Indian J. Genet., 78(3): 292-301 (2018)
DOI: doi.org/10.31742/IJGPB.78.3.3

Evaluation of genotypic variation for growth of rice seedlings under optimized hydroponics medium

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(Received: April 2018; Revised: July 2018; Accepted: August 2018)

Abstract

Although rice (*Oryza sativa* L.) is a semi-aquatic plant, its growth in solution culture is often problematic. In commonly used rice hydroponics media, plants exhibited mild nitrogen deficiency, leaf tip burn, salt deposition, along with zinc and iron deficiency. Therefore, we aimed to optimize the nutrient media for growing rice plants taking into consideration the nutrient concentration, pH and ratio of NH_4^+ to NO_3^- nitrogen (N) and named it as '*Pusa Rice* Hydroponics' (PusaRicH). PusaRicH contains higher amounts of macronutrients, significantly lower B, Mn and Cl and higher amounts of Zn than two commonly used hydroponics media, 'Yoshida' and 'Kumura B'. The optimal ratio of NH_4^+ to NO_3^- – N in PusaRicH medium was 0.5 mM NH_4^+ and 7.0 mM NO_3^- with pH 5.0. The PusaRicH medium was validated by growing 100 diverse rice genotypes and it significantly outperformed the widely cited 'Yoshida' and 'Kimura B'. Cluster analysis carried out on the squared Euclidean distance matrix of biomass and leaf area values of genotypes revealed four major clusters in all hydroponics medium. However, only PusaRicH medium resulted in ten genotypes as good performers in comparison to other two widely cited media. Therefore, the optimized PusaRicH medium can be used successfully to grow rice seedlings in hydroponic system which will aid in screening large number of genotypes in breeding and other physiological experiments.

Key words: Ammonium/nitrate ratio, elemental composition, hydroponics culture, media pH, *Oryza sativa*

Introduction

Growing plants in solution culture or hydroponics has important benefits in plant biology research as it provides better accessibility to roots and allow easy manipulation of nutrient profiles of the growth medium. This makes them most suitable for studying mechanisms of nutrient deficiency or toxicity, uptake, homeostasis and ion interaction or isotope labelling experiments. In addition, hydroponic system is now widely adopted for studying abiotic stresses like salinity and drought. A standard hydroponic medium was given by Hoagland (1938) and the nutrient composition of various media has essentially been derived from Hoagland's media (Hoagland and Arnon 1950). It is highly recommended that the concentration of macro- and micro-elements should be adjusted for every crop species as the nutrient requirement varies.

Rice being a typical model system for several monocot crops, it is widely used for physiological and molecular studies. Large genotypic variation exists in among rice genotypes for nutrient use efficiency as well as root traits which can be evaluated effectively at seedling stage using hydroponics system (Naher et al. 2014; Aluwihare et al. 2016; Chithrameenal et al. 2017). A range of specialized hydroponics medium has been published over the years by various laboratories such as Kimura B (Baba and Takahashi 1956), Yoshida (Yoshida et al. 1976), Alam's solution (Alam 1981), Makino 1988 (Makino et al. 1988), Kamachi (Kamachi et al. 1991), Yang 1994 (Yang et al. 1994), Mckeehan's solution (Mckeehan et al. 1996), Gui 2013 (Chen et al. 2013) and Ishimura (Ishimaru et al. 2006) (Suppl. Table 1). Growing rice in hydroponics system is challenging as reported by Kohl (2015). Also, the difficulty faced during our studies on nutrient responses and root behavior as well as frequent queries from other laboratories working on rice, compelled us

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Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by indianjournals.com; www.isgpb.org

to modify the nutrient solution used for rice hydroponics. The major problems encountered while growing rice seedlings in hydroponics were retarded root growth with very few lateral roots, occasional root browning, stunted and/or chlorotic shoot, and salt deposition on tips of young seedlings. The poor growth symptoms have been observed for many high yielding rice varieties with high nutrient demand as mentioned elsewhere (https://www. researchgate.net/post/Rice_plants_grown_in_ hydroponic_culture_turning_yellow). A comparison of elemental composition of standard Hoagland media with various published rice hydroponic solutions clearly showed that the ratio of nitrogen (N) to potassium (K), and calcium (Ca) to magnesium (Mg) was higher in Hoagland solution (Suppl. Table 1). In comparison to Hoagland, the concentration of N was 3.4 to 14.0 times less in other hydroponics solutions. Similarly, concentration of phosphorus (P) was 33 times, K up to 30 times and sulfur (S) 22 times lower in published hydroponic solutions for rice culture as compared to Hoagland solution. Rice requires higher K concentration (Wang et al. 1996) which also provides it tolerance against abiotic and biotic stresses (Rashid et al. 2016). In published hydroponic solutions, the ratio of Ca to Mg is less than 1.0 except for a few cases such as Alam's, Mckeehan's and Ishimaru's solution. The lower concentration of Ca leads to poor root development of seedlings.

Sub-optimal composition of nutrient elements, unbalanced ratio of ammonium (NH_4^+) and nitrate (NO_3^-) as N source, and unstable pH of the medium have been reported as the main cause for poor growth of rice seedlings. The ratio of NH_4^+ and NO_3^- N is critical in rice as addition of NO_3^- enhanced growth and yield (Duan et al. 2006; Shavrukov et al. 2012). Although lowland rice prefers NH_4^+, as a source of N, it grows better and utilizes N efficiently when both NO_3^- and NH_4^+ forms are supplied together (Ta et al. 1981). It has been suggested that rice roots may take up 15 to 40% of total N in the form of NO_3^- even from waterlogged paddy soil (Li et al. 2008). These reports suggest that an optimum ratio of NH_4^+ and NO_3^- is crop specific and under aerobic condition rice requires more NO_3^- than NH_4^+. But the preferential uptake of NH_4^+ over NO_3^- causes differential depletion of ions which significantly alters the pH of the media (Sasakava and Yamamoto 1978). So, stabilization of the pH of hydroponic culture medium is often required based on the concentration and form of salts used. Different pH (in the rage of 4.5-6.0) of the hydroponics medium has been used for growing rice such as 4.5 (Chen et al. 2006), 5.0 (Yoshida et al. 1976), 5.2 (Balasta et al. 1989), 5.5 (Murty and Ladha 1988), 5.7 (Kamachi et al. 1991), and 5.8 to 6.0 (Mckeehan et al. 1996). Even though the medium contains adequate amount of nutrient element, its pH plays an important role in determining nutrient availability to the plants. In general, most of the plant species grow well in nutrient solutions with slightly acidic to neutral pH (Munns and James 2003). The ratio of different forms of N, NH_4^+ and NO_3^-, also helps in maintaining pH of nutrient solution. For example, hydroponic culture of wheat and barley in nutrient solution containing equimolar concentration of ammonium nitrate (NH_4NO_3) and potassium nitrate (KNO_3) rapidly acidified the medium due to preferential uptake of NH_4^+ which is associated with release of protons to maintain charge balance (Shavrukov et al. 2012). Thus, maintaining the solution pH, providing adequate amounts of macro-nutrients and optimizing the ratio of NH_4^+ to NO_3^- N is key to growing plants successfully in hydroponic system. It is utmost important to reassess the nutrient composition of hydroponics media to meet the nutrient demand by modern rice cultivars. In this study, we optimized a hydroponics medium named '*Pusa Ric*e *H*ydroponics' (PusaRicH), to grow rice plants successfully under controlled conditions for various physiological, molecular and genotypic studies. A major difference between PusaRicH and other hydroponic media is the higher concentration of macro-nutrients (N, P, K, Ca and Mg), but lower than Hoagland solution, with 5.0 pH, same as that of Yoshida medium (Table 1 and Suppl. Table 1). Moreover, we used a higher ratio of NO_3^- to NH_4^+ as this medium can be used to grow and screen rice genotypes for upland or aerobic condition too.

Materials and methods

Plant material and growth conditions

Rice (*Oryza sativa* L.) variety MTU 1010 (Krishnaveni × IR-64) was selected for this study. It is a semi-dwarf variety suitable for both upland as well as irrigated mid-land regions and resistant to blast and tolerant to brown plant hopper. Seeds were surface sterilized with 0.1% $HgCl_2$ followed by 4-5 washings and soaking in double distilled water overnight. Imbibed seeds were then rolled in moist germination paper as 'cigar rolls', which facilitates vertical root growth and easy seedling transfer (Suppl. Fig. 1A). The basal end of cigar roll was dipped in distilled water contained in a magenta box and kept in incubator at 28°C in dark for

Table 1. Concentration and type of chemical compounds used in hydroponics media commonly used by researchers to grow rice seedlings. The composition of 'PusaRicH' (Pusa Rice Hydroponics) media was determined in the present study

Macronutrients	Yoshida's solution		Kimura B solution		PusaRicH solution	
	Source	Conc. (mM)	Source	Conc. (mM)	Source	Conc. (mM)
N	NH_4NO_3	1.43	$(NH_4)_2SO_4$	0.36	$(NH_4)_2SO_4$	0.5
			KNO_3	0.18	KNO_3	7.0
			$Ca(NO_{3)}$	0.35		
P	NaH_2PO_4	0.35	KH_2PO_4	0.14	H_3PO_4	0.5
K	K_2SO_4	0.51	KNO_3	0.18	KNO_3	7.0
			K_2SO_4	0.09		
			KH_2PO_4	0.14		
Ca	$CaCl_2$	1.0	$Ca(NO_{3)}$	0.35	$CaCl_2$	1.5
Mg	$MgSO_4$	1.64	$MgSO_4$	0.27	$MgSO_4$	2.0
S	K_2SO_4	0.51	$(NH_4)_2SO_4$	0.36	$(NH_4)_2SO_4$	0.5
	$MgSO_4$	1.64	K_2SO_4	0.09	$MgSO_4$	2.0
			$MgSO_4$	0.27		
Micronutrients		(µmol)		(µmol)		(µmol)
Fe	$FeCl_3$ + Citric acid	35.0	Fe-citrate	35.0	Fe-EDTA	40.0
B	H_3BO_3	18.89	H_3BO_3	18.89	H_3BO_3	1.0
Mn	$MnCl_2.4H_2O$	15.02	$MnCl_2.4H_20$	15.02	$MnCl_2.4H_20$	0.5
Cl	$MnCl_2.4H_2O$	15.02	$MnCl_2.4H_20$	15.02	$MnCl_2.4H_20$	0.5
Zn	$ZnSO_47H_2O$	0.15	$ZnSO_47H_20$	0.15	$ZnSO_47H_20$	1.0
Cu	$CuSO_45H_2O$	0.16	$CuSO_45H_20$	0.16	$CuSO_45H_20$	0.2
Mo	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.07	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.07	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.075
pH	5.0 (1 N HCl and 1 N NaOH)		4.5 (1 N HCl and 1 N NaOH)		5.0 (1 N HCl and 1 N KOH)	

germination. After emergence of coleoptiles, 5-6 days after sowing, the seedlings were transferred to half strength nutrient solution (respective media used in this study) for 3 days. From day four onwards, full strength nutrient media was supplied and renewed every fourth day. Plants were supported on thermocol sheet of 5 cm thickness fitted in black plastic tubs with 15 L capacity. Thick thermocol sheet provided strong mechanical support to the plants and barred light from entering into the rooting zone thus preventing algal growth in the nutrient medium. The nutrient solution was aerated continuously using aquarium pumps with PVC tubing and connectors. The experiment was setup in the glasshouse at the National Phytotron Facility (NPF), IARI, New Delhi. The temperature was maintained at 30^{o}C day/22^{o}C night under natural light and humidity (80-85%) in the glasshouse. Three sets of experiments were conducted to determine the (i) optimum concentration of nutrient elements, (ii) pH, and (iii) best combination of NH_4^+ and NO_3^- N, in the media. All experiments were conducted in completely randomized block design with five replications each. Throughout these experiments, deionized water was used for preparing nutrient solutions. Care was taken to adjust the pH of hydroponic media every day using 1.0 N HCl or 1.0 N KOH (NaOH was replaced with KOH to avoid any salt stress to plants that may occur due to sodium ions in the medium).

Experiment 1: Optimum nutrient composition of hydroponics media for growing rice seedlings

We used three different media compositions for growing rice hydroponically, out of which two compositions were commonly cited by several workers, *viz*., 'Yoshida' (Yoshida et al. 1976), 'Kimura B' (Baba and Takahashi 1956) while the third composition was modified from Hoagland, which we termed as 'PusaRicH'. A comparison between compositions of macro- and micro-nutrients in these media is presented in Table 1. Seedlings were transferred to 'Yoshida', 'Kimura B' or 'PusaRicH' medium and grown for 15 days only which suffices for the purpose of this study. Destructive sampling was done to measure total leaf area, shoot and root dry weight. Leaf area (cm^2 $plant^{-1}$) was measured using leaf area meter (Li-COR 3000, Lincon Nebraska, USA). For dry weight, plants were separated into root and shoot and dried in hot air oven at 65°C until a constant weight was obtained and expressed as g $plant^{-1}$.

Experiment 2: pH optimization for PusaRicH medium

Following the results of experiment 1, the pH of the PusaRicH solution was maintained at 4.5, 5.0, 5.5 and 6.0 to study the effect on growth. Yoshida and Kimura B media were included for comparison with their respective pH. Rice seedlings were grown for 15 days keeping all other growth conditions similar as in experiment 1.

Experiment 3: Optimizing NO_3^- and NH_4^+-N ratio in rice hydroponics medium

The plants were grown in PusaRicH medium (pH 5.0) with different concentration of NO_3^- and NH_4^+, supplied by KNO_3 and ammonium sulphate [$(NH_4)_2SO_4$] salts. Keeping total N concentration as 7.5 mM, as mentioned in Table 1 for PusaRicH medium, only the ratio of NH_4^+ and NO_3^- were varied. The treatments were: T1 – 7.0 mM NH_4^+ and 0.5 mM NO_3^-, T2 – 1.0 mM NH_4^+ and 6.5 mM NO_3^-, T3 – 2.0 mM NH_4^+ and 5.5 mM NO_3^-, T4 – 3.0 mM NH_4^+ and 4.5 mM NO_3^-, T5 – 5.0 mM NH_4^+ and 2.5 mM NO_3^-, T6 – 0.5 mM NH_4^+ and 7.0 mM NO_3^-, T7 – 7.5 mM NO_3^-, T8 – 7.5 mM NH_4^+. The seedlings were grown for 15 days before taking measurements as mentioned in experiment 1.

Validation of optimized rice hydroponics medium

To validate the optimized hydroponics medium, PusaRicH, a panel of 100 rice genotypes belonging to *Indica* type were grown and compared with Yoshida and Kimura B (Suppl. Table 2). The same procedure was followed as mentioned previously for raising the seedlings. Shoot and root biomass and leaf area were measured as mentioned in the experiment 1.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) and means were compared with critical difference (CD) at $P < 0.05$ significance. For validation of optimized media, Tukey's Multiple Comparison Test was carried out at significance level $P < 0.01$ to compare the PusaRicH medium with other media. Hierarchical cluster analysis based on Ward's method was performed on the squared Euclidean distance matrix of genotypic means for trait variables using the statistical software R version 3.1.2 (R Foundation for Statistical Computing, Vienna). Graphs were made using GraphPad Prism version 6.00 (GraphPad Software, La Jolla, CA).

Results and discussion

Initially, we followed the commonly used methods and well cited nutrient medium compositions for growing rice plants but encountered significant number of problems in growing rice plant. Visual symptoms similar to that of mild N deficiency, leaf tip burns and salt deposition on tips of young leaf could be observed (Fig. 1A). After 10 to 12 days of transplanting, plants were stunted and showed symptoms of zinc (Zn) and iron (Fe) deficiency. Similar problem was also reported by researchers working on rice in other laboratories (personal communication). Therefore, we felt it necessary to make necessary adjustment in the nutrient medium for optimal growth of rice plants. After testing several combinations/concentration of nutrients, we could successfully standardize the optimal hydroponics medium for rice.

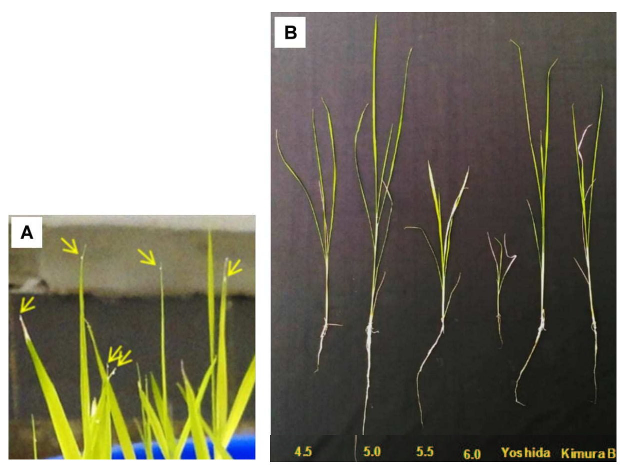


Fig. 1. (A) Rice (var. MTU1010) plants grown in commonly available hydroponics media (Yoshida) showing salt deposition on leaf tips (arrows) followed by necrosis. (B) Optimization of pH for rice hydroponics media. Rice plants grown in 'PusaRicH' medium with different pH and in 'Yoshida' with pH 5.0 and 'Kimura B' with pH 4.5

Optimized elemental composition for rice hydroponics

Experiment 1 with PusaRicH media revealed overall optimum plant growth (Suppl. Fig. 1B). A significant reduction in shoot biomass was observed in Yoshida (28%) and Kimura B (38%) medium as compared to that observed in PusaRicH medium (Fig. 2A). Similar trend was observed for root biomass which was significantly lower in Yoshida (47%) and 'Kimura B' (45%) in comparison to PusaRicH. Further, the total leaf area was also significantly lower for plants grown in Yoshida (33%) and Kimura B (44%) medium in comparison to PusaRicH grown plants (Fig. 2B). Improved growth of rice seedlings in 'PusaRicH' compared to other published media might be due to the higher concentration of essential nutrient elements which corroborate with the observations reported earlier in case of wheat and barley (Alam et al. 2001; Pandey et al. 2015). Comparison of the rice hydroponics media

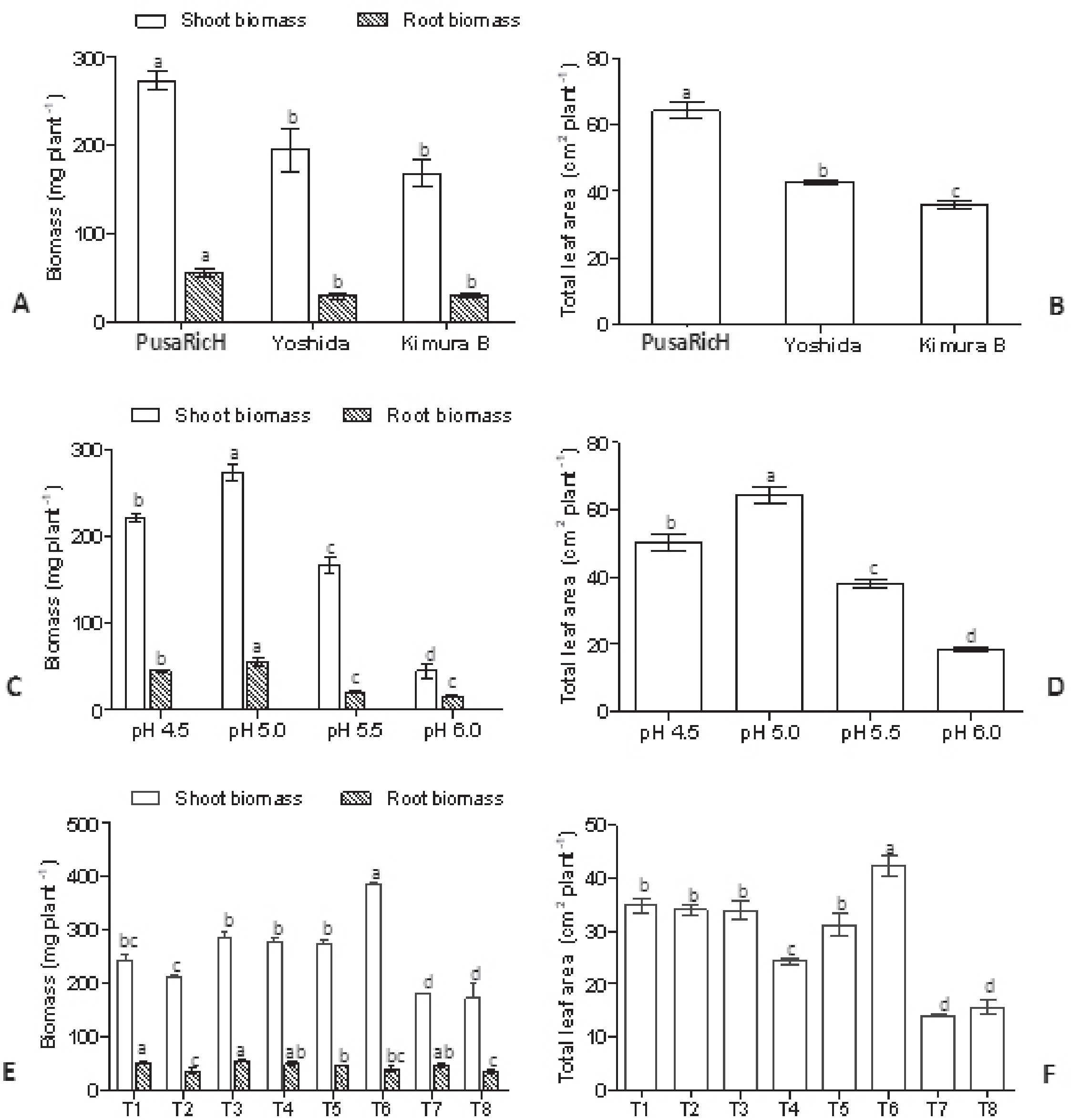


Fig. 2. Effect of different media, pH and nitrate-to-ammonium ratio on root and shoot biomass and leaf area of 15 days old rice plants grown in hydroponics. (A, B) Comparison of commonly citedrice hydroponics media with standardized PusaRicH medium; (C, D) Optimization of pH of rice hydroponics media, and (E, F) Optimization of ratio of NO_3^- and NH_4^+ for growing rice in hydroponics. Mean + SEm, n = 5. Mean with same letter are not significantly different at P < 0.05 according to least significant difference test. The basal nutrient solution used was 'PusaRicH' media. For explanation of treatments T1-T8, see text

PusaRicH with the widely cited, Yoshida and Kimura B, revealed a considerable difference in the concentrations of macro-nutrients and micro-nutrients (Table 1). The concentration of macro-nutrients of Yoshida and Kimura B hydroponics media are very low and might not be sufficient to support optimum growth of modern rice varieties.

pH of medium for rice hydroponics

Plants grown in PusaRicH medium with pH 5.0 showed significantly higher biomass in comparison to those grown at pH 4.5, 5.5 or 6.0 in PusaRicH medium. But even in Yoshida at pH 5.0 or Kimura B at pH 4.5, plants did not reach optimum growth as compared to PusaRicH (pH 5.0) (Fig. 1B). Shoot biomass was significantly lower by 19%, 39% and 84% at pH 4.5, 5.5 and 6.0, respectively as compared to pH 5.0 (Fig. 2C). Similarly, root biomass was also significantly reduced by 19%, 63% and 72% (Fig. 2C). Thus, plants maintained an optimum root to shoot ratio at pH 5.0. Total leaf area was drastically reduced for the plants grown at pH 5.5 (41%) and 6.0 (71%) but the reduction was less at pH 4.5 (22%) in comparison to those grown at pH 5.0 (Fig. 2D). Our result is in agreement with Alam (1981, 1984) who reported that rice plants exhibit normal growth and accumulated high biomass in nutrient medium with pH 4.5 to 5.0. Uptake of nutrients by plants results in alteration of pH of nutrient solution. This change in pH might be due to preferential uptake of ions, with the extrusion of protons or anions from the roots. Uptake of cations (Ca^{2+}, K^+, Mg^{2+}) from solution causes H^+ extrusion from root system to equalize the ratio of anions and cations in the root zone thus resulting in lowering the of pH of the medium (Sasakawa and Yamamoto 1978). Similarly, uptake of anions such as NO_3^- by plants leads to increase in pH due to release of OH^- into the media (Mengel and Schubert 1985; Sas et al. 2002). Under unfavourable conditions in rhizosphere such as pH extremes or nutrient deficiency, roots release compounds like protons, organic acids, siderophores, sugars, phenols and amino acids (Vengavasi and Pandey 2016a,b). Further, presence of cations such as Ca^{2+}, Mg^{2+}, K^+ or Na^+ in the external media resulted in extrusion of H^+ by maize roots thus lowering the pH of growing media (Mengel and Schubert 1985). Release of such compounds from plants aids in adjusting the pH of external media to a certain level which improves nutrient availability to roots. In the present study, while monitoring the pH of hydroponic solution on daily basis, it was observed that the media with pH 4.5, 5.5 and 6.0 were all changing towards pH 5.0 which might be the equilibrium pH resulting from preferential nutrient uptake and root exudation. This suggests that the optimal pH for rice growth in hydroponic media is likely to be5.0.

Ratio of NO_3^- and NH_4^+ for rice hydroponics medium

The combination of NH_4^+ and NO_3^- in nutrient a media for growing rice in hydroponics (Fig. 2E,F) showed that total biomass accumulation (426 mg plant^{-1}) and leaf area (42.2 cm) was maximal in T6 (0.5 mM NH_4^+ and 7.0 mM NO_3^-) as compared to N supplied either through NO_3^- (T7, 7.5 mM NO_3^-) or NH_4^+ (T8, 7.5 mM NH_4^+). Maximum root biomass was observed in T3 (2.0 mM NH_4^+ and 5.5 mM NO_3^-). An optimal ratio of NH_4^+ and NO_3^- ions in media helps in maintaining the pH within a favourable range whilst providing either forms of N alone disrupts the pH or creates toxicity (Mengel et al. 1983; Shavrukov et al. 2012). We observed T6 (0.5 mM NH_4^+ and 7.0 mM NO_3^-) to be the most favourable combination of NO_3^- and NH_4^+ ions in hydroponic medium, thus providing an opportunity to screen rice genotypes for growing under aerobic condition.

Validation of optimized rice hydroponics medium 'PusaRicH'

The optimized PusaRicH medium was validated by growing a diverse set of rice genotypes including landraces. Tukey's multiple comparison test showed that shoot and root biomass and total leaf area of plants grown in PusaRicH medium were significantly higher ($P < 0.01$) than those observed in Yoshida and Kimura medium (Table 2). No significant difference was observed between Yoshida and Kimura media for shoot biomass and leaf area, but root biomass did differ significantly ($P < 0.05$). The mean of genotypes for shoot and root biomass and leaf area was significantly higher in PusaRicH as compared to Yoshida or Kimura (Fig. 3A-C). This difference in growth indicates that the concentration of macro elements in hydroponics medium was not sufficient to adequately support rice plants in either Yoshida or Kimura. It is also evident from the concentration of macro elements (Table 1) that Kimura possesses the lowest concentration as compared to Yoshida. Similarly, Kohl (2015) also reported a lower concentration of N, P, K and Ca in all published rice hydroponics solution including Yoshida and Kimura B as compared to Hoagland. However, in PusaRicH medium, the concentration of N and P is 50% lower, while K is 14% and Mg is 50% higher in comparison

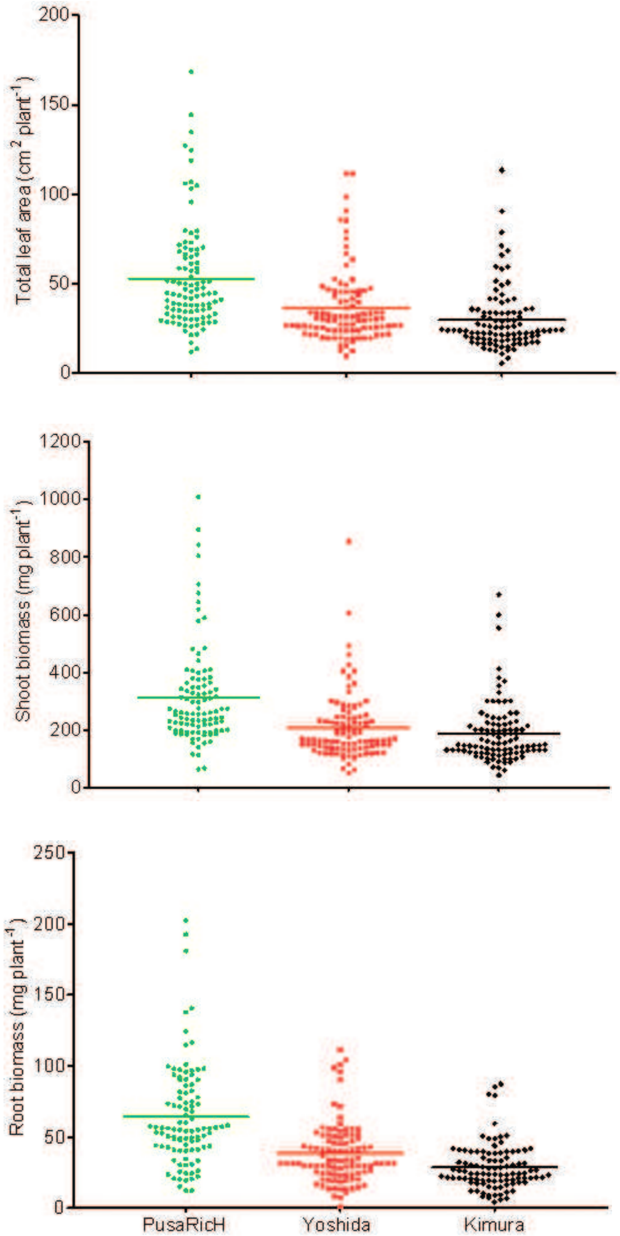


Fig. 3. Scatter dot plot of diverse rice genotypes (100 number) grown in PusaRicH, Yoshida and Kimura nutrient solutionsfor 15 days. (A) total leaf area, (B) shoot biomass, and (C) root biomass. Horizontal line represents mean value, and each dot represents a genotype

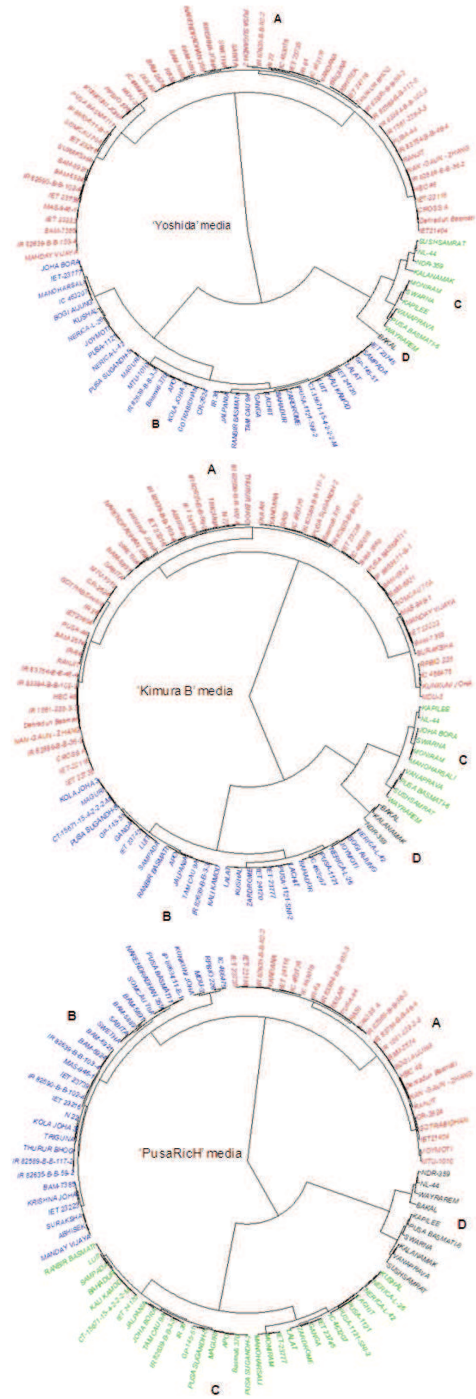


Fig. 4. Dendrogram (circular) depicting grouping of 100 rice genotypes into four distinct clusters based on total leaf area, shoot and root biomass. The genotypes were grown in popular rice hydroponics media namely, Yoshida, Kimura B and PusaRicH (optimised for rice). Cluster A (Red font) represents poor performer while genotypes in cluster D (black font) are best performers. Cluster B (blue font) and cluster C (green font) were the intermediates between best and poor performers

to Hoagland. Nevertheless, the concentrations of micro-elements were also substantially altered in PusaRicH compared to the other two media based on their requirement at seedling stage. We observed that requirement of zinc (Zn) was higher than boron (B), copper (Cu) or manganese (Mg) and the high concentration of chloride (supplied as $MnCl_2$) was not

Table 2. Tukey's Multiple Comparison Test for comparing the three different hydroponics media used for growing 100 diverse rice genotypes

Hydroponics media	Mean difference	Std. error of difference (q)	Significance level
Shoot biomass			
PusaRicH vs Yoshida	104	7.75	***
PusaRicH vs Kimura	125	9.35	***
Yoshida vs Kimura	21.4	1.6	ns
Root biomass			
PusaRicH vs Yoshida	26	10.1	***
PusaRicH vs Kimura	35.5	13.8	***
Yoshida vs Kimura	9.51	3.68	*
Leaf area			
PusaRicH vs Yoshida	16.4	7.07	***
PusaRicH vs Kimura	23.3	10	***
Yoshida vs Kimura	6.93	2.99	ns

ns -not significant; * significant at $P > 0.05$; *** significant at $P > 0.001$

required. Further, the pH was adjusted using KOH instead of NaOH which adds K ion to the media, and avoids any (Na^+) stress.

The rice genotypes were grouped into four clusters based on the Euclidean distance between genotypes significantly differing in growth traits (Fig. 4). Cluster A (red font) includes 30 genotypes common in all hydroponics media and are poor performer while cluster D (black font) possessed only one genotype (Bakal) which showed optimum growth in all media. The other genotypes performed best in PusaRicH were Pusa Basmati-6, Wayrarem, Swarna, NDR-359, NL-44, Kapilee, Kalanamak, Sushsamrat and Vanaprava which might be due to higher concentration of nutrient elements compared to that in the Yoshida and Kimura B media. The other two clusters B (blue font) and C (green font) consists of only two (Bogi Aijung and Joymoti) and one (Moniram) genotype, respectively which were common in all three growing medium (Supplementary Table 3). Similar clustering method was adopted for grouping of diverse genotypes of rice (Guduru et al. 2018), soybean (Vengavasi and Pandey 2016a) and maize (Ganie et al. 2015) for phosphorus use efficiency. In rice, existence of genotypic variation for nutrients such as nitrogen (Guduru et al. 2018; Rao et al. 2018), phosphorus (Aluwihare et al. 2016; Chithrameenal et al. 2017), zinc (Naher et al. 2014) and manganese (Fageria et al. 2008) have been reported however some of these studies were conducted using hydroponic system. Thus, the PusaRicH medium is a better nutrient medium for proper growth of rice plants under hydroponics which allows better experimentation under controlled condition to screen the genotypes. Further, this study also provides information on grouping of rice genotypes for aerobic cultivation as the major N source used in PusaRicH was nitrate-N.

Authors' contribution

Conceptualization of research (SS, RP); Designing of the experiments (SS, RP); Contribution of experimental materials (RP); Execution of field/lab experiments and data collection (SS, PB, MKM); Analysis of data and interpretation (RP, SS); Preparation of the manuscript (RP, SS, PB).

Declaration

The authors declare no conflict of interest.

Acknowledgements

This work was funded by Virtual Fertilizer Research Center, USA [Grant No. 02838/14]. The authors are thankful to Dr. H. Malhotra for revising the manuscript.

References

Alam S. M. 1981. Effect of solution pH on the growth and chemical composition of rice plant. J. Plant Nutr., **4**: 247-260.

Alam S. M. 1984. Effect of nutrient solution pH and N-Sources (NH_4/NO_3) on the growth and elemental content of rice plants. Agronomie, EDP Sciences, **4**: 361-365.

Alam S., Kamei S. and Kawai S. 2001. Amelioration of manganese toxicity in barley with iron. J. Plant Nutr., **24**: 1421-1433.

Aluwihare Y. C., Ishan M., Chamikara M. D. M., Weebadde C. K., Sirisena D. N., Samarasinghe W. L. G. and Sooriyapathirana S. D. S. S. 2016. Characterization and selection of phosphorus deficiency tolerant rice genotypes in Sri Lanka. Rice Sci., **23**(4): 184-195.

Baba S. and Takahashi Y. 1956. Water and sand culture, In: Togari Y., Matsuo T., Hatamari M., Yamada N., Horada T., Suzuki N., Sakumotsi-Shiken-ho (Laboratory Manual in Crop Science). Nogyo-Gijitsu, Kyokai, Tokyo pp. 157-185.

Balasta M. L. F., Perez C. M., Juliano B. O., Vlllareal C. P., Lott J. N. and Roxas D. B. 1989. Effects of silica level on some properties of *Oryza sativa* straw and hull. Can. J. Bot., **67**: 2356-2363.

Chen R. F., Shen R. F., Gu P., Dong X. Y., Du C. W. and Ma J. F. 2006. Response of rice (*Oryza sativa*) with root surface iron plaque under aluminium stress. Ann. Bot., **98**: 389-395.

Chen G., Guo S., Kronzucker H. J. and Shi W. 2013. Nitrogen use efficiency (NUE) in rice links to NH_4^+toxicity and futile NH_4^+cycling in roots. Plant Soil, **369**: 351-363.

Chithrameenal K., Vellaikumar S. and Ramalingam J. 2017. Identification of rice (*Oryza sativa* L.) genotypes with high phosphorus use efficiency (PUE) under field and hydroponic conditions. Indian Res. J. Genet. Biotech., **9**(1): 23-37.

Duan Y. H., Zhang Y. L., Shen Q. R. and Wang S. W. 2006. Nitrate effect on rice growth and nitrogen absorption and assimilation at different growth stages. Pedosphere, **16**: 707-717.

Fageria N. K., Filho M. P. B. and Moreira A. 2008. Screening upland rice genotypes for manganese use efficiency. Comm. Soil Sci. Plant Analysis, **39**: 2873-2882.

Ganie A. H., Ahmad A., Pandey R., Aref I. M., Yousuf P. Y., Ahmad S. and Iqbal M. 2015. Metabolite profiling of low-P tolerant and low-P sensitive maize genotypes under phosphorus starvation and restoration conditions. PLoS One, **10**(6): e0129520. doi: 10. 1371/journal.pone.0129520.

Guduru K. K., Pandit V., Palakurthi R., Konduru S., Kandi S. R., Banavath J. N., Akila C. S. and Puli C. O. R. 2018. Identification of nitrogen efficient *Indica* rice (*Oryza sativa* L.) genotypes: a physiological and multivariate statistical approach. Current Trends Biotech. Pharmacy, **12**(1): 16-32.

Hoagland D. R. and Arnon D. I. 1950. The water culture method for growing plants without soil. Berkeley, C. A. (ed.): University of California, Agricultural Experiment Station. Circular 347.

Hoagland D. R. 1938. The water-culture method for growing plants without soil, Berkeley, C. (ed.): University of California, College of Agriculture, Agricultural Experiment Station. Circular 347.

Ishimaru Y., Suzuki M., Tsukamoto T., Suzuki K., Nakazono M., Kobayashi T., Wada Y., Watanabe S., Matsuhashi S., Takahashi M., Nakanishi H., Mori S. and Nishizawa N. K. 2006. Rice plants take up iron as an Fe^{3+}-phytosiderophore and as Fe^{2+}. Plant J., **45**: 335-346.

Kamachi K., Yamaya T., Mae T. and Ojima K. 1991. A role for glutamine synthetase in the remobilization of leaf nitrogen during natural senescence in rice leaves. Plant Physiol., **96**: 411-417.

Kohl K. 2015. Growing rice in controlled environments. Ann. Applied Biol., **167**: 157-177.

Li Y. L., Fan X. R. and Shen Q. R. 2008. The relationship between rhizosphere nitrification and nitrogen-use efficiency in rice plants. Plant Cell Environ., **31**: 73-85.

Makino A., Mae T. and Ohira K. 1988. Differences between wheat and rice in the enzymic properties of ribulose-1,5-bisphosphate carboxylase oxygenase and the relationship to photosynthetic gas exchange. Planta, **174**: 30-38.

Mckeehan J. D., Mitchell C. A., Wheeler R. M., Bugbee B. and Neilsen S. S. 1996. Excess nutrients in hydroponic solutions alter nutrient content of rice, wheat and potato. Adv. Space Res., **18**: 73-83.

Mengel K., Robin P. and Salsac L. 1983. Nitrate reductase activity in shoots and roots of maize seedlings as affected by the form of nitrogen nutrition and the pH of the nutrient solution. Plant Physiol., **71**: 618-622.

Mengel K. and Schubert S. 1985. Active extrusion of protons into deionized water by roots of intact maize plants. Plant Physiol., **79**: 344-348.

Munns R. and James R. A. 2003. Screening methods for salinity tolerance: a case study of with tetraploid wheat. Plant Soil, **253**: 201-218.

Murty M. G. and Ladha J. K. 1988. Influence of *Azospirillum* inoculation on mineral uptake and growth of rice under hydroponic conditions. Plant Soil, **108**: 281-285.

Naher T., Sarkar M. R., Kabir A. H., Haider S. A. and Paul N. K. 2014. Screening of Zn-efficient rice through hydroponic culture. Plant Environ. Dev., **3**(2): 14-18.

Pandey R., Dubey K. K., Ahmad A., Nilofar R., Verma R., Jain V., Zinta G. and Kumar V. 2015. Elevated CO_2 improves growth and phosphorus utilization efficiency in cereal species under sub-optimal phosphorus supply. J. Plant Nutr., **38**: 1-22.

Rao I. S., Neeraja C. N., Srikanth B., Subrahmanyam D., Swamy K. N., Rajesh K., Vijayalakshmi P., Kiran T. V., Sailaja N., Revathi P., Rao P. R., Subba Rao L. V., Surekha K., Babu V. R. and Voleti S. R. 2018. Identification of rice landraces with promising yield and the associated genomic regions under low nitrogen. Scientific Reports, **8**: 9200. doi:10.1038/s41598-018-27484-0.

Rashid M. M., Jahan M. and Islam K. S. 2016. Impact of nitrogen, phosphorus and potassium on brown planthopper and tolerance of its host rice plants. Rice Sci., **23**: 119-131.

Sas L., Rengel Z. and Tang C. 2002. The effect of nitrogen nutrition on cluster root formation and proton extrusion by *Lupinus albus*. Ann Bot., **89**: 435-442.

Sasakawa H. and Yamamoto Y. 1978. Comparison of the uptake of nitrate and ammonium by rice seedlings.Influences of light, temperature, oxygen concentration, exogenous sucrose, and metabolic inhibitors. Plant Physiol., **62**: 665-669.

Shavrukov Y., Yusuf G. and Julie H. 2012. The use of hydroponics in abiotic stress tolerance research Hydroponics - a standard methodology for plant biological researches. Rijeka: InTech 39-66.

Singh B. and Pandey R. 2003. Differences in root exudation among phosphorus-starved genotypes of maize and green gram and its relationship with phosphorus uptake. J. Plant Nutr., **26**: 2391-2401.

Ta T. C., Tsutsumi M. and Kurihara K. 1981. Comparative study on the response of *Indica* and *Japonica* rice plants to ammonium and nitrate nitrogen. Soil Sci. Plant Nutr., **27**: 83-92.

Vengavasi K. and Pandey R. 2016a. Root exudation index: screening organic acid exudation and phosphorus acquisition efficiency in soybean genotypes. Crop Pasture Sci., **67**: 1096-1109.

Vengavasi K. and Pandey R. 2016b. Root acidification, a rapid method of screening soybean genotypes for low-phosphorus stress. Indian J. Genet. Plant Breed., **76**: 213-216.

Wang M. Y., Siddiqi M. Y. and Glass A. D. M. 1996. Interactions between K^+ and NH_4^+: Effects on ion uptake by rice roots. Plant Cell Environ., **19**: 1037-1046.

Yang X., Roemheld V. and Marschner H. 1994. Effect of bicarbonate on root growth and accumulation of organic acids in Zn-inefficient and Zn-efficient rice cultivars (*Oryza sativa* L.). Plant Soil, **164**: 1-7.

Yoshida S., Forno D. A., Cock J. A. and Gomez K. A. 1976. Laboratory Manual for Plant Physiological Studies of Rice, Ed 3. International Rice Research Institute, Manila, Philippines.

Supplementary Table1. A comparison of elemental composition of different nutrient media used for growing rice seedlings in hydroponic system

Hydroponics media	Concentration (mM L^{-1})								References
	N	P	K	Ca	Mg	S	Fe	pH	
Hoagland	14.00	1.00	6.03	5.00	1.00	2.00	0.018-0.065	~6.0	Hogland (1938)
Kimura B	1.64	0.18	0.55	0.36	1.67	0.72	0.025-0.065	4.5	Baba and Takahashi (1956)
Yoshida	2.86	0.32	1.03	1.00	1.67	2.15	0.036	5.0	Yoshida et al. (1976)
Alam Solution	4.00	1.00	2.00	1.50	1.00	3.01	0.010	5.5-6.5	Alam (1981)
Makino	2.00	0.60	0.30	0.20	0.40	0.30	0.045	5.5	Makino et al. (1988)
Murty and Ladha	1.05	0.03	0.20	0.02	0.04	0.09	0.040	5.5	Murty and Ladha (1988)
Kamachi	1.00	0.60	0.30	0.20	0.40	0.30	0.045	5.7	Kamachi et al. (1991)
Yang	1.43	0.32	1.32	1.00	1.64	2.96	0.035	6.0	Yang et al. (1994a)
Mckeehan	4.15	0.60	2.70	1.00	0.50	0.50	0.130	5.8-6.0	Mckeehan et al. (1996)
Ishimaru	4.00	0.10	0.90	2.00	0.50	1.20	0.100	5.5	Ishimaru et al. (2006)
Gui	-	0.32	1.02	1.43	1.65	0.45	0.350	5.5	Chen et al. (2013)

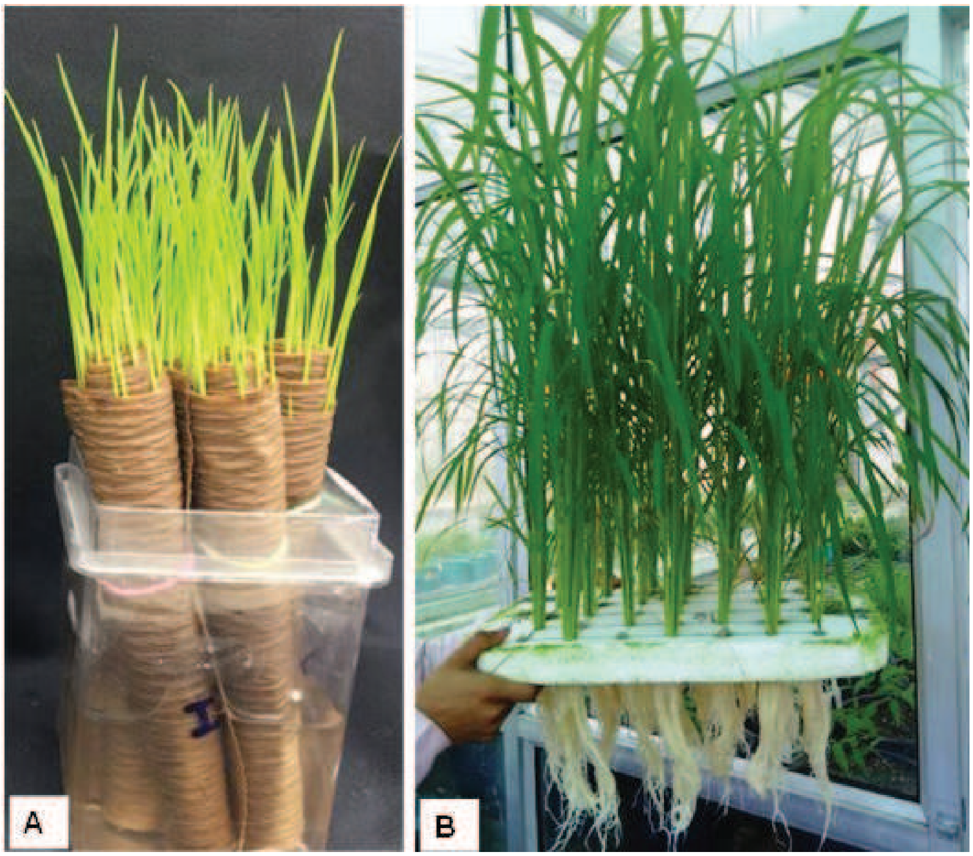
Supplementary Table 2. List of rice genotypes used for validation of optimized hydroponics media 'PusaRicH' in comparison with other media

S.No.	Name of genotype	S. No.	Name of genotype
1	BAM-2574	51	PUSA SUGANDH-2
2	ABHISEK	52	IR-64
3	MDU-3	53	CROSS A
4	MTU-1010	54	PUSA BASMATI-6
5	BAM-5891	55	APO
6	BAM-6921	56	GP-145-51
7	BAM-5889	57	PUSA-44
8	BAM-6924	58	WAYRAREM
9	BAM-7385	59	PUSA-1121-SNI-2
10	Basmati 370	60	SWARNA
11	CT-15671-15-4-2-2-2-M	61	PUSA SUGANDH-5
12	Dehradun Basmati	62	MAS-946-1
13	IR 1561-228-3-3	63	PUSA-1121
14	IR 82589-B-B-117-2	64	GANGA
15	IR 82639-B-B-103-4	65	GOTRABIDHAN
16	IR 82639-B-B-3-3	66	MAGURI
17	IR 83384-B-B-102-3	67	BOGI AIJUNG
18	IP 88634:11-B-1	68	KUNKUNI JOHA
19	KRISHNA JOHA	69	JALPANIA
20	NARENDRADHAN 359	70	JOHA BORA
21	RANBIR BASMATI	71	MANOHARSALI
22	RASI	72	BAHADUR

23	SABITA	73	LUIT
24	SOMCAU 70A	74	MONIRAM
25	SWETHA	75	LACHIT
26	TAM CAU 9A	76	NERICA-L-26
27	THURUR BHOG	77	NERICA-L-42
28	VANDANA	78	NL-44
29	PUSA BASMATI 1	79	KAPILEE
30	DULAR	80	JOYMOTI
31	HBC 46	81	SUSHSAMRAT
32	IC 463018	82	VANAPRAVA
33	IC 463176	83	KALANAMAK
34	IC 463207	84	BAKAL
35	IC 466475	85	IET-22116
36	IR 36	86	IET-23777
37	IR 82589-B-B-36-2	87	LALAT
38	IR 82590-B-B-102-4	88	SAMPADA
39	IR 82635-B-B-59-2	89	NDR-359
40	IR 82635-B-B-82-2	90	IET 24116
41	IR 83754-B-B-46-4	91	IET21404
42	KALI KAMOD	92	IET 23745
43	KOLA JOHA 3	93	IET 24120
44	MANDAY VIJAYA	94	IET 23223
45	NAGINA 22	95	IET 23216
46	NAN -GAUN - ZHANG	96	IET 23739
47	RPBIO 226	97	IET 23735
48	SURAKSHA	98	RANJIT
49	TRIGUNA	99	KUSHAL
50	ZARDROME	100	CR-2624

Supplementary Table 3. Rice genotypes belonging to different clusters based on the averages of shoot biomass, root biomass and leaf area in widely cited hydroponics media, 'Yoshida' and 'Kimura B' and compared with optimized media 'PusaRicH'. For more information on different clusters, see Fig. 4.

Cluster A (Poor performer)	Cluster B	Cluster C	Cluster D (Best performer)
Common in all three media			
ABHISEK	BOGI AIJUNG	MONIRAM	BAKAL
BAM-5889	JOYMOTI		
BAM-5891		***Only in PusaRicH***	***Only in PusaRicH***
BAM-6921	***Only in PusaRicH***	APO	PUSA BASMATI-6
BAM-6924	BAM-2574	BAHADUR	WAYRAREM
BAM-7385	CROSS A	Basmati 370	SWARNA
IC 466475	Dehradun Basmati	CT15671-15-4-2-2-2-M	NL-44
IET 23216	DULAR	GANGA	KAPILEE
IET 23223	HBC 46	GP-145-51	SUSHSAMRAT
IET 23739	IC 463018	IC 463207	VANAPRAVA
IP 88634:11-B-1	IC 463176	IET 23745	
IR 82589-B-B-117-2	IET 24116	IET 24120	
IR 82590-B-B-102-4	IET21404	IET-23777	
IR 82635-B-B-59-2	IET-22116	IR 36	
IR 82639-B-B-103-4	IR 1561-228-3-3	IR 82639-B-B-3-3	
KRISHNA JOHA	IR 82589-B-B-36-2	JALPANIA	
KUNKUNI JOHA	IR 82635-B-B-82-2	KALI KAMOD	
MANDAY VIJAYA	IR 83384-B-B-102-3	KUSHAL	
MAS-946-1	IR 83754-B-B-46-4	LACHIT	
MDU-3	IR-64	LALAT	
NAGINA 22	IET 23735	LUIT	
NARENDRADHAN 359	MTU-1010	MAGURI	
PUSA BASMATI 1	NAN -GAUN - ZHANG	NERICA-L-26	
RPBIO 226	PUSA-44	NERICA-L-42	
SABITA	RANJIT	PUSA SUGANDH-2	
SOMCAU 70A	RASI	PUSA SUGANDH-5	
SURAKSHA	VANDANA	PUSA-1121	
SWETHA		PUSA-1121-SNI-2	
THURUR BHOG		RANBIR BASMATI	
TRIGUNA		SAMPADA	
		TAM CAU 9A	
Only in PusaRicH		ZARDROME	
KOLA JOHA 3			



Supplementary Fig. 1. (A) Rice seedlings in 'cigar rolls' grown for 5 days in water and ready for transplanting to nutrient media. (B) Healthy rice plants (25 days old after transplanting, two plants per hole) grown in PusaRiceHydroponics (PuRicH) media in glasshouse