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Enriched Yoshida nutrient solution enhances rice growth under hydroponics

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Abstract

Growing rice (*Oryza Sativa*) in soil less culture, hydroponics, is challenging due to problems associated with inappropriate nutrient (s) availability limiting plants normal growth and development. The inorganic ingredients of the nutrient solution become available to plants only in the form of ions at a prescribed pH. Rice genotypes grown in standard Yoshida nutrient solution shows increased drying of the older leaf and reduced plant habit. Among the macronutrients, ammonium nitrate has lesser dissociation rate and acidifies the solution thereby inhibiting root elongation and lateral root formation. In this study, the nitrogen sources in Yoshida nutrient solution were modified accordingly, ammonium nitrate (1.14 M) was replaced by potassium nitrate (1.53 M) and potassium sulphate (0.41 M) by ammonium sulphate (0.50 M) on the basis that the NH_4^+ concentration was reduced by half and NO_3 - Level increased three times so as to reduce the problem of pH regulation. Further, the potassium level was increased two times considering its significant role in crop growth. Two rice elite rice cultivars, ASD16 and CO52, cultured under the modified Yoshida nutrient solution demonstrated positive outcomes which include, reduced senescence, improved plant habit, increased root and shoot biomass, proliferative lateral roots, increased leaf width, high photosynthetic efficiency (F_v/F_m) and increased numbers of productive tillers. The root architectural parameters were studied using GiA Roots software. The modified Yoshida nutrient solution demonstrates good growth and developmental features in rice under controlled conditions making it an ideal hydroponic nutrient solution for carrying out molecular and genetic studies.

Keywords: Yoshida, rice, hydroponics, nitrogen, ammonium, nitrate, potassium nitrate, NH_4^+/ NO_3^- ratio

Introduction

Hydroponics way of growing plants for experimental purposes under controlled environments such as greenhouse is widely in practice. As compared to hydroponics, the conventional field-based studies were challenging due to biotic and abiotic factors *viz*., problems of poor quality soil and irrigation water, nutrient deficiencies, nematodes and pests etc., (Kohl, 2015; Khan *et al.*, 2023) [24, 22]. The technique of soil-less culture known as hydroponics was originally coined by William Frederick Gericke, the term 'hydroponics' derived from a Greek word; "*hydro*" means water, and "*ponos*" means labour (Gericke, 1937) [15]. This technique involves raising the plants in trays with roots suspended in water containing nutrients essential for their growth (Maharana and Koul, 2011) [29]. In order to support the plants, soil was substituted by substrates such as perlite, sand, gravel etc., (Savvas, 2003) [36]. The first ever nutrient medium introduced was known as Hogland solution (Hoagland, 1938) [16]. Over the years, keeping Hoagland's solution as a reference medium, many other hydroponics solutions with different compositions were developed as per the plant requirements *viz*., Kimura B (Baba and Takahashi, 1956) [4], Yoshida (Yoshida *et al.*, 1976) [45], Alam's solution (Alam,1981) [1], Kamachi (Kamachi *et al.*, 1991) [21], Makino medium (Makino *et al.*, 1988) [30], Yang medium (Yang *et al.*, 1994) [43], McKeehan's solution (McKeehan *et al.* 1996) [32], Ishimaru (Ishimaru *et al.*, 2006) [18] and Gui (Chen *et al.*, 2013) [9].

Today hydroponics had become an important technique for crop research. Rice (*Oryza Sativa* L.) is an ideal model system for studying physiological, genetic and molecular mechanisms governing plants growth and development for monocots. Hydroponics system of rice culturing leads to a precise evaluation of the morphological changes while screening for abiotic stresses especially salt tolerance (Almeida *et al.*, 2016; Chithrameenal *et al.*, 2017; Sharma, 2018) [3, 10, 37]. Besides, this technique was found appropriate for studying allelopathic rice plants (Kim *et al.*, 2004) [23]. Towards culturing rice under hydroponics system, Yoshida and Hoagland nutrient solutions remain the mainstay in most of the experiments till date.

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This includes nutrient deficiency symptoms leading to pale green/yellow leaves, increased senescence of the older leaves, leaf tip rolling and drying, stunted shoot and reduced root growth. Thus, for a better growth and development, composition of the nutrients and their uptake is very crucial which in turn depends on right pH of the nutrient solution.

The pH of the hydroponic solution plays an important factor for regulating the nutrients availability and pH range varies depending upon the species. With an increase in pH between 5.5 and 6.5, the concentration of all the nutrients were higher in shoots except for iron. The iron being not translocated upwards resulted in chlorosis of leaves indicating the iron deficiency (Alam, 1981) [1]. Low pH in turn results in an excessive intake of micronutrients but reduced lateral root formation (Jones *et al.*, 1982) [20]. For growing rice, hydroponics at pH 4.5 (Yoshida *et al.*, 1976) [45], pH 4.5 (Chen *et al.*, 2006) [9], pH 5.5 (Murty *et al.*, 1988) [34] and pH 6.5 (Hogland, 1938) [16] were previously reported. But the right pH and its stability is a prerequisite for an effective nutrient uptake.

Maintaining a balanced nutrient composition helps to prevent nutrient imbalances, deficiencies, toxicity that could negatively impact plant growth and health. Further, plant requires nutrients at an appropriate quantity, hence it is important to maintain ionic balance in hydroponic nutrient solution. Among the macronutrients, nitrogen is the principal component for plant growth. Plant majorly take NH_4^+ and NO_3^- as the primary source of nitrogen (Cui *et al.*, 2017) [11]. The balance between these two nitrogen forms influences the overall efficiency of nitrogen utilization and plant growth and its ability to cope up with environmental stresses. The ratio of nitrate and ammonia (NH_4^+/NO_3^-) between pH of 5.0 to 6.0 plays a vital role in crop growth and yield (Alam, 1984; Ying-Hua *et al.*, 2006 and Zhu *et al.*, 2021) [2, 44, 46]. The equimolar distribution of NH_4NO_3 and K_2SO_4 in turn makes the medium acidic, since ammonium nitrate is acidic and potassium sulphate is neutral in nature as reported in wheat and barley (Shavrukov *et al.*, 2012) [38]. In order to achieve improvement in the nutrient uptake, earlier Yoshida nutrient solution was altered in its composition. The macronutrient stock $NaH_2PO_4.2H_2O$ was replaced with K_2HPO_4 and KH_2PO_4 to facilitate screening rice seedlings under salinity stress (Singh *et al.*, 2010) [39].

In India, ammonium nitrate is regulated as it used in manufacturing explosives thereby limiting the supply NH_4NO_3 even for laboratory purpose. As a consequence, the standard Yoshida nutrient solution could not be prepared as such without NH_4NO_3. In this context, the present investigation intended to replace ammonium nitrate as well as to address other compounding issues such as acidification of the nutrient solution and pH instability affecting root and shoot growth (Bloom *et al.*, 2010; Lima *et al.*, 2010) [5, 27]. In this context, the present study intended to modify primarily the nitrogen source by replacing the ammonium nitrate (NH_4NO_3) by potassium nitrate (KNO_3) and potassium sulphate (K_2SO_4) by ammonium sulphate ($(NH_4)_2SO_4$) and evaluate the performance of the modified medium as compared to standard Yoshida nutrient solution.

Materials and Methods

Plant material and growth condition

Two elite rice cultivars, ASD16 and CO52 were used in this study. ASD16 is a mid-early, bold grain type and cv. CO52 is medium in duration and slender grain type. The experiment was performed with three biological and three technical replicates for each of the genotypes under greenhouse conditions. The temperature was maintained at 30 ºC day at 80-85% relative humidity.

Preparation of hydroponics medium and experimental set up

Seeds were raised in two-inch net pots filled with perlite and fitted on a thermocol sheet. The above was placed on a mini plastic tank of eight litre capacity. Yoshida stock solutions were prepared as per the table 1 and, working solution as per the table 2 using reverse osmosis (RO) water. The initial pH of the medium would be around 6.2 - 6.6 and it was brought down to 4.5 by adding 1N Hydrochloric acid (HCl). Mixed the solution thoroughly after adding a few drops HCl and waited until the ions dispersed uniformly. The above step was repeated until the pH of hydroponics medium was stabilized at 4.5. While culturing, the drop in the level of the nutrient solution due to high transpiration was adjusted with fresh nutrient solution every two days and the solution completely changed at an interval of seven days.

Table 1: Chemical components and their concentration in standard and modified Yoshida nutrient solutions.

Elements	Yoshida medium Components	Stock (g/l)	Modified Yoshida medium for rice Components	Stock (g/l)	Concentration of element (ppm)
Macronutrients					
Stock I (N)	NH_4NO_3	91.4	$(NH_4)_2SO_4$	66.07	40
Stock II (P)	$NaH_2PO_4.2H_2O$	35.6	$NaH_2PO_4.2H_2O$	35.6	10
Stock III (K)	K_2SO_4	71.4	KNO_3	155	40
Stock IV (Ca)	$CaCl_2.2H_2O$	117.35	$CaCl_2.2H_2O$	117.35	40
Stock V (Mg)	$MgSO_4.7H_2O$	324	$MgSO_4.7H_2O$	324	40
Stock VI					
	$MnCl_2.2H2O$	1.5	$MnCl_2.2H2O$	1.5	0.5
Mn	$(NH_4)_6MoO_{14}.4H_2O$	0.074	$(NH_4)_6MoO_{14}.4H_2O$	0.074	0.05
Mo	$ZnSO4.7H_20$	0.035	$ZnSO4.7H_2O$	0.035	0.01
Zn	H_2BO_3	0.0934	H_2BO_3	0.934	0.2
B	$CuSO_4.5H_2O$	0.031	$CuSO_4.5H_2O$	0.03	0.01
Cu	$FeCl_3.6H_2O$	7.7	$FeCl_3.6H_2O$	7.7	2.00
Fe	Citric acid monohydrate ($C_6H_3O_7.H_2O$)	11.9	Citric acid monohydrate ($C_6H_3O_7.H_2O$)	11.9	0.12

Modified Yoshida with altered nitrogen sources for growing rice

Here, we used two different nutrient solutions for growing rice in hydroponics *viz.*, the standard Yoshida nutrient solution (Yoshida *et al.*, 1976) [45] and modified Yoshida nutrient solution. In the modified Yoshida nutrient solution, the modifications include, KNO_3 (1.53 M) and $(NH_4)_2SO_4$ (0.50 M). Three weeks old seedlings of rice cultivars CO52 and ASD16 were transferred to the hydroponic tray assembly containing Yoshida and modified Yoshida nutrient solutions and cultured till seed set.

Table 2: Preparation of working stock as described by Yoshida *et al.* (1976) [44].

Stock No.	Element	Amount of stock solution needed for 20 l in ml	Amount of stock solution needed for 40 l ml	Amount of stock solution needed for 120 l ml	Amount of stock solution needed for 240 l in ml
1	N	25	50	150	300
2	P	25	50	150	300
3	K	25	50	150	300
4	Ca	25	50	150	300
5	Mg	25	50	150	300
6	Mn	25	50	150	300
7	Mo				
8	B				
9	Zn				
10	Cu				
11	Fe				

pH optimization for modified rice medium

The pH of the modified Yoshida nutrient solution was maintained between 4.5 to 4.8 to study the effect on plant growth. The standard Yoshida nutrient solution was taken as a comparison at pH range of 4.5-4.8. The rice seedlings were grown till its reproductive stage keeping all other growth conditions similar as mentioned above.

Estimating the photosynthetic efficiency using chlorophyll fluorescence meter

Chlorophyll fluorescence vide the F_v/F_m ratio is a direct estimate of the efficiency of photosystem II (PSII) and the observations made as described by Van Kooten, (1990) [41]. The F_v/F_m measurements were made on 50 DAS, the second leaf was dark adapted for 10 mins. The initial fluorescence (F_0) under non photosynthetic condition was recorded using handheld chlorophyll fluorometer Opti-Sciences (model no. OS30P+). Then maximum fluorescence (F_m) was measured by applying a range of 525-6,000 µE saturation pulse that is detected and filtered by IN photodiode with a 700 nm - 750 nm band pass filter. The duration of the test (Fv/Fm) would take around 0.1-1.5 seconds.

Phenotyping rice genotypes grown in hydroponics

The roots of 90 days old rice plants were uprooted from the experimental set up and washed thoroughly in water to remove the adhered perlite particles. For each of the cultivars, plants in replicates were subjected to root and shoot length measurements in cm. Further, the root and shoot portions were excised, dried and dry weight (DW) recorded.

Quantification of root architecture using GiA software

Prior to drying of roots for calculating the biomass, the root portion below the collar region of the shoot was excised and used for quantification of root architectural parameters The roots were spread evenly in an inverted cone shaped manner as described by (Perween *et al.*, 2021) [35] and imaged using a high-resolution camera. The parameters governing the architecture of rice roots in modified and original Yoshida medium were analysed using GiA Roots software.

Statistical analysis

The standard deviation and standard error were calculated for the root length, shoot length, root and shoot biomass and Fv/Fm values between the varieties. Ducan's Multiple Range Test (DMRT) was performed to find statistically significant differences between pair of treatment means using WASP – Web Agri Stat Package.

Results and Discussion

Culturing rice in modified Yoshida medium under hydroponics

Yoshida nutrient solution (Yoshida *et al.*, 1976) [45] is a predominantly used medium in rice hydroponics due to its balanced composition of the macro and micronutrients which is very essential to support the growth and development of rice crop from seedling to seed set. Nitrogen is one of the most important macronutrients required by the plants for proper growth and development. It is highly indispensable and supports vital biological functions such as leaf area development, photosynthesis, tillering and grain filling. Any deficiency in nitrogen under field situations lead to a constrained rice production (Ladha *et al.*, 2016) [25]. The roots of higher plants generally prefer NO_3 and NH_4 as the predominant inorganic nitrogen forms under *in situ* and *in vitro* conditions (Marschner, 1995) [31].

In this experiment, we had modified the original Yoshida nutrient solution for nitrogen sources. The ammonium nitrate (NH_4NO_3) component in the Yoshida nutrient solution was replaced by potassium nitrate (KNO_3) and potassium sulphate (K_2SO_4) by ammonium sulphate $(NH_4)_2SO_4$), respectively. The modified Yoshida nutrient solution comprised of the following concentrations of $(NH_4)_2SO_4$ (0.5 M) and KNO_3 (1.53 M). The molar concentrations of $(NH_4)_2SO_4$ and KNO_3 salts were arrived based on the proportions of ammonium and nitrate between NH_4NO_3 and $(NH_4)_2SO_4$ and K_2SO_4 and KNO_3, respectively on molecular weight basis. Finally, the modified Yoshida nutrient solution comprised of three times more NO_3, two times more potassium while NH_4 - levels were reduced by half with reference to standard Yoshida.

Influence of optimized nitrogen sources on pH regulation

Nutrient uptake by plants is different for each element under specific pH. One of the key factors affecting the pH of the hydroponics solution is the ratio of ammonium (NH_4^+) and nitrate (NO_3). Presence of more NH_4^+ in the nutrient solution releases more protons (H^+) around the roots thereby creating acidic environment resulting in a pH drop whereas, more of NO_3^- releases hydroxide ions (OH^-) creating an alkaline environment and increase in pH emphasizing the importance of maintaining a balance in nitrate and ammonium in turn pH stabilization. According to Bretler and Smith (1974) [6], presence of ammonia decreased the pH of nutrient solution even in the presence of nitrate. Contrary, potassium and nitrate were reported to have positive correlation and there exists a relation between K^+ and NO_3^- absorption by roots of higher plants (Dong *et al.*, 2004) [13]. Hence, in this study NH_4^- concentration was reduced by half and NO_3^- level increased by three times. KNO_3 being a neutral salt will help in stabilizing the pH of the nutrient solution as well as the plants benefit from an enhanced absorption of the nutrients in the modified Yoshida nutrient solution. Besides, a twofold higher potassium concentration in modified Yoshida nutrient solution contributed to an improved rice growth and development. Potassium is an important macronutrient generally available as free ions in wide range of pH say, 2-9 and rarely forms complexes with SO_4 and NO_3 (De Rijck and Schrevens, 1999) [12].

Morpho-physiological parameters demonstrates the role of modified Yoshida in improved performance

Two elite rice cultivars, ASD16 and CO52, were evaluated for their performance under standard and modified Yoshida nutrient solutions in a replicated experimental set up (Fig 1).

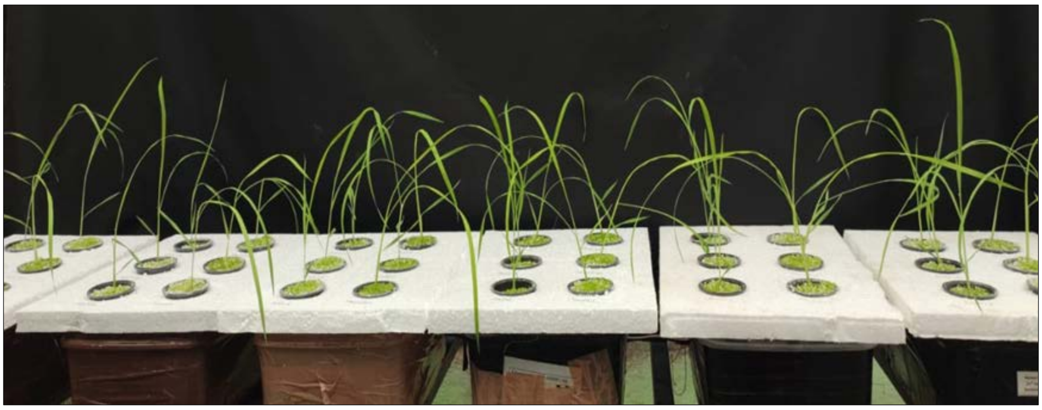

Fig 1: Experimental set up for culturing rice under hydroponics system under greenhouse conditions

Both the cultivars performed better in modified Yoshida compared to the standard Yoshida nutrient solution in terms of seedling vigour, root length, plant height, number of tillers, number of productive tillers, photosynthetic ability, root and shoot biomass and grain yield. In addition, the leaves were wider and exhibited reduced senescence in the older leaves however, the grain filling was good in both the media (Fig 2).

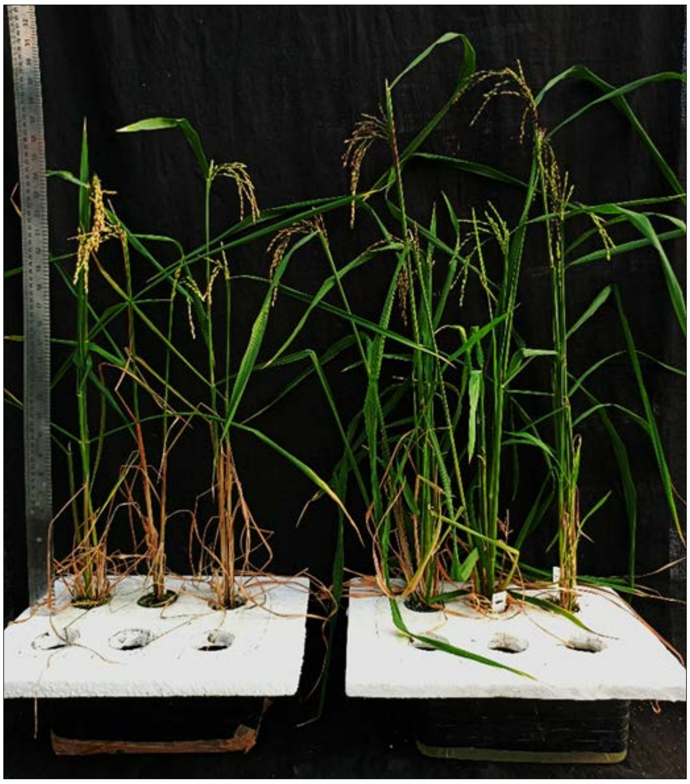

Fig 2: Representative image of rice cultured in standard Yoshida (left) and modified Yoshida (right) nutrient solutions

Rice seedlings of ASD16 and CO52 raised in modified Yoshida nutrient solution showed an increased root length (Fig 3) and shoot length (Fig 4) as compared to standard Yoshida. A statistically significant difference was evident in the shoot length in favour of the modified medium.

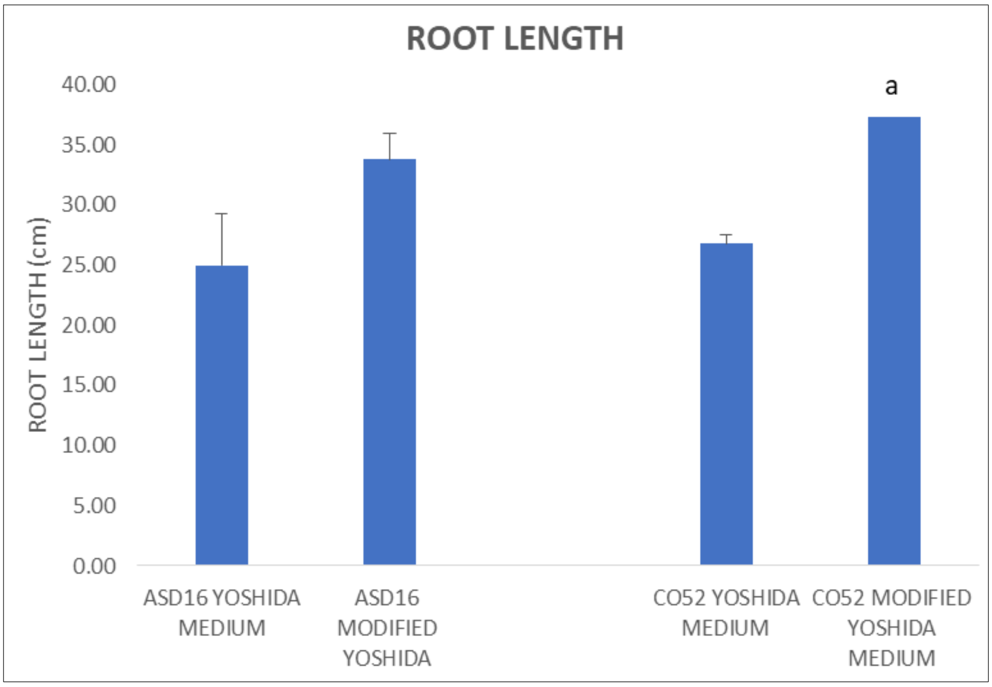

Fig 3: Rice cultivars ASD16 and CO52 showing significant difference in root length between modified Yoshida and original Yoshida nutrient solutions. Different letters indicate significant differences between treatment means, with ‘a’ as best treatment and ‘c’ as poor treatment, tested by DMRT (p<0.05).

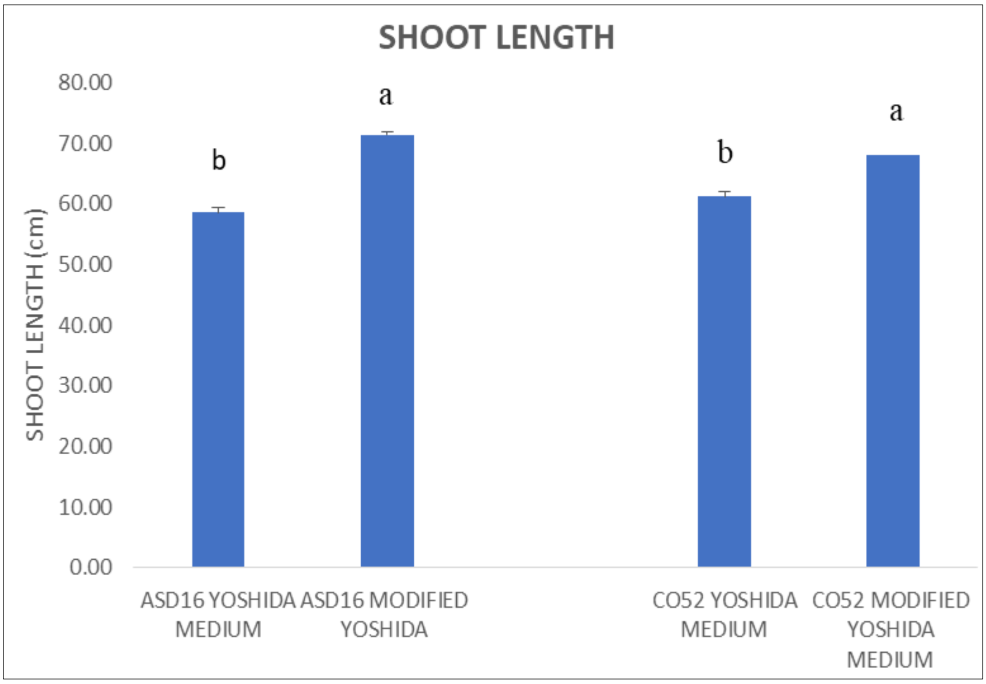

Fig 4: Rice cultivars ASD16 and CO52 showing significant difference in shoot length between modified Yoshida and original Yoshida nutrient solutions. Different letters indicate significant differences between treatment means, with ‘a’ as best treatment and ‘b’ as poor treatment tested by DMRT (p<0.05)

Rice under limiting potassium is likely to hinder the photosynthetic as well as electron transfer efficiencies of photosystem II (PSII) reaction centre (Cakmak, 2005 and Lu *et al.*, 2016) [7, 28]. As the concentration of potassium is higher in the modified Yoshida nutrient solution, rice cultured in modified Yoshida showed an increased PSII photosynthetic efficiency (F_v/F_m) when analysed with chlorophyll Fluorometer (Fig 5).

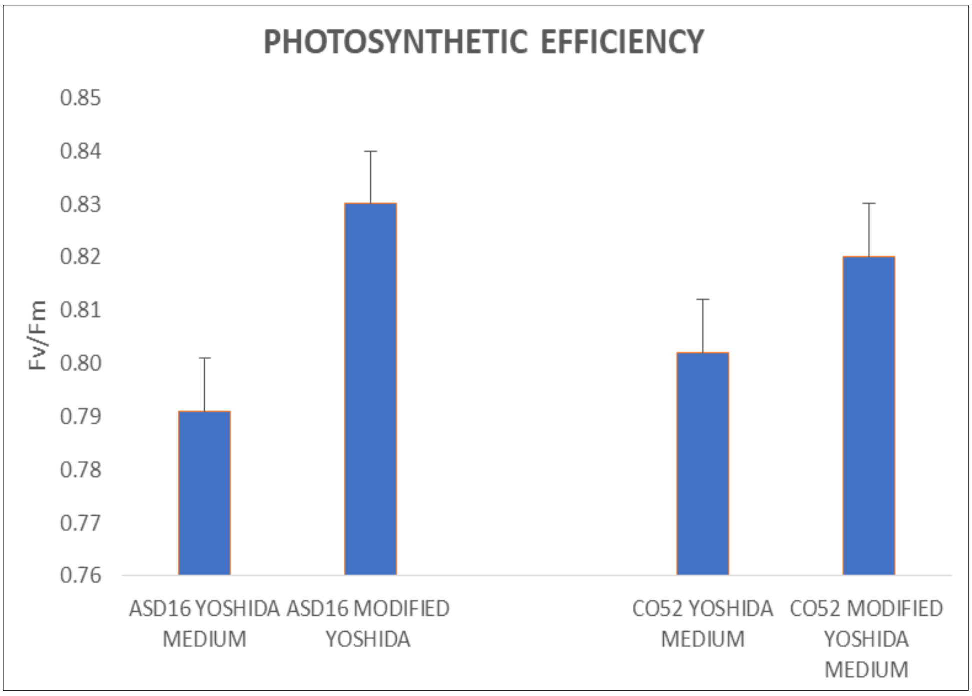

Fig 5: Fv/Fm values depicting the photosynthetic efficiency of rice seedlings grown in Yoshida and modified Yoshida nutrient solutions. Different letters indicate significant differences between treatment means, with ‘a’ as best treatment and ‘b’ as poor treatment tested by DMRT (p<0.05)

Role of NO_3^- and NH_4^+ levels in hydroponics on root and shoot biomass in rice

Rice grown in the presence of potassium nitrate exhibited an increased root and shoot biomass (Table 3). Nitrogen triggered root response was previously reported in rice (Xuan *et al.*, 2013) [42]. In this experiment, rice crop when supplemented with 1.53 M potassium nitrate showed an increased root length with more lateral root formation when compared to 1.14 M ammonium nitrate as shown in Fig. 6. When ammonium nitrate concentration is high, it creates an acidic environment near the roots and inhibits its growth. On the other hand, potassium nitrate treatment promoted root growth in both the varieties. Potassium nitrate being a neutral salt have more dissociation rate compared to ammonium nitrate, so that both K^+ and $NO3^-$ ions become easily available for uptake by the plants. Potassium deficiency mediated root growth inhibition was already reported by (Sustr *et al.*, 2019) [40]. Further, roots showed less browning in modified medium. The combined effect of potassium and nitrate alleviated ammonium toxicity and showed positive effect on rice root and shoot biomass as reported by (Fang *et al.*, 2021) [14].

Table 3: The root and shoot biomass of rice cultivars cultured in Yoshida and modified Yoshida nutrient solutions. Different letters indicate significant differences between treatment means, with ‘a’ as best treatment and ‘b’ as poor treatment tested by DMRT (p<0.05).

Cultivar	**Root biomass (g)**		**Shoot biomass (g)**	
	Yoshida	Modified Yoshida	Yoshida	Modified Yoshida
ASD16	0.6 ± 0.16^b	1.4 ± 0.27^a	5.08 ± 0.08^b	9.89 ± 1.87^a
CO52	$0.6+0.03^b$	$1.1+0.35^a$	4.46 ± 0.32^b	9.63 ± 1.29^a


(a) (b) (c) (d)

Fig 6 Representative Root images of rice cv. CO52 (a, b) and ASD16 (c, d) grown in modified Yoshida nutrient solutions (top panel) and standard Yoshida (bottom panel)

Nutritional response on root architecture

In rice, the roots are broadly classified into seminal roots (embryonic roots), crown roots (adventitious roots) and lateral roots (Hochholdinger *et al.*, 2004) [17]. In *Arabidopsis*, in the presence of two inorganic nitrogen forms, nitrate and ammonia, root architecture showed differential response based on the nutrient availability (Li *et al.*, 2014) [26].

The root architecture was analysed using GiA Roots software. Both the cultivars, ASD16 and CO52, showed significant difference ($p<0.05$) in average root width, network length distribution specific root length and maximum number of roots between Yoshida and modified Yoshida nutrient solution. Rice cultivar, CO52 showed significant difference in network volume and network length. Similar results were observed in cultivars on comparing t stat value with t critical values (two tailed test) for each parameter in table 5.

Table 4: List of six different root parameters analysed using GiA software

Parameter	Units	Description
Average root width	dm	The mean value of the root width estimation computed for all pixels of the medial axis of the entire root system.
Network Length Distribution	cm	The fraction of network pixels found in the lower 2/3 of the network. The lower 2/3 of the network is defined based on the network depth
Network length	cm	The total number of pixels in the network skeleton.
Network volume	cm^3	The sum of the local volume at each pixel of the network skeleton, as approximated by a tubular shape whose radius is estimated from the image.
Maximum Number of Roots	n	After sorting the number of roots crossing a horizontal line from smallest to largest, the maximum number is considered to be the 84th-percentile value (one standard deviation).
Specific root length	cm/cm^3	Total network length divided by network volume. Volume is estimated as the sum of cross-sectional areas for all pixels of the medial axis of the root system. The total root length is the number of pixels in the medial axis of the root system.

Table 5: Selected root architectural parameters analyzed in rice cultivars ASD16 and CO52 using GiA Roots software

Varieties	Average root width		Network length distribution		Network length		Network volume		Maximum number of roots		Specific root length	
	T stat	**P**	**T stat**	**P**	**T stat**	**P**	**T stat**	**P**	**T stat**	**P**	**T stat**	**P**
ASD16	-2.97*	0.03*	-4.24*	0.01*	-0.45	0.35	-0.29	0.40	-6.27*	0.00*	-2.44*	0.03*
CO52	-3.18*	0.03*	-2.34*	0.04*	-3.15*	0.02*	-3.12*	0.02*	-2.92*	0.03*	-4.94*	0.00*

Significant difference at 5% level

Conclusion

Growing rice for experimental purpose requires an appropriate culture system. Hydroponics method of rice cultivation is widely practised in such research environments and Yoshida nutrient solution was commonly used. Given the deficiencies in the standard Yoshida nutrient solution, we developed a modified version of hydroponic nutrient solution for rice with changes made on the nitrogen source. Replacement of ammonium nitrate by potassium nitrate improved the overall growth and development, the latter as K^+ and NO_3^- ions were effectively absorbed by the plants. The plant habit was healthier showing reduced senescence, increased root and shoot biomass and more of reproductive tillers. According to Camut *et al.*, (2021) [8], nitrate is one of the key elements involved in modulating the expression of genes involved in growth of plants. Similarly, increase in NO_3^- levels by three times resulted in an enhanced plant growth as evinced in this study. Recent studies showed that, at molecular level, pH dependent root growth retardation in plants was associated with auxin mediated signalling in root tips under ammonium trigerred low pH (acidic) condition since auxin is responsible for modulating root growth direction (Positive Gravitropism), (Jia *et al.*, 2020) [19]. Thus, the optimized medium will be better for growing rice under controlled conditions leveraging genetic and molecular investigations.

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