 ppm), $NaH_2PO_4{\cdot}2H_2O$ (10 ppm), K_2SO_4 (40 ppm), $CaCl_2$ (40 ppm), $Mg_2SO_4{\cdot}7H_2O$ (40 ppm), $MnCl_2{\cdot}4H_2O$ (0.5 ppm), $(NH_4)_6{\cdot}Mo_7O_{24}{\cdot}4H_2O$ (0.05 ppm), H_3BO_3 (0.2 ppm), $ZnSO_4{\cdot}7H_2O$ (0.01 ppm), $CuSO_4{\cdot}5H_2O$ (0.01 ppm), $FeCl_3{\cdot}6H_2O$ + citric acid (monohydrate) (2 ppm), and $Na_2SiO_3{\cdot}5H_2O$ (0.1 mM). Nutrient solution pH was

maintained at 5.0 (SympHony® pH/conductivity meter, VWR International, Arlington Heights, IL 60004, USA); pH was adjusted every other day for the first week, then daily. Water that evaporated from the system was replaced by deionized water daily. Nutrient solution was replaced weekly, using half-strength solution for 2 weeks; full-strength nutrient solution was used thereafter.

2.3. Hydroponics Culture Conditions

The trays described previously had 57 mm deep wells with five drain holes. Each seedling was placed on a plastic 3 mm mesh. Each tray contained 12 plants; each rice type was grown in four trays under greenhouse conditions from August to September (day temperature: 22˚C - 39˚C; night temperature: 21˚C - 30˚C) and from April to May the following year (day temperature: 21˚C - 27˚C; night temperature: 19˚C - 27˚C). The greenhouse was set to a day:night length of 14:10 h using supplemented lighting from 400 W metal halide lamps (Philips 34415-0, Philips Electronics, NY 10020, USA). Temperature and relative humidity were monitored (HOBO® Temperature Data Logger H01-001-01, Onset Computer Corp., MA 02532, USA). Plant growth stages were designated using a growth staging system as a guide [36]. Since the rate of development for rice grown in the greenhouse has not been documented, four extra plants per tray served as control for destructive sampling to check for the "green ring" inside the shoot meristem, which marks the R_0 stage [36] when weedy red rice and cultivated rice demonstrated differential accumulation of fertilizer N in field experiments [7]. In both plant types, R_0 was at V_8 (eight leaves with visible collar on main stem).

2.4. N Treatments

To simulate N-deficient conditions, defined as N sufficiency index (NSI) < 95% [11], plants were subjected to four treatments at R_0 stage: T_1 (complete nutrient solution; control); T_2—Nutrient solution without NH_4NO_3 until NSI < 95%; T_3—24 h supply of complete nutrient solution post-N deficiency; and T_4—48 h supply of complete nutrient solution post-N deficiency (**Figure 1**).

To assess both early and late molecular responses for subsequent microarray experiments, 24 h and 48 h time points for N supplementation, respectively, were selected. At R_0, T_1 plants were transferred into tubs with fresh nutrient solution, while $T_{2\text{-}4}$ plants were transferred to fresh nutrient solution without NH_4NO_3 and grown until NSI < 95%. In both years, it took 3 - 5 d without NH_4NO_3 to drop NSI below 95%. Following published procedures [37], NSI was monitored daily at mid-morning using a chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., USA); NSI was calculated for each rice type using the **Formula** (**a**) where average reading was calculated from all plants under similar growth stages, with three readings per plant. Readings were taken from the same spot in the mid-region of the youngest fully expanded leaf on the main culm of each plant [37]. T_1 and T_2 plants were harvested when the latter reached NSI < 95%; T_3 and T_4 plants were transferred to fresh nutrient solution containing NH_4NO_3 and harvested after 24 h and 48 h, respectively.

2.5. Experimental Design

A split-plot design was employed, in which whole plot factor was rice type (Stf-3, Wells) and split-plot factor was N treatment (Full, N-starvation, 24 h and 48 h N-

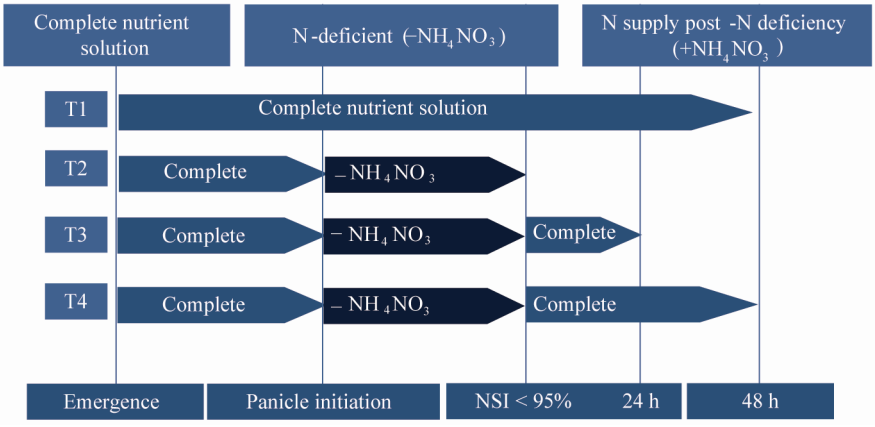

Figure 1. Schematic diagram of the N treatments.

$$\text{NSI, \%} = \frac{\text{Average reading of plants in N-starved tub } (T_2 - T_4)}{\text{Average reading of plants } (T_1)} \times 100 \quad \text{(a)}$$

readdition). There were four replications, with three plants per replication per N treatment. Randomization was constrained by the following: each rice type was placed at both sides of the greenhouse, on two benches; each N treatment was randomly assigned to a row of three plants within each tub.

2.6. Data Collection and Statistical Analyses

To determine inherent morphological and growth rate differences between red rice and Wells rice, height and tiller number were measured weekly. After imposition of N treatments, biomass and root characteristics were recorded at harvest. To determine biomass production, plant samples were separated into roots and shoots and oven-dried to a constant weight at 60˚C. Prior to oven-drying, root samples were gently washed with deionized water, blotted dry with paper towels, stained with methylene blue in 10% ethanol, and stored at 4˚C until scanned (Epson Twain Pro, Seiko Epson Corp., Japan) for length, surface area, average diameter and number of root tips. Scanned images were analyzed using WinRHIZO 5.0 (Regent Ltd., Canada).

To determine physiological responses at N stress, shoot tissue concentrations of total N and sugars were quantified. Concentrations of glucose, fructose, and sucrose in leaf tissue were analyzed because these would indicate changes in carbohydrate metabolism in response to imposed nutrient stress. To measure total N concentration, shoots from one plant per replication were oven-dried as described for biomass determination, and their dry weights recorded prior to grinding in a rice mill (3383 L-10, Thomas Scientific, USA). Ground shoot tissues were analyzed for total N by the Dumas combustion method at the Agriculture Diagnostic Laboratory of the University of Arkansas, Fayetteville.

To determine concentration of total sugars, youngest fully expanded leaves from one plant per replication were freeze-dried to a constant weight at –70˚C in a lyophilizer (Freezemobile 25SL, Virtis, USA) before grinding. Three 100 mg samples of ground tissue from each plant sample were then extracted for total sugars following a modified procedure [38]. Sugar extracts (1 mL) were analyzed for fructose, glucose and sucrose concentrations ([Fruc], [Glu] and [Suc]) by high performance liquid chromatography (Alliance 2690 Separation Module, Waters, USA) using acetonitrile: 2-propanol:water (825:35:140) as solvent and passed through 250 mm × 2.0 mm columns (Phenosphere 5 μ NH_2 columns, Phenomenex, CA 90501, USA) at a flow rate of 0.6 ml min^{-1} at 40˚C. Sugar concentrations were calculated using the **Formula** (**b**) where 500 = factor for a 1 mL extraction volume. Data were subjected to analysis of variance using SAS® (v8.2, SAS Institute, Inc., Cary, NC, USA). When F-tests were significant, means were separated using Fisher's protected LSD at a significance level of 0.05.

3. Results and Discussion

3.1. Developmental Differences between Rice Types

The two rice types reached R_0 within 2 d of each other. There was no difference in Year 1, but the time lag extended to 2 d in Year 2. This year difference may be attributed to greenhouse temperatures, as experiments were established at different periods of the year. In general, indica varieties require higher minimum temperatures than japonica varieties [39-41]; red rice is an indica, while Wells is a japonica. Optimum germination of japonica rice seeds is at a 20˚C day temperature, and at 30˚C - 35˚C day temperatures for an indica [42]. The optimum temperature range for photosynthesis in indica rice varieties was reported to be 25˚C - 35˚C, higher than that of japonica (18˚C - 33˚C) [43]. During vegetative growth, indica varieties were more sensitive to lower temperatures than japonica varieties when partial regression of days to heading on mean temperatures was done [44-46]. Air temperature was the most important factor which affected yields of indica varieties, followed by day length [47]. Red rice ecotypes also differ in maturation period [32].

3.2. Overview of Data Analysis Results

There were significant differences in plant responses to N treatments between years, thus data were analyzed separately (**Table 1**). In Year 1, only shoot tissue [N] showed significant interaction effect of rice type and N levels. In Year 2, shoot tissue [N] and [Suc] as well as root length and surface area showed a strong evidence of rice type and N level interaction (**Table 1**).

3.3.Morphological Differences

Aboveground traits. In both years, Stf-3 grew taller and produced more tillers than Wells under full N supply (**Table 2**). Rice type effect on shoot biomass production was evident only in Year 2, with Stf-3 producing more than Wells (**Table 3**). Aboveground morphological differences in the greenhouse reflected those in field conditions, where Stf-3 can grow up to 130 cm at flowering [4] while Wells can be as tall as 100 cm at maturity [33].

$$\text{Sugar concentration } (\mu g \cdot g^{-1}) = \frac{\text{Total amount in a 2 } \mu l \text{ injection} \times 500, \mu g}{\text{Total weight of sample, g}} \quad \text{(b)}$$

Table 1.Table of p-values of ANOVA f-tests. Bold values followed by * are significant at $\alpha = 0.05$.

Response variables	Source of variation					
	Year 1			Year 2		
	Rice type (R)	N level (N)	R × N	Rice type (R)	N level (N)	R × N
Plant height	**0.0133***	0.6716	0.1801	**0.0010***	**0.0001***	0.1750
Number of tillers	**0.0034***	0.9508	0.1484	**0.0017***	**0.0148***	0.1125
Root length	0.0687	0.6556	0.7216	**0.0005***	**0.0034***	**0.0206***
Root surface area	0.1116	0.7715	0.6817	**0.0008***	**< 0.0001***	**0.0009***
Average root diameter	**0.0039***	0.0848	0.2078	**0.0006***	**< 0.0001***	0.4216
Number of root tips	0.0634	0.2932	0.7969	**0.0014***	0.0979	0.1961
Shoot dry weight	0.1169	0.4765	0.0843	**0.0074***	0.3724	0.9356
Root dry weight	0.0706	0.4019	0.1825	**0.0042***	**0.0261***	0.9346
Total dry weight	0.1044	0.5876	0.0895	**0.0065***	0.3030	0.9763
Shoot tissue total N	**0.0069***	**< 0.0001***	**0.0275***	**0.0110***	**< 0.0001***	**0.0100***
Shoot tissue total sugars						
Fructose	0.0754	0.0698	0.5310	0.1358	**0.0021***	0.0858
Glucose	0.3030	0.0841	0.5385	0.2030	**0.0034***	0.0545
Sucrose	**0.0075***	**0.0285***	0.1456	0.3801	**< 0.0001***	**0.0162***

Table 2. Growth characteristics affected by rice type, grown in complete nutrient solution (T_1).

Variable	Year	Stf-3[a]	Wells[b]	LSD[c]
Height (cm)	1	90.31	68.66	10.35
	2	70.05	50.28	6.60
Tiller number	1	7	3	1
	2	8	3	2
Ave. root diameter (mm)	1	0.361	0.446	0.022
	2	0.365	0.453	0.025

[a]Weedy red rice, n = 4. [b]Cultivated rice, n = 3. [c]Means were separated using Fisher's protected LSD at $\alpha = 0.05$.

Table 3. Growth characteristics as affected by rice type, grown grown in complete nutrient solution (T_1), Year 2.

Rice type	No. of root tips ($\times 10^3$)	Shoot DW[c] (g)	Root DW (g)	Total DW (g)
Stf-3[a]	56.124	3.73	1.09	4.82
Wells[b]	10.836	1.41	0.41	1.82
LSD[d]	16.906	1.54	0.37	1.90

[a]Weedy red rice, n = 4. [b]Cultivated rice, n = 4. [c]DW = dry weight dMeans were separated using Fisher's protected LSD at $\alpha = 0.05$.

Changing N supply resulted in detectable differences in whole-plant aboveground characteristics in Year 2 (**Table 1**). The findings that Wells generally has lower response to N compared with red rice agree with findings in field conditions, with respect to biomass accumulation [7].

Belowground traits. Average root diameter in both years also differed between rice types (**Table 2**). In Year 2, red rice had 6 times more root tips and 3 times more shoot and root biomass than Wells (**Table 3**). Differences in root length and surface area due to the interaction of rice type and N treatment was also evident, particularly in Stf-3 (**Table 4**). T_2 plants had visible, but not sig- nificant, retardation in root growth and expansion of root surface area in Stf-3 relative to plants grown in T_1, but the change in Wells was imperceptible (**Table 4**). At T_4, Stf-3 significantly increased root length and root surface area, but not Wells. N treatment effect on root biomass was also evident in Year 2, with the greatest root dry weight observed at T_1 (**Table 5**). Thus, red rice response to restoration of full N supply after starvation was evident in root morphology within 48 hr, but not in Wells rice.

Table 4. Growth characteristics affected by the interaction of rice type and N treatment, Year 2[a].

N treatment	Root length (m)		Root surface area (m^2)	
	Stf-3	Wells	Stf-3	Wells
T_1 (complete)	88.17	14.54	1006	203
T_2 ($-NH_4NO_3$)	62.90	22.70	609	298
T_3 (24 h complete post-N deficiency)	85.63	23.37	1010	346
T_4 (48 h complete post-N deficiency)	131.43	29.22	1700	446
[b]LSD_1	54.28		570	
LSD_2	27.97		319	

[a]Rice types were weedy red rice (Stf-3) and cultivated rice (Wells). Means were separated using Fisher's protected LSD at $\alpha = 0.05$ (n = 4). [b]LSD_1 separates means within same rice type; LSD_2 separates means for different rice types.

Table 5. Growth characteristics and shoot nutrient concentrations affected by N treatment, averaged over rice types, Year 2[a].

N treatment	Root DW (g)	Fructose ($\mu g \cdot g^{-1}$)	Glucose ($\mu g \cdot g^{-1}$)
T_1 (complete)	0.52	38.61	62.73
T_2 ($-NH_4NO_3$)	0.89	51.43	87.63
T_3 (24 h complete post-N deficiency)	0.78	32.90	61.71
T_4 (48 h complete post-N deficiency)	0.81	29.98	58.34
LSD[b]	0.24	10.39	15.56

[a]Rice types were weedy red rice (Stf-3) and cultivated rice (Wells), n = 8. [b]Means were separated using Fisher's protected LSD at $\alpha = 0.05$.

Root characteristics are correlated with nutrient access to and uptake from the rhizosphere and are significant factors in underground competition. Changes in root architecture are typical responses in plants during nutrient stress as an adaptive mechanism to increase nutrient access [8]. The effect of N supply on root growth of Stf-3 observed in Year 2 confirmed similar findings in cultivated rice [49] which showed that NO_3^- stimulates root elongation and growth of root hairs. On the other hand, N supply effect on root morphology was not evident in other studies [50] as was observed with Wells in this current research. Since differences in root morphology between Stf-3 and Wells had been consistent regardless of N supply, genotypic effect was strongly evident. Stf-3 responded more to N supplementation than Wells, producing longer roots and greater root surface area after some recovery period, which equate to greater N uptake capacity than that of Wells. While Stf-3 had visibly longer and finer root hairs, Wells had consistently thicker roots compared with Stf-3. Larger roots offer stronger plant support, but have smaller surface areas and fewer root tips for nutrient absorption. The number of root tips is indicative of the ability of plants to absorb nutrients [51]. Therefore, more root tips and greater root surface area in Stf-3 than in Wells must have contributed to greater leaf tissue [N] in Stf-3 than in Wells. Root characteristics of Stf-3 indicate that, at the whole-plant level, an extensive root system and a faster root growth response to N supplementation contribute greatly to the nutrient uptake advantage of weedy rice over cultivated rice.

3.4. Physiological Differences

Shoot tissue [*N*]. Differences in shoot tissue [N] as affected by the interaction of N treatment and rice type were significant in both years (**Table 6**). Stf-3 had higher [N] in its shoot tissue than Wells when grown under complete nutrient solution. The [N] in Stf-3 and Wells declined by 32% and 30%, respectively, in Year 1 and 38% and 37%, respectively in Year 2 at NSI < 95% (T_2), relative to plants grown in T_1. At T_4, Stf-3 showed a significant increase in shoot tissue [N] in Year 1. This was observed even earlier (T_3) in Year 2. Although Wells did not show a significant increase in [N] even at T_4 in Year 1, it showed full recovery of shoot tissue [N] under the same N conditions in Year 2. Within 48 h of post-N deficiency (T_4), both rice types had lesser shoot tissue [N] than plants grown in T_1 in Year 1, but in Year 2 both rice types recovered faster from N stress than in Year 1, showing similar shoot tissue [N] as those grown in T_1.

Table 6. Shoot tissue N concentrations ($mg \cdot kg^{-1}$) affected by the interaction of rice type and N treatment.

N treatment	Year 1		Year 2	
	Stf-3[a]	Wells[b]	Stf-3	Wells
T_1 (complete)	42.15	31.63	53.90	46.28
T_2 ($-NH_4NO_3$)	28.48	22.20	33.20	29.05
T_3 (24 h complete post-N deficiency)	32.53	26.47	43.28	36.90
T_4 (48 h complete post-N deficiency)	36.30	26.43	46.50	50.33
[c]LSD_1	5.02		9.81	
LSD_2	3.10		4.77	

[a]Weedy red rice, n = 4. [b]Cultivated rice, n = 3. [c]Means were separated using Fisher's protected LSD at $\alpha = 0.05$. LSD_1 separates means within same rice type; LSD_2 separates means for different rice types.

Differences in shoot tissue [N] as affected by interaction of N treatment and rice type confirmed that exogenously applied N at varying levels was absorbed at different amounts by Wells and Stf-3, and that accumulation in shoot tissue also varied according to N supply. Generally, shoot tissue [N] was greater in Stf-3 than in Wells at control and treated conditions, except in Year 2 when both plants had similar concentrations at T_2 and T_4 (**Table 6**). This corroborated reports of higher N uptake capacity of Stf-3 as indicated by its inherently more extensive root system and its apparent root growth response to added N compared to Wells. There is evidence, therefore, supporting our hypothesis that red rice is able to accumulate N better than cultivated rice, considering its biomass production and shoot [N]. Both plants attained [N] similar to unstressed plants (T_1) after 48 h of N supply post-N deficiency (T_4).

Total sugars. [Fruc] and [Glu] were affected by N treatments in Year 2 (**Table 5**), where the greatest concentration was observed at T_2. Differences were most detectable in [Suc], considering that in higher plants, sucrose is the major sugar for transport throughout the

plant. Sucrose concentrations differed by rice type ($LSD_{0.05}$ = 0.036), with Stf-3 having greater [Suc] (0.194 $mg \cdot g^{-1}$) than Wells (0.114 $mg \cdot g^{-1}$). The effect of N treatment on [Suc] was also evident in Year 1, when the lowest [Suc] was observed at T_1, averaged over rice type (**Table 7**). In Year 2, the interaction effect of rice type and N treatment on [Suc] was evident, when [Suc] in Stf-3 was greatest at T_2 and declined with duration of N supply post-N deficiency (**Table 8**). A similar trend was observed in Wells, except that the change in [Suc] from one treatment to another was not significant.

Varying shoot tissue [Suc] indicate that Stf-3 responded to N treatments to a greater extent than Wells in Year 2, since Wells [Suc] at optimum N concentrations was not different from that at 0 N (**Table 8**). Moreover, [Suc] in Stf-3 declined quickly with time of recovery, approaching its baseline level at full N, whereas [Suc] in Wells hardly changed regardless of N treatment. Increased [Suc] in red rice under N deficiency corroborates evidence for the involvement of soluble sugars in stress response and their role as nutrient and metabolite signaling molecules [52]. Thus, under N deficiency, increased [Suc] in both Stf-3 and Wells, albeit comparatively lower in the latter, may be a stress signaling mechanism for the plant to stimulate N uptake [53-55]. In this case, the signaling mechanism of Stf-3 may be more efficient than that of Wells. For example, plants are able to adapt to cold stress by accumulating sugars [56]. However, much remains to be done in characterizing the many signaling pathways of sugar-induced responses to stress, considering that most investigations have been limited to sugar-induced stress responses in relation tohormones and growth regulators [57].

Table 7. Shoot sucrose concentrations as affected by N treatment, averaged over rice types, Year 1[a].

N treatment	Sucrose ($mg \cdot g^{-1}$)
T_1 (complete)	0.079
T_2 ($-NH_4NO_3$)	0.198
T_3 (24 h complete post-N deficiency)	0.163
T_4 (48 h complete post-N deficiency)	0.200
LSD[b]	0.041

[a]n = 7. [b]Means were separated using Fisher's protected LSD at α = 0.05.

Table 8. Shoot sucrose concentrations affected by the interaction of rice type and N treatment, Year 2[a].

N treatment	Sucrose ($mg \cdot g^{-1}$)	
	Stf-3	Wells
T_1 (complete)	0.292	0.386
T_2 ($-NH_4NO_3$)	0.626	0.432
T_3 (24 h complete post-N deficiency)	0.443	0.274
T_4 (48 h complete post-N deficiency)	0.181	0.237
[b]LSD_1	0.304	
LSD_2	0.242	

[a]Rice types were weedy red rice (Stf-3) and cultivated rice (Wells). Means were separated using Fisher's protected LSD at α = 0.05 (n = 4). [b]LSD_1 separates means within the same rice type; LSD_2 separates means for dif- ferent rice types.

4. Implications and Recommendations

Our findings corroborate earlier reports on red rice accumulating more N than cultivated rice. Differences in response to N treatments between Stf-3 and Wells rice suggest different adaptive mechanisms within the N metabolic pathway, as well as the role of sucrose as a stress signaling molecule. For instance, the stimulatory effect of N supply, particularly NO_3^-, on root elongation, has been demonstrated to regulate the transcription of many genes in rice, including those involved in signal transduction, transcription regulation, auxin transport and ethylene synthesis. Genomic analysis to identify genes involved in these pathways in response to N stress conditions would help answer these questions.

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