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EFFECT OF INORGANIC NITROGEN FORMS AND CONCENTRATIONS ON GROWTH OF RICE GENOTYPES UNDER SEVERE SALINE CONDITION

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ABSTRACT

Hydroponic and field experiments were conducted to evaluate the effect of three nitrogen (N) supplies and two N forms - ammonium nitrate and urea - on growth of rice plant under severe saline conditions. In hydroponics, two salt tolerant cultivars, Cuom and A69-1, were examined at tillering stage. Three concentrations of N - 1.4, 2.1 and 2.8 mM were applied in the Yoshida solution supplemented with NaCl at 113 mM. In paddy field under severe saline condition, six cultivars, viz. Cuom, Ngoi, Hom rau, A69-1, FL478, and IR28 were used to evaluate growth and yield at three N supplies, 45, 68 and 90 kg N ha^{-1}. In hydroponic conditionplant dry weight, number of root tips, total root length, and SPAD value increased with ammonium nitrate concentration.In contrast, all these parameters as well as leaf specific area and N use efficiency decreased with urea concentration in both of cultivars. In the paddy field experiment, N forms and levels had no effect on grain yield, but N forms affected 1000-grain weight and number of panicles per plant, whereas N levels increased dry matter weight at dough-ripening stage with N applied. The use of reduced level of N fertilizer is suggested for rice under severe saline condition.

Keywords: Ammonium nitrate, N use efficiency, *oryza sativa* L., salinity, urea.

Ảnh hưởng của các dạng đạm bón và lượng đạm bón đến sinh trưởng của một số giống lúa trong điều kiện mặn cao

TÓM TẮT

Các thí nghiệm được tiến hành trong điều kiện thủy canh và đồng ruộng nhằm đánh giá ảnh hưởng của ba liều lượng đạm và hai dạng đạm bón khác nhau là urê và amon nitrat đến sinh trưởng của các giống lúa trong điều kiện mặn cao. Thí nghiệm thủy canh được tiến hành với hai giống lúa chịu mặn là Cườm và A69-1, sử dụng dung dịch dinh dưỡng Yoshida có bổ sung muối NaCl với nồng độ 113 mM, xử lý đạm ở ba nồng độ là 1,4 mM N, 2,1 mM N, và 2,8 mM N. Cây lúa được đánh giá khả năng sinh trưởng ở thời điểm 4 tuần sau xử lý mặn. Ở thí nghiệm đồng ruộng, ba giống lúa địa phương gồm Cườm, Ngoi, Hom râu, và ba giống lúa cải tiến gồm A69-1, FL478 và IR28 được sử dụng để đánh giá sinh trưởng và năng suất dưới ảnh hưởng của ba liều lượng đạm bón là 45 kg N, 68 kg N, 90 kg N/ha ở điều kiện đất nhiễm mặn cao. Kết quả thí nghiệm thu được ở điều kiện thủy canh cho thấy các dạng đạm và liều lượng đạm bón ảnh hưởng theo chiều hướng khác khau đến sinh trưởng của cây lúa. Cụ thể, khi tăng lượng đạm bón, khối lượng chất khô tích lũy, số lượng rễ, tổng chiều dài rễ và giá trị SPAD tăng ở dạng đạm amon nitrat nhưng lại giảm ở dạng đạm urê cùng với chỉ tiêu chỉ số độ dày lá và hiệu suất sử dụng đạm ở cả hai giống lúa chịu mặn. Ở thí nghiệm đồng ruộng, việc thay đổi liều lượng và dạng đạm bón không ảnh hưởng đến năng suất thực thu. Tuy nhiên, dạng đạm bón có ảnh hưởng đến khối lượng 1.000 hạt và số bông/khóm, còn liều lượng đạm làm tăng khối lượng chất khô tích lũy ở giai đoạn chín sáp khi tăng lượng đạm bón. Các giống lúa địa phương có số bông/khóm và tỉ lệ hạt chắc thấp nhất ở liều lượng đạm bón 45 kg N/ha, không có sự khác biệt ở hai liều lượng bón 68 kg N/ha và 90 kgN/ha. Trong khi đó, các giống cải tiến chịu mặn không bị ảnh hưởng bởi việc thay đổi liều lượng đạm bón, giống nhiễm mặn IR28 lại giảm về năng suất thực thu, số hạt/bông, tỉ lệ hạt chắc và khối lượng 1.000 hạt khi giảm lượng đạm bón từ 90 kg N/ha xuống 45 kg N/ha.

Từ khóa: Amon nitrat, hiệu suất sử dụng đạm, lúa (*Oryza sativa* L.), mặn, urê.

1. INTRODUCTION

Rice is the staple food for more than half of the world population, however, its cultivation has widely been suffered from salinity. Salt stress has been shown to reduce grain yield by 18 to 25% in salt tolerant rice cultivars, and by 93% in sensitive ones (Moradi and Ismail, 2007, Tang *et al.*, 2011). Vietnam is one of the five countries most influenced by climate change (Nguyen, 2012). The annual sea level rises 2.15 mm, dry season extends, and agricultural production is significant depressed (Tran and Nguyen, 2013). Soil salinity has been occurring both from underground and in surface by sea water intrusion.

Rice plants resist to salt stress by decreasing in concentration of toxicity ions such as Na^+ and Cl^- in plants by different pathways. On the one hand, the toxic ions could be reduced in absorption decline or translocation adjustment inside plants, and finally located in vacuoles of leaves (Yeo and Flowers, 1984). On the other hand, the salt tolerance was enhanced with the provision of supporters. For example, supplying some ions antagonising with Na^+ and Cl^- such as K^+ and NO_3^- performed effective inhibition of toxic ion absorption to rice plants (Hu and Schmidhalter, 2005; Wu and Wang, 2012). Besides that, various rice genotypes could show different responses to salt condition. In our previous research, Tang *et al.* (2011) and Pham *et al.* (2012a, 2012b) demonstrated that salt tolerant local rice and improved rice performed differently in photosynthesis, proline accumulation, dry matter and N uptake in salt stress condition.

Nitrogen (N) is one of the most important nutrients for growth and grain yield of rice. To maximize yields, large amount of inorganic N fertilizer are usually applied. However, only 50% or less of the applied N is readily absorbed by plants. The other 50% or more is wasted by volatilization, leaching, surface runoff and the denitrification (Anjana and Iqbal, 2007). N uptake and assimilation decreases under salt stress condition. However, results of our survey showed that the amount of N fertilizer applied under saline conditions is as high as under non-saline ones.

Although ammonium-nitrogen has been considered as main nitrogen nutrition for rice, it has been shown that the lowland rice is exceptionally efficient in uptake nitrate nutrition. Moreover, with changeable irrigation by “alternate wet and drainage” in lowland, aerobic soil conditions were more frequent, thus, the role of nitrate-nitrogen form in paddy rice attracted more attention. Previous results indicated that partial replacement of NH_4^+ by NO_3^- stimulated biomass of rice plants. Besides that, nitrate-nitrogen was demonstrated less volatilizable than ammonium and urea-nitrogen. To avoid excessive losses, N fertilization strategy needs to be adapted to the N demand of crops. Therefore, improved N use efficiency of rice should be searched for by decreasing inorganic N application or changing forms of N applied.

2. MATERIALS AND METHODS

2.1. Experiment in hydroponic solution

Two genotypes were used, one local salt tolerant (Cuom) and one improved salt tolerant cultivar (A69-1).

Imbibed seeds were sown in sandy loamy soil in greenhouse. Ten seedlings of each variety at the four-leaf stage were transplanted to tanks containing 25L of Yoshida solution (Yoshida *et al.*, 1976) at a density of one plant per hole, two cultivars - thus 20 holes - per tank. The solution was renewed every two weeks. The pH was adjusted daily at 5.0 - 5.5 with KOH 2M or HCl 1M. At transplanting , N was applied in two different forms NH_4NO_3 and urea at three different concentrations, i.e. 1X (the standard concentration in the Yoshida solution: 2.8 mM N), 2/3X (2.1 mM N), and 1/2X (1.4 mM N). Starting from the same period, NaCl was applied in all treatments at the severe concentration of 113 mM (≈11.5 dS/m of EC). Each of the six treatments was repeated twice, thus amounting to 12 tanks, 120 plants per genotype, 240 plants in total. The plants were examined after four weeks of treatment. The experimental design corresponded to a completely randomized design (CRD) by rearranging the tank position every week, with three fixed and crossed factors-genotype, N concentrations and N forms.

Four weeks after treatment, the number of tillers per plant, the chlorophyll content index (CCI), root system development and dry weight were evaluated on five plants of each genotype in each treatment. The CCI was recorded at three points of the upper surface of the two youngest fully expanded leaves by using a SPAD meter (SPAD 502, Minolta, Japan). The number of roots, total root length, total root surface area, total root volume, and root a verage diameter were determined by using the root scanner WinRHIZO software package (v. 5.0, Regent Instruments Inc., Canada). Plant dry weight was determined after drying the samples in an oven at 80°C till constant weight (approximately 48h).

Nitrogen use efficiency (NUE) was calculated using the formula as follow:

Agronomical NUE (agNUE) = Total dry weight ($g.plant^{-1}$)/ Total N applied (g)

2.2. Experiment in paddy field

Six rice cultivars including three local cultivars (Cuom, Hom rau, and Ngoi), two improved cultivars (A69-1 and FL478), and one salt sensitive cultivar (IR28) were used in this experiment.

The experiment was conducted in paddy field under severe saline condition in coastal area in Nam Dien commune, Nghia Hung district, Nam Dinh province of Vietnam from June 2015 to November 2015 to evaluate agronomical and physiological traits related to NUE of rice under salt condition. Two N sources - urea and NH_4NO_3 - were applied at three levels, 90, 68 and 45 kg ha^{-1}. Thus there were six different treatments applied to six cultivars and total of 36 experimental plots. The experiment was laid out in a split-split-plot design with three replications; each plot size was 10 m^2. Phosphate and potassium fertilizers were used at dose of 90 kg P_2O_5 ha^{-1} and 90 kg K_2O ha^{-1} in all treatments.

At harvesting time, grain yield and yield components (number of panicles per hill, number of grains per panicle, filled-grain rate and 1000-grain weight) were determined.

The chlorophyll content index (SPAD value), leaf area and plant dry matter weight were measured at the three growth stages:active tillering stage (four weeks after transplanting), heading stage, and dough-ripening stage (two weeks after heading).

Soil salinity was measured every week by the electrical conductivity meter HI 8633 (HANNA instruments, USA).

Data were statistically analyzed using the "R" software.

3. RESULTS AND DISCUSSION

3.1. Hydroponic experiment

N forms, N concentrations and their interaction significantly affected dry matter weight, total root length, number of root tips, SPAD value, leaf specific area, and N use efficiency of rice plants (Table 1). Plants generally performed better with urea (U) than with ammonium nitrate (AN). It may be because urea requires less energy to be assimilated than nitrate which needs firstly to be reduced to ammonium. With AN treatment, plant dry weight, root system development (data only shown for number of root tips and total root length, not for root volume, root average diameter, and root surface area), SPAD value and leaf specific area increased with increasing applied N in both cultivars (Figure 1). In contrast, with U treatment, rice plants performed better under the lowest N concentration and dramatically decreased under the highest one. It could be that, whereas in soil condition urea is hydrolysed by urease - a nickel-dependent enzyme produced by soil microorganisms - into NH_4^+, this enzyme is unavailable in nutrient solution, hence, urea cannot hydrolysed (Merigout *et al.*, 2008) and excess urea may become toxic. Tan *et al.* (2000) reported that in tomato, at the seedling stage, the absorption of urea-N was only 25% of nitrate-N, but in subsequent stages it was up to 80%. When using ^{15}N urea as a tracer in Arabidopsis, Merigout *et al.* (2008) proved that part of urea was absorbed by roots and was transferred to shoots before its hydrolysis and assimilation, whereas NH_4^+ seemed to be assimilated directly in roots and NO_3^- was rapidly translocated to the shoots.

Cuom cv. performed better that A69-1 under the saline conditions of the experiment. This result was consistent with our previous studies (Pham *et al.* 2012a, 2012b). In addition, photosynthesis, leaf area, dry matter weight, chlorophyll content and proline accumulation increased when increasing ammonium nitrate from 1.4 to 4.3 mM N in both Cuom and A69-1 cultivars.

3.2. Field experiment

The experiment was conducted under severe saline condition in a field which conductivity varied from 4 to 8 mS cm^{-1} (Figure 2), which is higher than the sensitivity threshold reported as 3 mS cm^{-1} for *indica* cultivars (Singh *et al.*, 2010). Neither N forms nor N affected the salinity all through the experiment. At transplanting time, salinity was around 4.5 mS cm^{-1} and it reached a peak at 8 mS cm^{-1} 3 weeks after transplanting (WAT) (tillering stage). Hence, it resulted in a strong decrease in numbers of tillers, and consequently number of panicles per plant and grain yield. Subsequently, it decreased at 4 mS cm^{-1} at 8 WAT because of the rain. At this time, panicle initiation started in three local varieties, namely Cuom, Hom rau and Ngoi, whereas IR28 and FL478 were already at the flower differentiation stage. Salinity then steadily increased and picked up to 6.7 mS cm^{-1} at the heading stage. Such a high salinity level at this stage and subsequent ones could have a strong effect on pollination, grain filling and finally grain yield in all the varieties.

The ANOVA results (Table 2) indicated that N forms only significantly affected 1000-grain weight (1000 GW), and N levels only dry weight and SPAD value at dough-ripening. The interaction between these factors was not significant. In contrast, the effect of the cultivars was significant for all parameters. The interaction between N forms and cultivars, N levels and cultivars, and between the three factors were significant on number of panicles, number of grains and 1000 GW.

Thus, under these prevailing severe saline conditions, increasing N levels did not significantly increase grain yield, except in IR28 cultivar (Figure 4). There was a positive correlation between N applied and filled-grain rate for both N forms (Table 3). N application correlated with SPAD value at active tillering stage for U form, and with SPAD value at dough-ripening stage for AN form. Grain yield correlated positively with 1000GW and number of grains for both N forms, as well as with number of panicles for U form only. In addition, grain yield correlated positively with LAI and dry matter weight at the reproductive stage, but negatively with SPAD at this stage. Number of grains correlated with LAI and dry weight at the reproductive stage for both N forms, whereas number of panicles correlated with LAI and dry weight only for U form.

Table 1. Analysis of variance with three fixed factors for agronomical and physiological parameters in the hydroponic experiment

Source of variation	df	DM	TRL	NR	SPAD	LSA	NUE
F	1	***	***	***	***	**	***
L	2	***	**	***	***	**	***
C	1	***	***	***	***	*	***
F*L	2	***	***	***	***	***	***
F*C	1	***	***	*	***	ns	***
L*C	2	***	***	ns	**	ns	***
F*L*C	2	***	***	***	***	ns	***

Note: F: N form, L: N concentration, C: cultivar, DM: dry matter weight, TRL: total root length, NR: number of root tips, SPAD: SPAD value, LSA: leaf specific area, NUE: N use efficiency, df: degrees of freedom, ns not significant,
$^* P < 0.05$, $^{**} P < 0.01$, $^{***} P < 0.001$

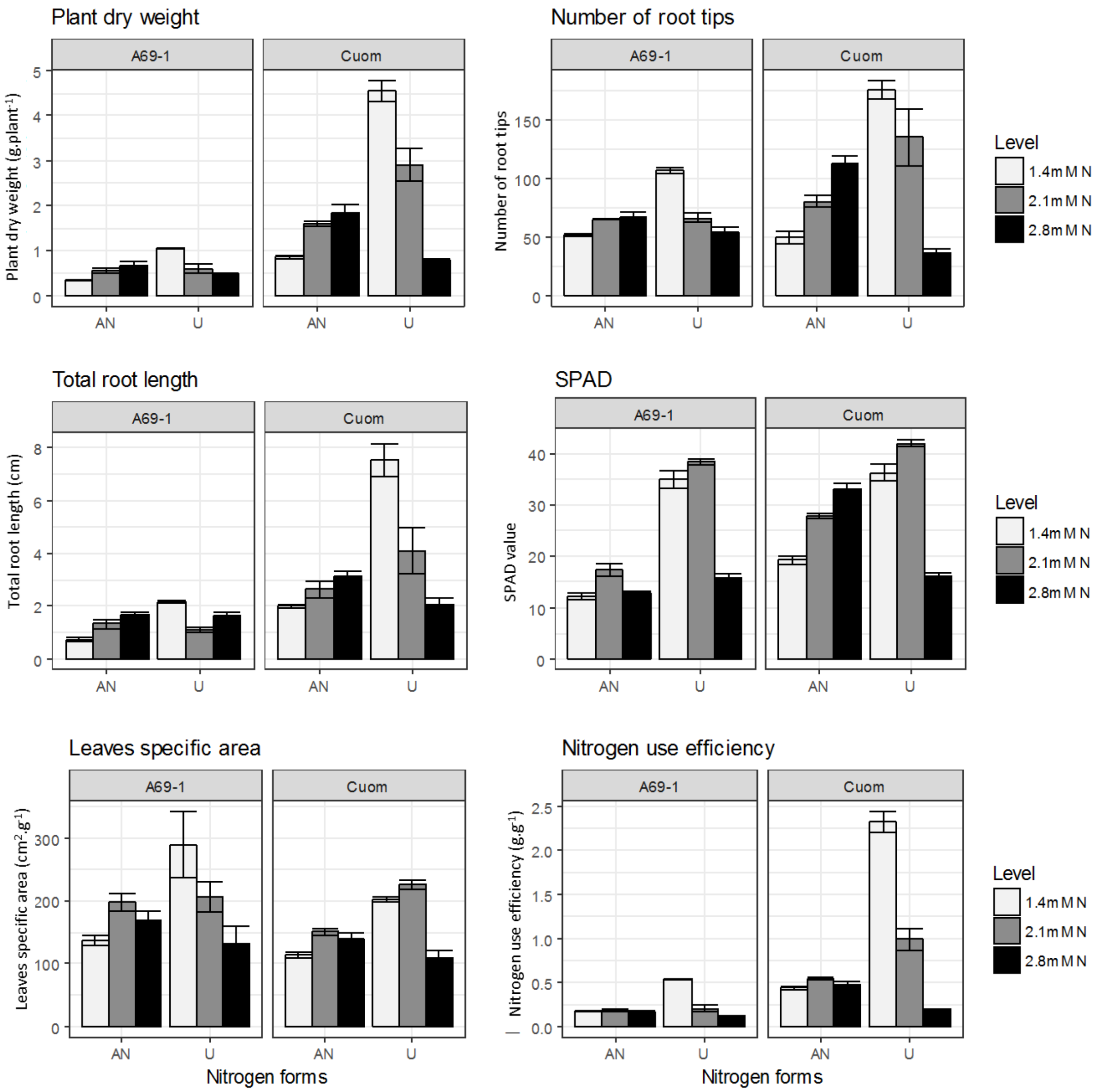

Figure 1. Plant dry weight, number of root tips, total root length, SPAD value, leaf specific area and N use efficiency of rice plants under different concentrations and forms of N

Note: AN: ammonium nitrate, U: urea

Decreasing N level from 90 to 68 and even 45 kg N ha^{-1} caused no effect on SPAD value (data not shown), leaf area index (LAI, data not shown), and dry weight at tillering stage, but caused a decline in these parameters at heading and dough-ripening stages (Figure 3). Differential response to N forms and levels was also found at heading and subsequent stages, but no clear tendency could be highlighted for plant dry weight. For example, Cuom cultivar produced the highest dry weight at heading time with NH_4NO_3 application at 90 kg ha^{-1}, but at dough-ripening stage with urea at 90 kg ha^{-1}. Besides, Ngoi and IR28 cultivars at ripening stage had predominant dry weight when they were applied urea of 68 kg N ha^{-1}, and NH_4NO_3 at 90 kg N ha^{-1}. Other cultivars had the highest dry weight at high dose of N application.

N forms or N levels showed no significant effect on grain yield but affected several yield components, i.e. number of panicles, number of grains per panicles, filled-grain rate and 1000-grain weight (Figure 4, 5). A69-1, an improved salt tolerant cultivar, showed the highest grain yield, followed by Cuom (traditional) and FL478 (improved cultivar). Among the three improved cultivars, A69-1, Fl478, and IR28, A69-1 was affected neither by N forms nor N levels, whereas FL478 showed higher number of panicles, higher number of grains under lower N levels for both forms. IR28, on the contrary, showed the lowest number of grains, filled-grain rate and grain yield at 45 kg N ha^{-1}. In the local cultivars, lowest values of yield components were obtained under the lowest N level and there were generally no significant differences between the other N levels.

4. CONCLUSION

In hydroponics, lower concentration of urea or higher concentration of NH_4NO_3-N were suitable form for rice in severe saline condition. In paddy field, N sources and N levels did not show any effect on grain yield, but showed effect on the number of panicles, number of grains, grain weight, SPAD, LAI and dry weight at the reproductive stage. Local rice cultivars showed the lowest number of panicles and filled-grain rate at the level of 45 kgN ha^{-1}, whereas there was no significant difference between the two other levels. In improved salt tolerant cultivars (A69-1 and FL478), there was no effect of N level on grain yield, while in the improved salt susceptible cultivar IR28, a significant decrease of grain yield was observed under the lower N supply. Thus, N fertilizer application should be decreased consistently with the low demand of the rice plants under saline condition. If so, N fertilizer should be reduced at basal dressing and first top-dressing (5 to 10 days after transplanting) because of the low growth of rice under saline condition at these stages, and maintained at the second top-dressing (at the middle of panicle initiation) to enhance growth and subsequent development stages.

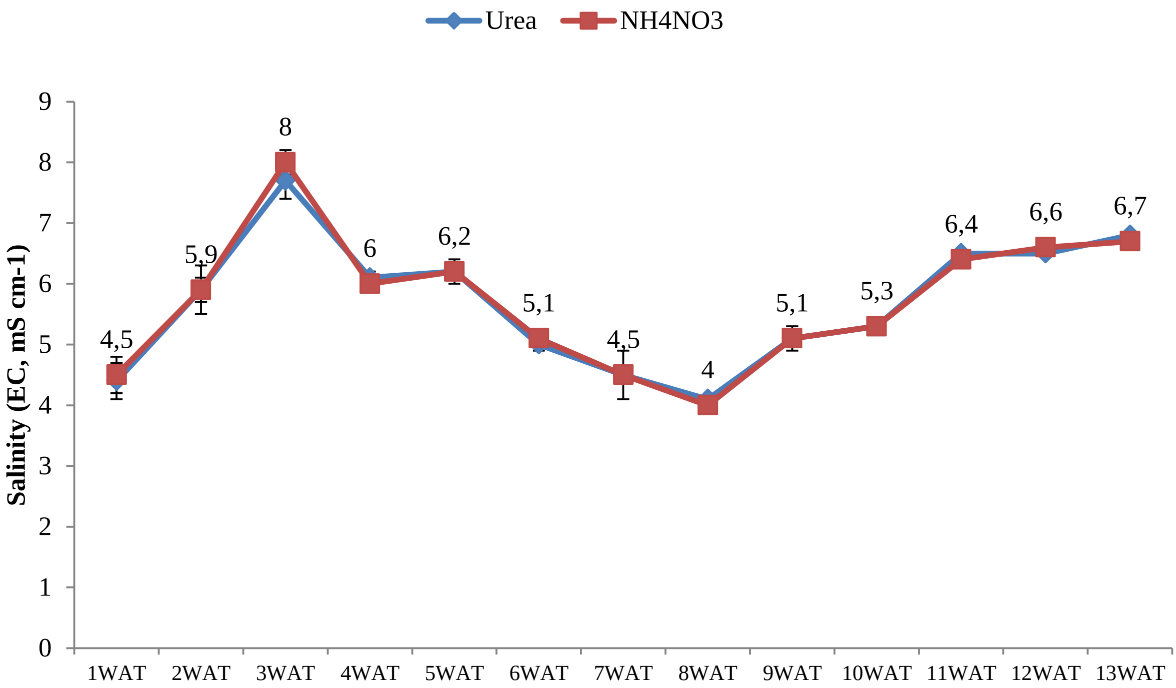

Figure 2. Salinity of soil in paddy field experiment

Note: WAT = weeks after transplanting

Table 2. Analysis of variance with three fixed factors for agronomical and physiological parameters in the paddy field experiment

Source of variation	df	GY	NP	NG	1000GW	AT DM	HD DM	DR DM	AT SP	HD SP	DR SP
F	1	ns	ns	ns	***	ns	ns	ns	ns	ns	ns
L	2	ns	ns	ns	ns	ns	ns	**	ns	ns	*
C	5	***	***	***	***	***	***	***	**	***	***
F*L	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
F*C	5	***	***	***	ns	ns	*	ns	ns	ns	ns
L*C	10	ns	***	***	ns	ns	ns	ns	ns	ns	ns
F*L*C	10	ns	*	**	*	ns	ns	ns	ns	*	ns

*Note: F: N form, L: N level, C: cultivar, GY: grain yield, NP: number of panicles per hill, NG: number of grains per panicle, 1000GW: weight of 1000 grains, AT DM: dry matter weight at active tillering stage, HD DM: dry matter weight at heading stage, DR DM: dry matter weight at dough ripening stage, AT SP: SPAD value at active tillering stage, HD SP: SPAD value at heading stage, DR SP: SPAD value at dough ripening stage, df: degrees of freedom, ns not significant, * P<0.05, ** P<0.01, *** P<0.001*

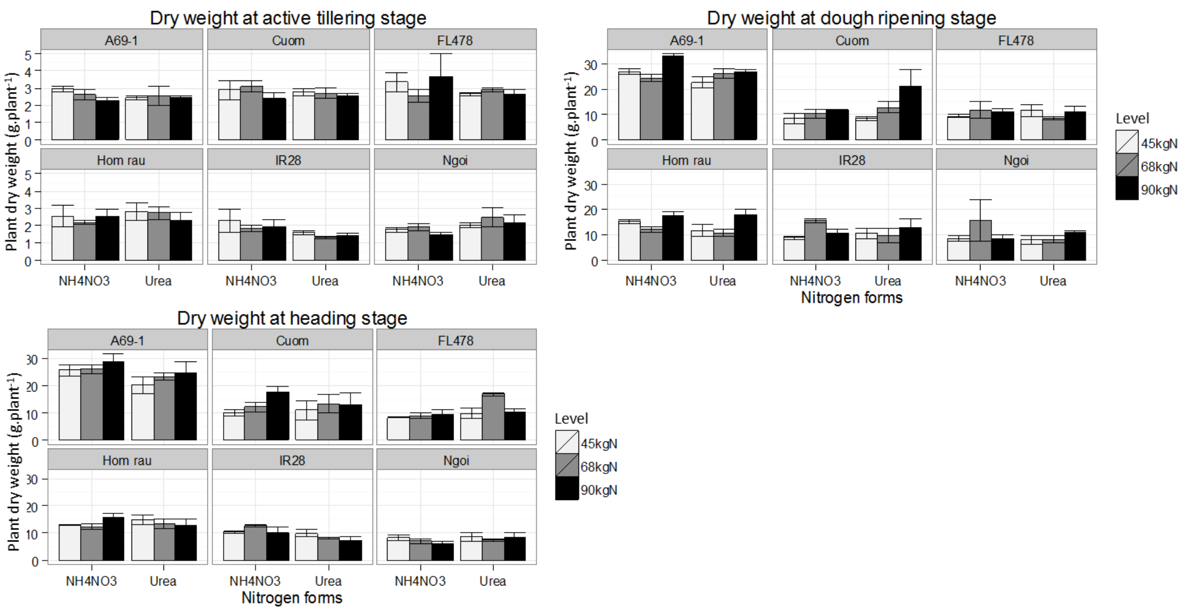

Figure 3. Dry matter weight of rice plants under different N forms and N levels in saline condition

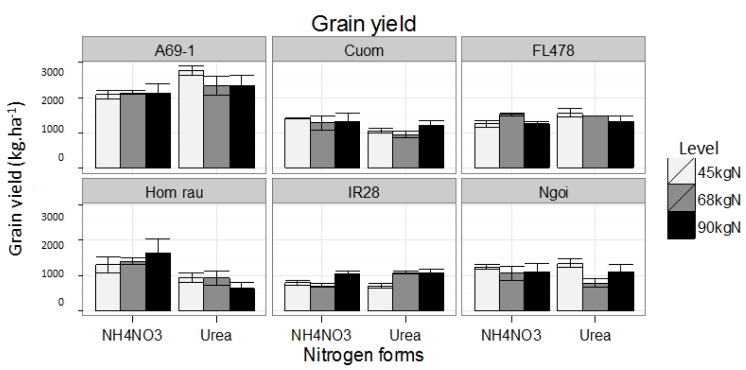

Figure 4. Grain yield of rice plants under different N forms and N levels in saline condition

Table 3. Correlation between measured parameters in ammonium nitrate (AN) treatment and urea (U) treatment in paddy field experiment

		GY	1000GW	NP	NG	%FG	ATSP	HDSP	DRSP	ATLAI	HDLAI	DRLAI	ATDM	HDDM	DRDM	NUE
GY	AN	-	0.73***	0.23	0.40	-0.07	-0.13	-0.77***	-0.21	0.43	0.77***	0.81***	0.32	0.81***	0.77***	0.21
	U		0.51*	0.50*	0.49*	-0.29	0.07	-0.48*	-0.48**	0.66**	0.69**	0.72***	0.20	0.76***	0.70**	0.28
1000GW	AN		-	0.40	-0.03	0.18	0.016	-0.51*	0.04	0.25	0.38	0.38	0.45	0.43	0.43	0.30
	U			0.63**	0.29	-0.19	0.23	-0.47*	-0.35	0.66**	0.51*	0.42	0.77***	0.53*	0.42	0.43
NP	AN			-	0.04	0.27	0.12	0.14	-0.19	0.25	0.08	0.20	0.27	0.10	0.22	0.07
	U				0.13	-0.07	0.31	-0.07	-0.25	0.34	0.44	0.53*	0.29	0.42	0.51*	0.14
NG	AN				-	-0.23	-0.39	-0.51*	-0.34	0.08	0.65**	0.54*	-0.27	0.54*	0.43	0.11
	U					0.11	-0.25	-0.65**	-0.09	0.32	0.58*	0.73**	0.18	0.71**	0.70**	0.03
%FG	AN					-	-0.08	0.22	0.45	-0.56*	-0.15	-0.07	-0.35	-0.14	0.11	-0.38
	U						0.03	0.23	0.02	-0.18	-0.33	-0.13	0.10	-0.27	-0.12	-0.40
ATSP	AN						-	0.24	-0.23	0.43	-0.08	-0.21	0.61**	-0.15	-0.17	-0.08
	U							0.40	-0.36	0.04	-0.04	-0.02	0.21	0.07	0.05	-0.29
HDSP	AN							-	0.03	-0.23	-0.77***	-0.70***	-0.11	-0.82***	-0.70**	-0.11
	U								-0.09	-0.60**	-0.70**	-0.66**	-0.37	-0.65**	-0.56*	-0.37
DRSP	AN								-	-0.44	-0.22	-0.09	-0.20	-0.11	0.04	-0.45
	U									-0.50*	-0.24	-0.12	-0.45	-0.43	-0.1	-0.07
ATLAI	AN									-	0.35	0.33	0.082***	0.34	0.18	0.35
	U										0.74***	0.54*	0.68**	0.73***	0.49*	0.40
HDLAI	AN										-	0.91***	0.15	0.96***	0.84***	0.11
	U											0.89***	0.30	0.91***	0.87***	0.08
DRLAI	AN											-	0.08	0.96***	0.93***	0.03
	U												0.15	0.84***	0.97***	-0.02
ATDM	AN												-	0.14	0.04	0.36
	U													0.41.	0.11	0.35
HDDM	AN													-	0.90***	0.06
	U														0.80***	0.13
DRDM	AN														-	0.03
	U															-0.11

*GY: grain yield, 1000GW: weight of 1000 grains, NP: number of panicles per hill, NG: number of grains per panicle, %FG: rate of filled-grain, ATSP: SPAD value at active tillering stage, HDSP: SPAD value at heading stage, DRSP: SPAD value at dough ripening stage, ATLAI: leaf area index (LAI) at active tillering stage, HDLAI: LAI at heading stage, DRLAI: LAI at dough ripening stage, ATDM: dry matter weight at active tillering stage, HDDM: dry matter weight at heading stage, DRDM: dry matter weight at dough ripening stage, NUE: N use efficiency, df: degrees of freedom, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$*

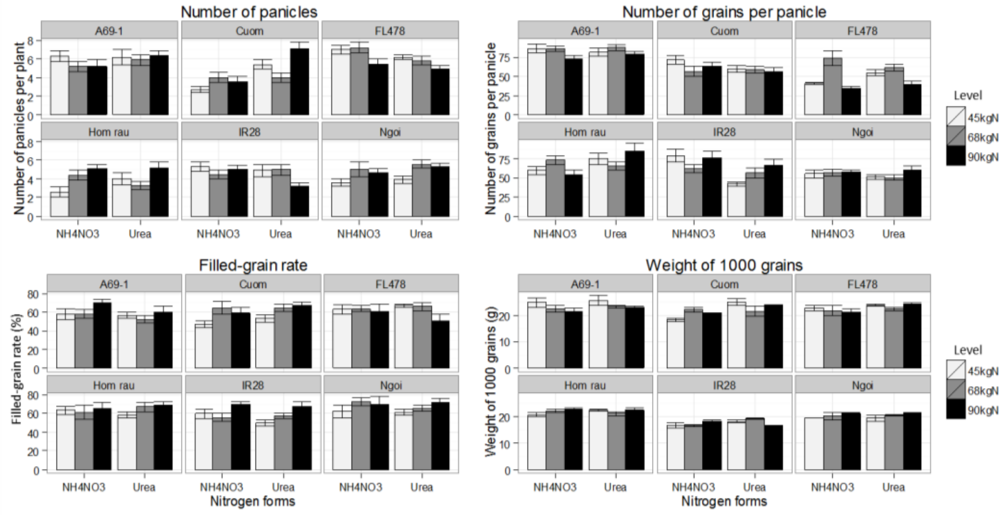

Figure 5. Yield components of rice plants under different N forms and N levels in saline condition

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