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APPLICATION TIME OF NITROGEN AND PHOSPHORUS FERTILIZATION MITIGATES THE ADVERSE EFFECT OF SUBMERGENCE IN RICE (*ORYZA SATIVA L.*)

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SUMMARY

Large areas of rainfed lowlands of Asia annually experienced flash flooding during the rice-growing season, which is an important abiotic stress that adversely affect grain yield of rice (*Oryza sativa L.*) crop. Submergence stress is a common environmental challenge for agriculture sustainability in these areas because lack of high-yielding, flood-tolerant cultivars. In this study, IR64-Sub1 and IR64 were compared for their tolerance to submergence at active tillering (AT), panicle initiation (PI) and heading (H) stages with nitrogen and phosphorus application time. We evaluated the role of cultivars, stage of submergence and N and P application on phenology, leaf senescence (LS), photosynthetic (Pn) rate, yield attributes and yield. Under non-submerged conditions, no difference was observed in phenology, Pn rate and yield of both cultivars. Submergence substantially reduced biomass, Pn rate, yields attributes and yield across cultivars with more drastic reduction in IR64. Submergence at H stage proves to be most detrimental. Nitrogen application after desubmergence with basal P improved the Pn rate resulting in significantly higher yield and yield components. Nitrogen application before submergence resulted in increased LS and ethylene accumulation in shoots leading to drastic reduction in growth, Pn rate and yield. Crop establishment and productivity could therefore be enhanced in areas where untimely flooding is anticipated by avoiding N application before submergence and applying N after desubmergence with basal P (phosphorus).

INTRODUCTION

Rice (*O. sativa* L.) is at the heart of the culture and the staple food of 557 million people in Southeast Asia (Manzanilla *et al.*, 2011). Amid this demand, rice productivity in Southeast Asia is seen to suffer serious losses mainly because of erratic rainfall patterns and increasing risks from typhoon and rainfall-induced flood. Plants require water for growth but excess water that occurs during submergence is harmful or even lethal (Nishiuchi *et al.*, 2012). Prolonged submergence is a major production constraint and affects 22 million ha of rainfed lowland rice in South and Southeast Asia

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Abbreviation: AS, after submergence; AT, active tillering; BS, before submergence; DAT, days after transplanting; Gs, stomatal conductance; LAI, leaf area index; LS, leaf senescence; N, nitrogen; P, phosphorus; PI, panicle initiation; Pn rate: photosynthetic rate.

(Khush, 1984; Mackill *et al.*, 2012), of which over 6 million ha in India (Khush, 1984; Sarkar *et al.*, 2006). Flooding is the third most important abiotic stress affecting rice productivity in Asia, surpassed only by drought and weeds (Widawsky and O'Toole, 1990). The adverse effects of flooding constitute a complex phenomenon that varies with genotype and pretreatments, stage of the plant when flooding occurs, duration and severity of flooding (Setter *et al.*, 1987), carbohydrate status during submergence in seeds (Ella and Setter, 1999) and shoots (Palada and Vergara, 1972; Sakagami and Kawano, 2011), Pn rate of submerged rice (Mazaredo and Vergara, 1982; Panda *et al.*, 2008; Setter *et al.*, 1989) and degree of turbidity of floodwater (Das *et al.*, 2009; Setter *et al.*, 1995). Underwater photosynthesis can improve survival by enhancing internal aeration, increasing carbohydrate reserves or both and supported by the observation, that blocking early ethylene-induced chlorophyll degradation improves survival (Ella *et al.*, 2003). Ethylene production and accumulation occur during stress such as submergence and that ethylene triggers LS, which consequently reduces Pn rate during and after submergence (Bradford and Yang, 1980; Ella *et al.*, 2003; Jackson *et al.*, 1987; Voesenek *et al.*, 1993). Submergence subjects plants to the stresses of low light, limited gas diffusion, effusion of soil nutrients, mechanical damage and increased susceptibility to pests and diseases (Greenway and Setter, 1996; Ram *et al.*, 1999; Setter *et al.*, 1997).

Progress has been made in developing more tolerant germplasm for flood-prone ecosystems, but fewer efforts have been devoted to identifying proper nutrient management options (Ella and Ismail, 2006), but are gradually gaining ground with increasing attention of the research community to unfavourable environments. Rice grain yield may decrease about 21% when water depth increased from 30 to 90 cm (Kupkanchanakul, 1979). The reduction in yield has been attributed to a decrease in the proportion of ripened grains due to fertilization failure (Matsushima, 1962; Reddy and Mittra, 1985a; Singh *et al.*, 2009). In rice, the reproductive stage is the most sensitive to complete submergence, followed by the seedling and the maximum tillering stages (Gautam *et al.*, 2014a; Matsushima, 1962; Pearson and Jacobs, 1986). Rice genotypes tolerant of complete submergence at the vegetative stage, such as the Indian landrace FR13A, were identified that can survive submergence for over 2 weeks, and a single gene responsible for tolerance (*SUB1A*) was cloned and its role in conferring tolerance established (Bailey-Serres *et al.*, 2010; Mazaredo and Vergara, 1982; Setter *et al.*, 1997; Xu *et al.*, 2006). Low yield of *FR13A* hindered its use by farmers (Mackill *et al.*, 1996, 2012; Mohanty *et al.*, 2000). However, developing cultivars with submergence tolerance matching that of *FR13A*, coupled with high yield and good grain quality, could have enormous impact through increasing and stabilizing the productivity of rice in rainfed areas, since 25–30% of the world's rice-growing areas are prone to submergence (Singh *et al.*, 2009). The varieties developed through marker assisted back crossing system and by transferring *Sub1* gene into popular rice varieties showed a yield advantage of 1 to >3 t/ha over the original varieties following submergence for a few days to 18 days (Mackill *et al.*, 2012; Neeraja *et al.*, 2007).

Optimal nutrition before flooding is necessary to equip plants with cellular and metabolic requirements essential for survival of flooding, and also for fast recovery

Table 1. Fertilizer application schedule followed in the experiment.

Nutrient combination	Submergence time (DAT)	Nitrogen*application time		
		2nd split	3rd split	4th split
Control	—	—	—	—
P	—	—	—	—
N_{BS}	21	19 DAT	PI stage (45 DAT)	H stage (65 DAT)
	45	AT stage (21 DAT)	43 DAT	H stage (65 DAT)
	65	AT stage (21 DAT)	PI stage (45 DAT)	63 DAT
N_{AS}	21	36 DAT	PI stage (45 DAT)	H stage (65 DAT)
	45	AT stage (21 DAT)	61 DAT	H stage (65 DAT)
	65	AT stage (21 DAT)	PI stage (45 DAT)	81 DAT
NP_{BS}	21	19 DAT	PI stage (45 DAT)	H stage (65 DAT)
	45	AT stage (21 DAT)	43 DAT	H stage (65 DAT)
	65	AT stage (21 DAT)	PI stage (45 DAT)	63 DAT
NP_{AS}	21	36 DAT	PI stage (45 DAT)	H stage (65 DAT)
	45	AT stage (21 DAT)	61 DAT	H stage (65 DAT)
	65	AT stage (21 DAT)	PI stage (45 DAT)	81 DAT

AT: active tillering, PI: panicle initiation, H: heading, BS: before submergence, AS: after submergence, N-nitrogen, P- phosphorus.

*Nitrogen was applied in equal amounts (25% of total dose) in each split, full dose of P and K and first split of N was applied as basal at the time of transplanting.

after floodwater recedes (Ella and Ismail, 2006; Gautam *et al.*, 2014a; Lal *et al.*, 2014). The farmers' practice of applying only nitrogenous fertilizers in the nursery to get taller and greener seedlings may not have positive consequences in flood-prone areas because high N content in seedlings before flooding adversely affects survival after flooding and results in poorer recovery (Ella and Ismail, 2006). However, when high N is accompanied by high P supply on P-deficient soils, seedling survival seems to improve, with effectively better recovery after submergence (Gautam *et al.*, 2014b; Jackson and Ram, 2003). Here, we quantified the impact of IR64-Sub1 with IR64 under non-submerged conditions as well as under complete submergence (14 days) at AT, PI and H stage with different nutrient combination applied before submergence and after desubmergence on growth, Pn rate and yield. This information is essential in providing insights into the usefulness of application time of fertilizers in typical farmers' fields.

MATERIALS AND METHODS

Plant material and growth conditions

The experiment was conducted under natural conditions during 2012–13 with two *Indica* rice (*O. sativa* L.) cultivars i.e. IR64-Sub1 having submergence tolerant *Sub-1* gene and IR64 without *Sub-1* gene; six schedules of nutrient application *viz.*, control, P (phosphorus), N_{BS} (nitrogen before submergence), N_{AS} (after submergence), NP_{BS} and NP_{AS} (details of fertilizer application schedule given in Table 1) and three different stages of submergence *viz.*, AT (21 days after transplanting (DAT)), PI (45 DAT) and H stage (65 DAT). The experiment was arranged in a factorial randomized block design with four replications. One 15 days old seedling of each cultivar was transplanted in the pots containing 8 kg of farm soils (Sandy clay loam, pH 6.4, EC-0.079 dSm^{-1}, available N, P and K-5.89, 0.45 and 6.47 mg kg^{-1} of soil, respectively). 1.75 g $(NH_4)_2SO_4$, 0.95 g KH_2PO_4 and 0.52g KCl were applied to each pot as per the treatments (dose of fertilizer was kept as 100:50:50 kg ha^{-1} for NPK, respectively). The amount of K applied through KH_2PO_4 was adjusted while applying KCl as source of K. Plants were irrigated with fresh water to maintain 2 cm standing water except during the period when rice plants were subjected to submergence. Potted plants were completely submerged in a concrete tank containing fresh water and the water depth was maintained at 30 cm above the top of the plant canopy for 14 days at three different times of crop duration i.e. 21, 45 and 65 DAT, which coincides with AT, PI and H stages of crop, respectively.

After desubmergence, plants were allowed to recover for 7 days, and plant survival was recorded. Plant samples were collected just before submergence and soon after desubmergence for various measurements of plant responses during submergence. Light transmission (photosynthetically active radiation) through the floodwater, water temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), redox potential (ORP), total dissolved salts (TDS) were determined. Light intensity was measured at 12:00 h using LICOR light meter (LI-COR, USA), whereas temperature and other water quality parameters were determined by using U-50 multiparameter water quality meter (HORIBA, Japan).

Assessment of phenology, yield attributes and yield

Plant height and dry matter was determined at maturity, total number of tillers was counted during different crop growth stages, whereas number of tillers producing panicles out of maximum number of tillers was represented as percentage productive tillers. Total leaf area was recorded by putting every fresh leaf of one plant from each treatment in digital leaf area meter just before submergence and 7 days after desubmergence. The area thus obtained was divided by the area of ground to get leaf area index (LAI). Percent change in LAI was recorded after desubmergence over control/non-submerged plants. Number of panicles was counted from each treatment, five panicles per plant were randomly selected and panicle length and panicle weight was determined. The crop was harvested and sun dried, then total produce was weighed and recorded as total biomass. The produce was then threshed and grains were separated, dried (up to 14% moisture content with grain moisture meter (Model-MB 45, OHAUS grain moisture meter, Switzerland)) and weighed.

Measurement of photosynthetic (Pn) rate

Net photosynthetic rate rate and stomatal conductance (Gs) of rice plants 7 days after submergence and non-submerged plants were measured with an infrared gas analyser (LI-6400, LI-COR, Lincoln, NE) around 11:00 AM. The conditions in the assimilation chamber were kept as follows: air humidity, 70%; leaf temperature, 35°C; light intensity (PAR), 1200 μmol m^{-2} s^{-1}, CO_2 concentration of 380 μmol CO_2

mol^{-1}. Middle portion (3 cm long) of the fully expanded and not senescent leaf blade was selected for Pn rate measurement rate.

Measurement of Leaf senescence (LS)

LS senescence is characterized by dramatic yellowing resulting from chloroplast degeneration. Submergence tolerant cultivars were able to retain green leaves for longer time than intolerant cultivars. LS was assessed immediately after submergence on per plant basis using a visual scale of 1 to 5. This visual score was based on the yellow proportion of leaves: 1 = all leaves green; 5 = all leaves completely yellow or degenerated (Toojinda *et al.*, 2003). A chlorophyll meter (Minolta SPAD-502) was used to confirm the LS based on the measurement of amount of chlorophyll or greenness of leaves.

Measurements of ethylene concentration

Ethylene was measured according to procedure described by Kende and Hanson (1976). The internodes (2 cm long) of plant (2 from each treatment) were placed in 30 ml test tubes with 2 ml of water or test solution. The tubes were stoppered with serum vial caps and kept horizontally. Ethylene was sampled by first injecting 1 ml of air into each tube with a tuberculin syringe, pumping the syringe several times, and then withdrawing 1 ml for analysis. Ethylene was determined by gas chromatography (GC Model Chemito CERES 800 Plus, Thermo Scientific) equipped with Porapak-Q column (6 feet long, 1/8 inch outer diameter, 80/100 mesh size, stainless steel column) and flame ionization detector. The oven, injector and detector temperatures were set at 100, 300 and 150°C respectively and the flow of carrier N_2 gas, air and H_2 were maintained at 15, 285, 30 ml per minute, respectively. The amount of ethylene produced from samples was expressed by comparing with the standard curve of pure ethylene standard gas (9.12 ppm in N_2, Matheson Tri Gas) and under afore mentioned GC conditions, ethylene was detected at retention time of 2.247 minutes.

Statistical analysis

The statistical analysis was carried out for each parameter studied based on factorial randomized block design model using SAS software, 2012 (version 9.2). Associations between parameters were studied using correlation and linear regression analysis. Means were compared by least significant difference (LSD) test if the *f* value is significant.

RESULTS

Floodwater characteristics

The temperature of floodwater during the crop growth period ranged from 22.6 to 27.1 °C in the morning (06:30 AM) and from 24.5 to 29.5 °C in the afternoon (02:00 PM). The temperature was slightly lower during submergence at AT stage (21 DAT), as compared to 45 and 65 DAT, due to prevailing weather conditions. The pH, DO and EC of floodwater ranged from 8.3–8.5, 7.3–8.2 mg l^{-1} and 0.24–0.27 dSm^{-1},

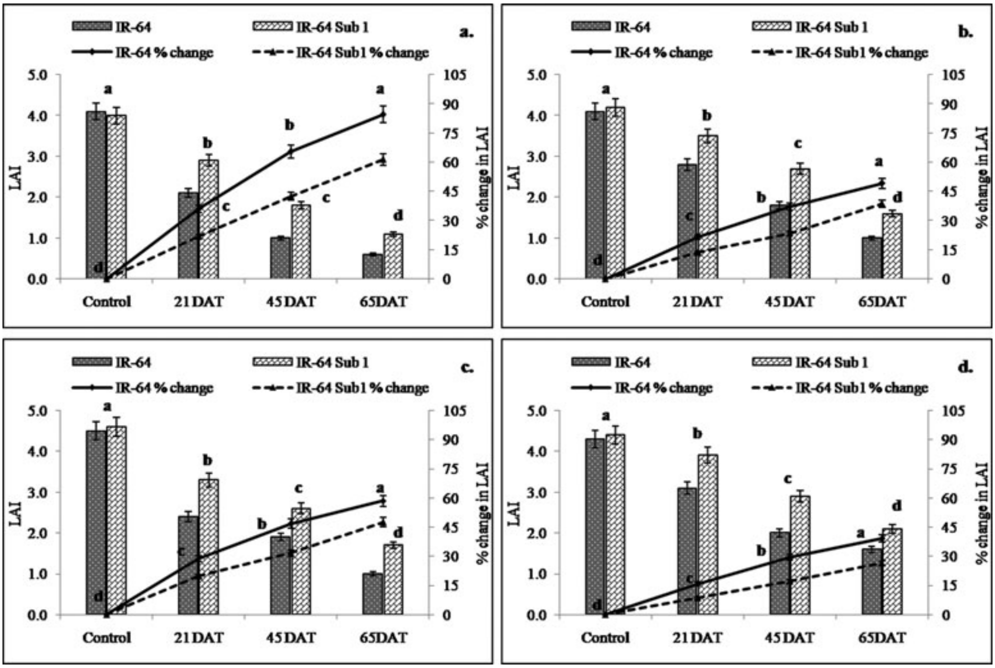

Figure 1. Leaf area index (LAI) and percentage change in LAI of IR64 and IR64-Sub 1 under non-submerged conditions and submergence at 21, 45 and 65 days after transplanting (DAT) (vertical bars in each line and column represents standard error). (a) pre-submergence N application, (b) post-submergence N application, (c) basal P and pre-submergence N application and (d) basal P and post-submergence N application. Vertical bars on primary axis represent LAI and lines on secondary axis represent percentage change in LAI.

respectively. Water depth did not have significant effects on the temperature, pH, DO and EC, but substantially affect light penetration. When calculated as the percentage of total incidence irradiance above the water surface, under-water light intensity decreased by 35.9% at 5 cm below the water surface, and by an additional 53.7% decrease at 50 cm and 77% at 75 cm.

Growth and metabolic activities affected due to submergence

Leaf area index. LAI before submergence was almost similar in both the cultivars but decreased significantly after submergence with more devastating effects in IR64, and percentage change in LAI was higher when submergence induced at H stage, as compared to other stages and non-submerged plants (Figure 1). The decrease in LAI after submergence was 54.2% in IR64 and 14.7% in IR64-Sub1 as compared to non-submerged conditions. N application before submergence (48 h before submergence) resulted in highest percentage change in LAI (96%) which indicates that before submergence N application contributed to more chlorophyll degeneration, damage was more severe when basal P was not applied. Minimum percentage change in LAI (23.1%) was observed when N applied after desubmergence (48 h after submergence) along with basal P (Figure 1).

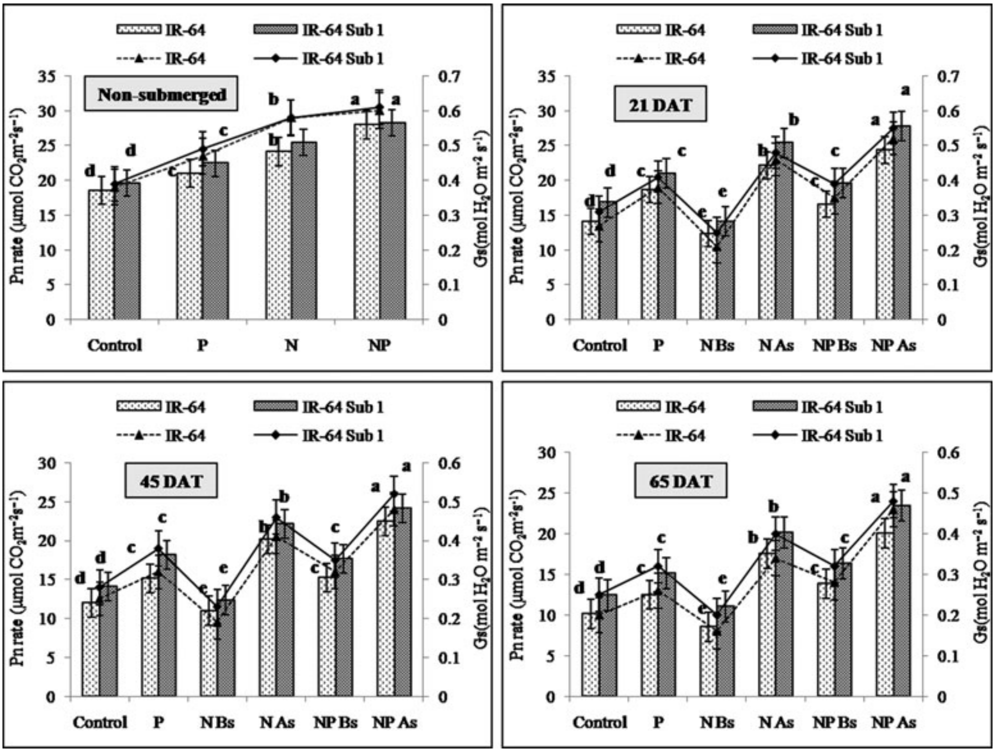

Figure 2. Photosynthetic (Pn) rate (μmol $CO_2m^{-2}s^{-1}$) and stomatal conductance (Gs) (mol H_2O m^{-2} s^{-1}) of IR64 and IR64-Sub 1 under non-submerged conditions and submergence at 21, 45 and 65 days after transplanting (DAT) (vertical bars in each line and column represents standard error). Vertical bars on primary axis represent Pn rate and lines on secondary axis represent Gs. Control - no N and P application, P-basal P application only, N Bs-pre-submergence N application, N As - post-submergence N application, NP Bs - Basal P and pre-submergence N application and NP As - Basal P and post-submergence N application.

Photosynthetic rate and stomatal conductance. Pn rate and Gs of both cultivars was almost similar under non-submerged conditions. The leaf Pn rate decreased in both the cultivars with the progression of time of submergence but decrease was more in IR64 (44.1%) than in IR64-Sub1 (17.8%) as compared to non-submerged conditions (Figure 2). Higher value of leaf Gs in IR64-Sub1 after submergence resulted in 27.7% higher Pn rate over IR64 (Figure 2). (Pn) rate and the factor controlling Pn rate varied significantly with time of submergence and stage of crop. Pn rate remained highest when plants were submerged at AT stage whereas, minimum Pn rate and Gs was observed during H stage submergence. Nitrogen application after desubmergence considerably improved Pn rate and Gs, this improvement was more prominent when basal P was applied along with N. Application of N after desubmergence along with basal P resulted in 78.7% higher Pn rate as compared to control. Pn rate was lowest when N was applied before submergence, and much less when no basal P was applied with N (Figure 2).

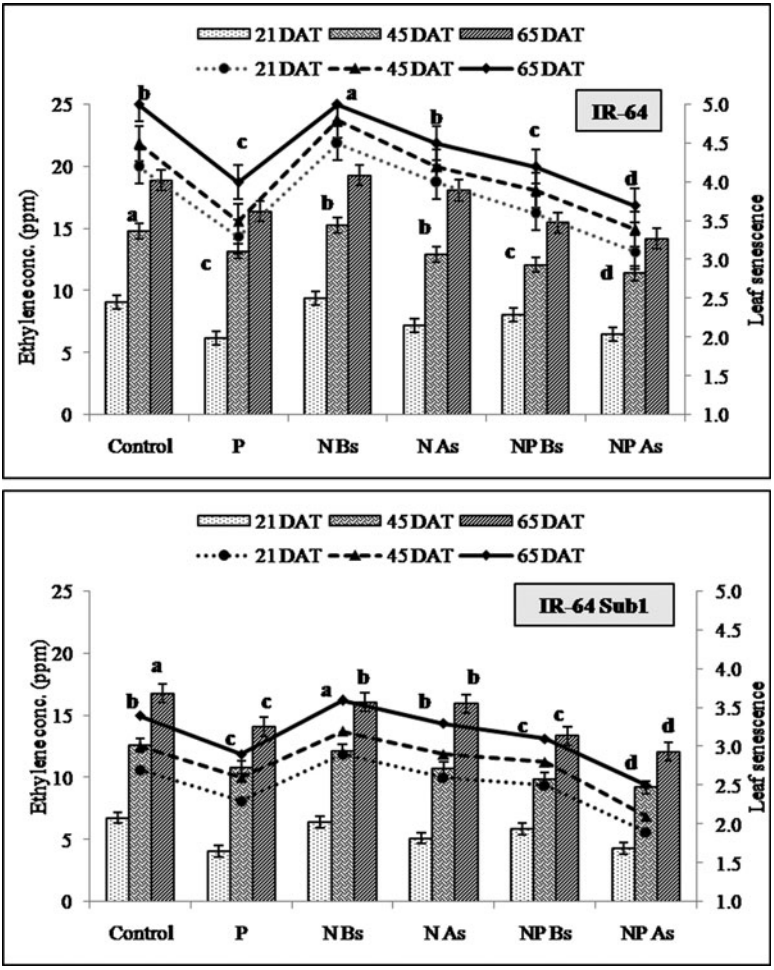

Figure 3. Ethylene concentration (ppm) and leaf senescence score of IR64 and IR64-Sub1 after submergence at 21, 45 and 65 days after transplanting (DAT) (vertical bars in each line and column represents standard error). Vertical bars on primary axis represent ethylene concentration and lines on secondary axis represent leaf senescence. Control - no N and P application, P-basal P application only, N Bs - pre-submergence N application, N As - post-submergence N application, NP Bs - Basal P and pre-submergence N application and NP As - Basal P and post-submergence N application.

Ethylene accumulation and leaf senescence. Submergence enhanced the ethylene accumulation in both the cultivars, and accumulation was 22.5% higher in IR64 over IR64-Sub1 (Figure 3). Stage of submergence also significantly influenced the ethylene accumulation irrespective of the cultivars; the order of ethylene accumulation was H > PI > AT stage. Plants enriched with nutrients reduced the accumulation of ethylene; P has the major role in low ethylene production and accumulation. Basal P application resulted in 22.1% lower accumulation of ethylene as compared to no P application.

LS occurs when plants were exposed to complete submergence for 14 days submergence at AT, PI and H stage (Figure 3). LS increased significantly after

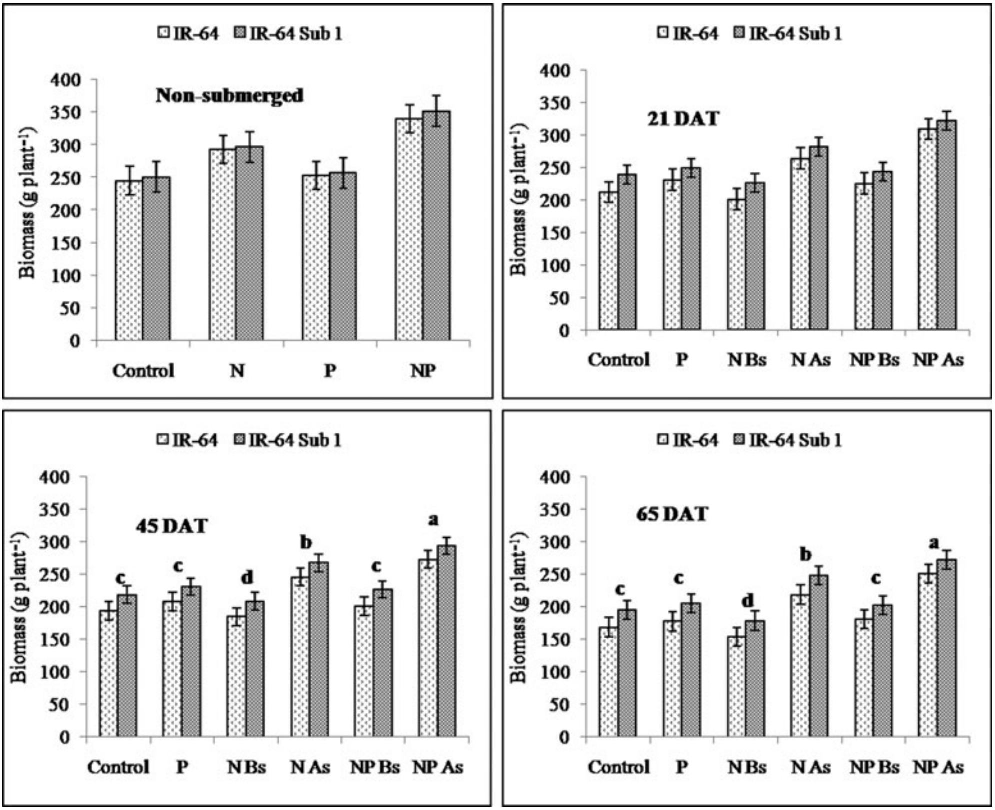

Figure 4. Biomass production (g plant^{-1}) in IR64 and IR64-Sub 1 at harvest, under non-submerged conditions and submergence at 21, 45 and 65 days after transplanting (DAT) (vertical bars in each line represents standard error). Control - no N and P application, P - basal P application only, N Bs - pre-submergence N application, N As - post-submergence N application, NP Bs - Basal P and pre-submergence N application and NP As - Basal P and post-submergence N application.

submergence and effect was higher in IR64. IR64-Sub1 was superior in terms of more number of green leaves after desubmergence. Submergence at H stage resulted in 24.1 and 18.9% higher LS IR64 and IR64-Sub1 respectively, over submergence at AT stage. LS was accelerated by N application before submergence; with more damaging effect when basal P was not applied.

Phenology, yield attributes and yield affected due to submergence

Biomass and phenology. Under non-submerged conditions, biomass accumulation remained at par in both the cultivars. After introduction of submergence, significant reduction in biomass over non-submerged plants was observed in both the cultivars but more in IR64. Biomass accumulation in IR64 decreased to extent of 13.2%, and in IR64-Sub1 decreased only by 1.8%, over non-submerged conditions (Figure 4). Biomass decreased variably with time of submergence compared to non-submerged conditions with progression of time of submergence, biomass reduced consistently from

Table 2. Phenology as affected by cultivar, time of submergence and nutrient application.

Treatments	Tillers/plant	Percentage productive tillers	Days to flowering	Days to maturity
Variety (V)				
IR64	22.8b	78.9b	118.5a	149.5a
IR64-Sub1	28.4a	85.5a	108.1b	137.1b
Stage of submergence (S)				
Non-submerged	36.4a	90.2a	98.7d	127.4d
Active tillering	28.0b	84.2b	108.8c	140.5c
Panicle initiation	20.9c	79.5bc	118.5b	149.2b
Heading	17.2d	75.1c	127.3a	156.7a
Nutrient application (N)				
Control	20.9d	76.3c	114.1bc	144.3bc
P	26.4c	80.7bc	110.8c	138.8c
N_{BS}	19.3d	75.4c	119.9a	153.8a
N_{AS}	29.3b	88.5a	112.4bc	141.4c
NP_{BS}	23.8c	81.0b	115.4b	147.8b
NP_{AS}	33.5a	91.5a	108.3d	136.4d
$LSD_{0.05}$ (VxS)	2.03	2.87	3.42	2.09
$LSD_{0.05}$ (VxN)	3.10	4.36	5.40	2.60
$LSD_{0.05}$ (SxN)	4.16	6.41	6.19	3.26
$LSD_{0.05}$ (VxSxN)	5.24	7.82	8.55	4.57

In a column, values followed by a common letter are not significantly different at $p < 0.05$ using least significance difference test.

BS-before submergence, AS-after submergence, N-nitrogen, P-phosphorus.

AT, PI to H stages. Application of N and P and application time of N significantly contributed to the biomass production at the time of harvest. Basal P and N application after desubmergence resulted in 36.5 and 45.1% higher biomass production in IR64 and IR64-Sub1, respectively over control. Whereas, N before submergence and no P application reduced biomass production up to 59.7 and 43.2% in IR64 and IR64-Sub1, respectively over basal P and N application after desubmergence (Figure 4).

Under non-submerged condition, IR64 and IR64-Sub1 flowered at the same time. Delay in flowering was observed after submergence in both the cultivars and more in IR64. Flowering was substantially delayed for both the genotypes, by about 30 days in IR64 and 18 days in IR64-Sub1 (Table 2). The apparent delay in maturity was mostly because of the delay in flowering, which was more affected when submergence induced at reproductive stage. Submergence for 14 days delayed flowering and maturity by 29, 20 and 10 days when submergence induced at H, PI and AT stage, respectively. Application of N prior to submergence substantially delayed flowering (27 days) and delay was more (33 days) when basal P was not applied with N. Flowering and maturity in both the cultivars were took less time (18 days) when N was applied after desubmergence with basal P as compared to control (Table 2).

Yield attributes and yield. Cultivar, stage of submergence and nutrient application had significant effect on yield attributes (Table 2). IR64-Sub1 produced maximum number of productive tillers (85.5%), panicles per plant (20.9), spikelets per panicle (81.2) and fertility percentage (75.6%) over IR64 (Tables 2 and 3). H stage submergence resulted

Table 3. Yield attributes as affected by cultivar, time of submergence and nutrient application.

Treatments	No. of Panicles/plant	Panicle length (cm)	Panicle weight (g)	Spikelets/ panicle	Grain filling (%)	1000-seed weight (g)
Variety (V)						
IR64	16.8b	21.2b	1.85b	69.2b	69.6b	22.6b
IR64-Sub1	20.9a	21.7a	2.09a	81.2a	75.6a	23.4a
Stage of submergence (S)						
Non-submerged	29.6a	22.2a	2.18a	89.3a	81.9a	23.4a
Active tillering	20.5b	21.9b	2.04b	77.2b	77.6b	23.3a
Panicle initiation	14.4c	21.2b	1.96c	72.1c	69.7c	22.8b
Heading	10.8d	20.8c	1.71d	62.3d	61.6d	22.2b
Nutrient application (N)						
Control	14.3d	20.8de	1.83d	67.9de	66.9c	22.4bc
P	18.1c	21.4cd	1.94c	72.8cd	71.4bc	23.2ab
N_{BS}	13.8d	20.2e	1.79d	65.7e	65.8c	21.6c
N_{AS}	22.2b	22.1ab	2.12b	81.9b	76.8b	23.6a
NP_{BS}	19.0c	21.8bc	1.90c	76.7c	72.3b	22.6b
NP_{AS}	25.8a	22.9a	2.26a	86.7a	83.3a	23.8a
$LSD_{0.05}$ (VxS)	1.34	ns	ns	4.51	3.04	ns
$LSD_{0.05}$ (VxN)	1.81	ns	ns	5.23	4.12	ns
$LSD_{0.05}$ (SxN)	1.92	ns	ns	6.72	5.86	ns
$LSD_{0.05}$ (VxSxN)	2.42	ns	ns	7.12	7.04	ns

In a column, values followed by a common letter are not significantly different at $p < 0.05$ using least significance difference test.

BS-before submergence, AS-after submergence, N-nitrogen, P-phosphorus.

in maximum damage of all the yield attributes, whereas, submergence at AT stage reflected the least damage. Nitrogen application before submergence resulted in less number of productive tillers (75.4%), panicles (13.8), spikelets (65.7) and grain filling (65.8%), these attributes affected more when basal P was not applied. Maximum number of tillers (28.2), panicles (25.8), spikletes (86.7) and highest grain filling (83.3%) was achieved with post-submergence N and basal P application (Table 3). Yield of the *Sub 1* introgression line were similar to that of its recurrent parent under non-submerged condition. The exposure to submergence had left detrimental effects on yield of both the cultivars, grain yield decreased up to 35.5 and 13.8% in IR64 and IR64-Sub1, respectively as compared to non-submerged condition (Table 4). The *Sub 1* genotype did not have any apparent negative effect on grain yield under controlled conditions, but considerably a yield advantage of 21.8% over IR64 under submerged condition. Submergence at different stages of crop growth caused a significant reduction in grain yield in both the genotypes. Fourteen days complete submergence at H, PI and AT stage resulted in 58.7, 29.8 and 7.6% yield loss, respectively, as compared to non-submerged conditions (Figure 5). Grain yield was influenced by application of both N and P, positive influence was reflected in case of basal P and post-submergence N application whereas, negative effect was seen when N applied before submergence. When N applied after desubmergence without basal P resulted in 16.7% yield reduction but when it combined with basal P then yield loss was only 4.2% as compared to non-submerged condition. Whereas, N application

Table 4. Grain yield (g plant^{-1}) influenced by interaction effect of cultivar, time of submergence and nutrient application.

	IR64			IR64-Sub1		
Treatment	Active tillering	Panicle initiation	Heading	Active tillering	Panicle initiation	Heading
Control	86.5cd	67.6c	54.3d	104.4d	94.5c	74.8d
P	96.7c	78.6c	69.8bc	113.7c	104.2c	91.2c
N_{BS}	79.7d	52.4d	43.3d	103.0d	89.2d	71.3d
N_{AS}	109.8b	93.4b	79.6b	127.7b	119.8b	102.3b
NP_{BS}	94.5c	75.6c	65.4c	110.1cd	101.3c	88.7c
NP_{AS}	121.6a	112.4a	92.3a	143a	131.2a	118.4a
Mean	98.1	80.0	67.4	116.9	106.7	91.1
$LSD_{0.05}$ (VxS)			9.68			
$LSD_{0.05}$ (VxN)			15.81			
$LSD_{0.05}$ (SxN)			19.36			
$LSD_{0.05}$ (VxSxN)			27.38			

In a column, values followed by a common letter are not significantly different at $p < 0.05$ using least significance difference test.

BS- before submergence, AS-after submergence, N-nitrogen, P- phosphorus, V-variety, S-stage of submergence, N-nutrient.

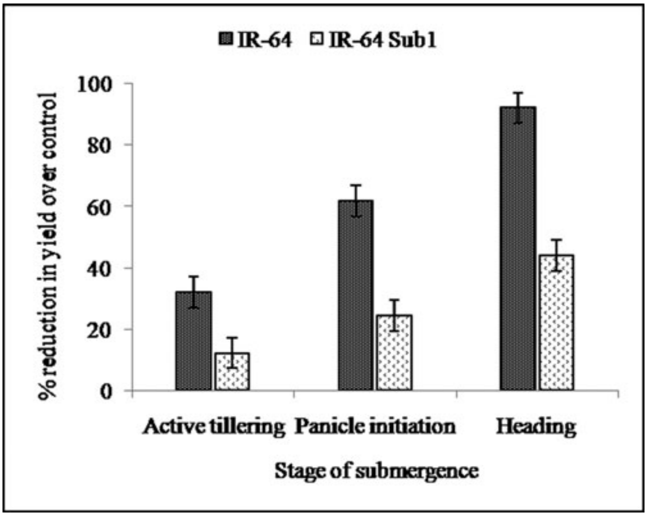

Figure 5. Percentage reduction in yield of IR64 and IR64-Sub 1 when subjected to submergence at active tillering, panicle initiation and heading stage (which coincides with 21, 45 and 65 days after transplanting (DAT)) over non-submerged conditions (vertical bars in each column represents standard error).

before submergence along with basal P resulted in yield reduction of 35.3% but when no basal P was supplied, yield subdued up to 44.7% indicating maximum damage (Table 4).

DISCUSSION

Submergence substantially decreased LAI, Pn rate, biomass, yield because chlorophyll contents decreased during submergence, and failed to recover following a return to non-submerged conditions. One of the reasons for the decreased Pn rate may be ethylene accumulation in shoots. Complete submergence enhances the accumulation of ethylene due to increased synthesis which triggers chlorophyll degradation and LS of the submerged plants through suppression of abscisic acid synthesis but enhanced synthesis and sensitivity to gibberellins (Fukao and Bailey-Serres, 2008) resulting in lower Pn rate and yield. Another reason may be low light reaching at canopy level is injurious resulting in slower photosynthesis, reduction in production of respirable assimilates (Jackson and Ram, 2003) and low LAI, biomass and yield. The depletion of Pn rate under submergence has been documented previously and primarily it was based on the loss of chlorophyll fluorescence (Panda *et al.*, 2008), lowering of Gs, intercellular CO_2 concentration (Ismail *et al.*, 2012) as well as denaturing of the Pn machineries (Mackill *et al.*, 2012). Survival of the *Sub 1* lines was substantially higher after submergence than that of the non-*Sub 1* varieties, and this is consistently reflected in a yield advantage of 1 to over 3.5 t ha^{-1} based on the stage at which submergence occurs, duration of submergence and the conditions of floodwater (Das *et al.*, 2009; Gautam *et al.*, 2014c; Ismail *et al.*, 2013).

LAI, Pn rate, biomass and yield was higher but LS and ethylene accumulation was lower in IR64-Sub1 compared to IR64 after submergence. This might be because *Sub 1* genotype increases the chances of survival and after growth in two ways: first, less energy is wasted on elongation (Das *et al.*, 2009); second, the intact chlorophyll helps rice plants to generate more energy during submergence (Singh *et al.*, 2009) and to perform better photosynthesis after desubmergence. Under submergence, plants receive less light and oxygen compared to control conditions, Pn apparatus suffered greater damage when plants exposed to air. The tolerant cultivar, however, adjusted to the new environment quickly, and the susceptible cultivar failed to adjust with the upcoming conditions (Panda *et al.*, 2008) and Pn rate decreased further.

Introgression of *Sub 1* gene narrowed the delay in flowering and suppressed ethylene induced LS caused by submergence, possibly by maintaining healthier plants that can resume faster, as *Sub1* is known to enhance chlorophyll retention and conserve carbohydrate reserves through reducing leaf and stem elongation (Das *et al.*, 2005; Fukao *et al.*, 2006) and by halting ethylene accumulation. In all cases, submergence induced reductions in yield attributes were more severe in IR64 because the ability for faster recovery and early tiller formation following submergence is probably one of the most important traits of *Sub 1* genotype for higher grain yield because only early tillers will become effective in contributing to grain yield.

Submergence at H stage proved most detrimental and resulted in lowest LAI, Pn rate, biomass and yield but maximum LF and ethylene accumulation, reverse was the case for AT stage submergence. Plants at active tillering stage are tender and able to recover fast, as plant age increases hardiness, decreases the ability of recovery or regeneration, which reflected in grain yield. Reddy and Mittra (1985a) reported that decrease in grain yield on submergence at reproductive stage was due to impaired anthesis causing high sterility as evidenced by higher number of unfilled spikelets. Moreover, the growth of the endosperm is suppressed after fertilization and this finally leads to abortive kernels with under developed endosperm (Matsushima and

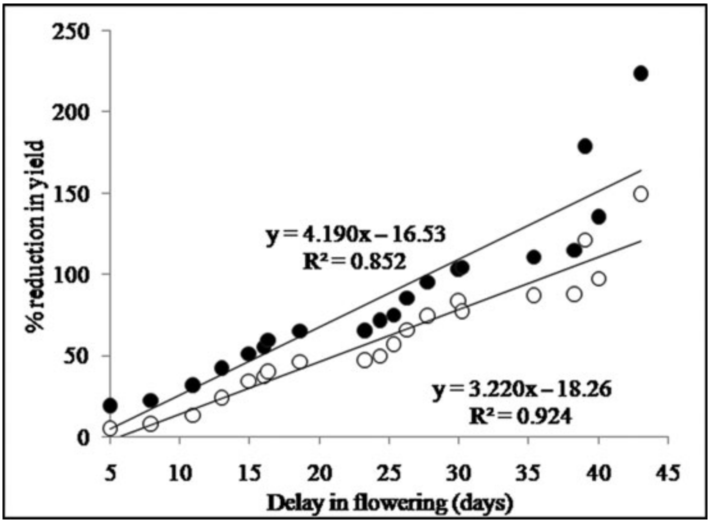

Figure 6. Association of delay in flowering time with corresponding percentage reductions in grain yield caused by submergence at 21, 45 and 65 DAT (coinciding with active tillering, panicle initiation and heading stage, respectively) in IR64 (closed circles) and IR64-Sub 1 (open circles).

Wada, 1966). Panicle weight and filled spikelets are less due to improper grain filling after submergence. Improper grain filling following submergence might attribute to a reduction in source capacity to provide sufficient carbohydrate, as well as a reduced translocation of assimilates to the sink (Palada and Vergara, 1972). Delay in flowering might also have contributed to loss in grain yield as compared to non-submerged conditions, strong positive correlation between delay in flowering and percentage reduction in yield supported the statement (Figure 6). Singh *et al.* (2009) also reported that delay in flowering and maturity could result in severe damage during grain formation and filling caused by stresses such as terminal drought and cold weather at reproductive stage.

Nitrogen application before submergence resulted in higher LS and ethylene accumulation but lower Pn rate because of chlorosis and degeneration of leaves and this damage was fatal without basal P application. Application of basal P alone delayed LS as evident from higher SPAD values, more green leaves or less senescence in *Sub1* genotype was also confirmed by the SPAD values, which was higher in *Sub1* cultivars (Figure 7). The strong negative correlation between leaf senescence and SPAD values but positive correlation between ethylene accumulation and LS (Figure 7); and negative correlation between ethylene accumulation and Pn rate (Figure 8) confirmed the above findings.

Nitrogen application after desubmergence along with basal P resulted in more number of green leaves, higher Pn rate, biomass and yield. It might be due to leaf sheath N could have a major accumulative and supportive role during submergence, perhaps by protein acting as respirable reserves. Addition of P to soil prior to seed sowing can stimulate overall growth in height, dry mass and it also helps in reducing

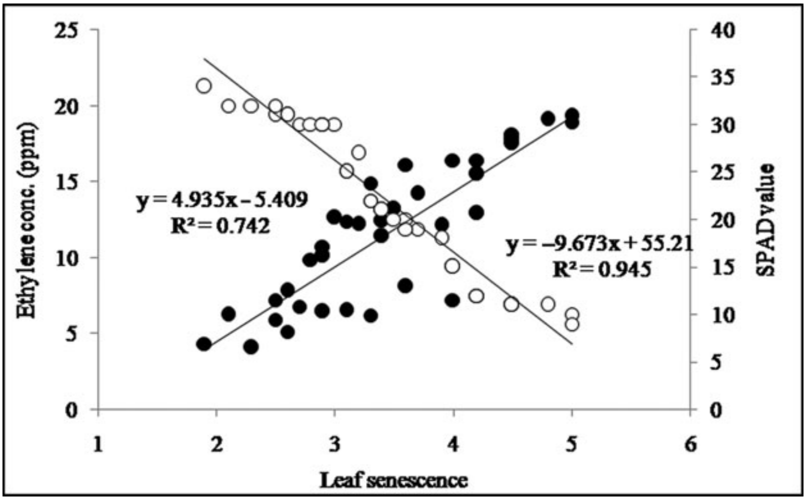

Figure 7. Relationship between leaf senescence and ethylene accumulation (closed circles); leaf senescence and SPAD values (open circles).

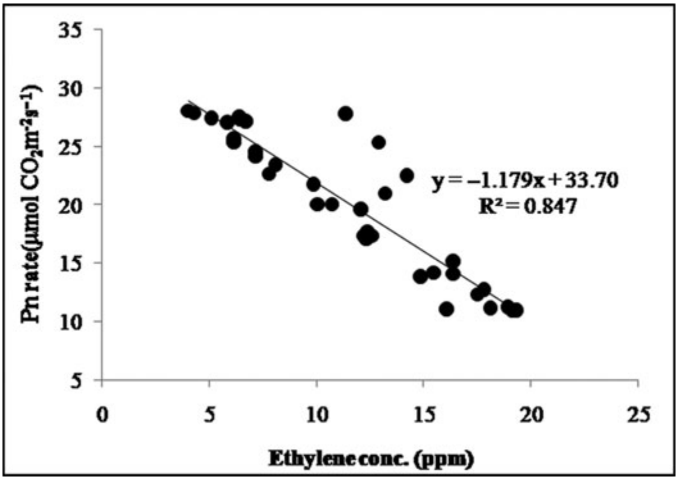

Figure 8. Relationship between ethylene accumulation and photosynthetic rate.

under water elongation and more strong recovery after desubmergence (Jackson and Ram, 2003). Nitrogen application after desubmergence along with basal P contributed to fast recovery of surviving plants leading to early flowering and maturity and this is of particular relevance to actual field condition in some areas (Gautam *et al.*, 2014b; Lal *et al.*, 2014). Reddy and Mittra (1985b) reported that the N requirement being more during vegetative stage, the N and P fertilization resulted in higher number of tillers and ultimately the number of panicle bearing tillers. This indicates the crop fertilized with these two nutrients could withstand better the onslaught of complete plant submergence than that of unfertilized crop and resulted in the grain yield 9–14%

Table 5. Correlation coefficients for the association among yield attributes, Pn rate, elongation and survival as affected by nutrient application with grain yield.

Parameters	Pn rate (μmol CO_2m^{-2} s^{-1})	Percentage productive tillers	LAI	Spikelets panicle	Panicles/ plant	Panicle wt. (g)	Biomass (g $plant^{-1}$)	Grain yield (g $plant^{-1}$)
Pn rate	1	0.788	0.859	0.873	0.943	0.909	0.836	0.859
Percentage productive tillers		1	0.906	0.900	0.907	0.747	0.657	0.670
LAI			1	0.919	0.958	0.904	0.799	0.809
Spikelets /panicle				1	0.961	0.877	0.866	0.873
Panicles/hill					1	0.941	0.879	0.894
Panicle weight						1	0.946	0.956
Biomass							1	0.998

Pn rate: photosynthetic rate, LAI: leaf area index.

over N alone under deepwater conditions. Grain yield is the ultimate result of various interacting growth factor, photosynthesis, interdependence on growth, development and yield attributing characters and is reflected in the strong positive correlation among yield attributing characters ($r = 0.89$), LAI ($r = 0.81$) and photosynthetic rate rate ($r = 0.74$) with grain yield (Table 5).

CONCLUSIONS

The results of this study showed that the IR64-Sub1 could maintain better phenology and yield attributes after submergence, which subsequently reflected higher yields after submergence. The tolerant genotypes followed quiescence strategy during submergence period had higher Pn rate, and lower ethylene concentration during recovery period than genotypes followed escape strategy. Submergence at H stage resulted in poor phenology and lowest grain yield. Application of P helped in improving the phenology, Pn rate and yield attributes, post-submergence N application along with basal P proved its superiority in terms of better leaf area, photosynthesis and led to higher grain yield. This study suggests that productivity could be enhanced in areas where untimely flooding is anticipated by applying basal P and adjusting the time of N application, if combined with tolerant germplasm, this approach could contribute to enhanced productivity and production of rice in flood-prone lowlands.

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