ROLES OF OXIDISED AND REDUCED NITROGEN AEROSOLS ON PRODUCTIVITY OF WINTER RICE (Oryza sativa L.) CROP

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Abstract— *In a pot culture (2011), aerosols of oxidized nitrogen (NaNO2) @20kgha-1yr-1 (≈200 ppm), reduced nitrogen (NH4Cl) @10kg-1hayr-1 (≈100 ppm) and distilled water (control) were sprayed (1500 cm3plant-1) weekly at different days after sowing (DAS) to study their impacts on physiology of rice varieties (Bishnuprasad, Kanaklata, Joymati, Jyotiprasad and Mulagavaru). In a field trial (2012), the simulated N-aerosols @ 20 & 40 kg ha-1y-1 of each (1000 cm3m-2) along with a control were misted to population of the rice variety, Kanaklata at four different growth stages viz., germination and tillering (0-30 DAS), maximum tillering, (30-60 DAS), panicle initiation (60-90 DAS) and reproductive development (90-120 DAS). In both pot and field experiments, the N- aerosols brought about wide differences in net photosynthesis rates, nitrate reductase activity, nitrogen use efficiency (NUE), membrane permeability in the crop. The aerosols with lower doses at earlier growth stages influenced NUE and economic yield of the crop varieties. Mechanisms of altering membrane permeability either by depletion of Ca2+, or acceleration of peroxidase activity of cell in presence of the aerosols have been elucidated.*

Keywords— atmospheric pollution, rice, aerosols, ammonia, cations, nitrite, nitrogen, photosynthesis, membrane, permeability, peroxidase.

I. INTRODUCTION

Crop plants suffer from oxides of nitrogen (NO, NO2 and N2O or NOx) and reduced ammonia (NH3 or NH4+) in rapidly growing urban and peri-urban areas (McClean et. al. 2011, Bharali et al., 2012). Nitrogen dioxides form week acids in the extra-cellular water and dissociate into HNO2 and HNO3 to produce nitrate, nitrite and protons (Zeevart, 1976). A concentration of NOx >10μll-1 alter physiological processes including N assimilation (Darrall, 1989). Ammonia at concentration >1mM in plants causes death of tissues in plants (Mehrer and Mohr, 1989).

In field, farmers apply fertilizers in nitrate and ammonium forms. These are very mobile in soil. Crop plants are able to utilize only 30-40% of applied nitrogen (Raun and Johnson, 1999). We have discussed here the tolerance mechanism(s) of rice to aerosols of nitrogen.

II. MATERIALS AND METHODS

A. **Cultural practices:** Rice genotypes (viz., Bishnuprasad, Kanaklata, Joymati, Jyotiprasad and Mulagavaru in pots were grown from seeds in plastic pots. Kanaklata was selected for field studies. In both pot and field, N,P,K @ 40:20:20 ha-1 in the form of Urea, Single super phosphate and Muriate of Potash were applied as basal. The crop was irrigated during the germination stage to avoid desiccation effects and a level of 2-3 cm water was maintained in the pots and field plots. The experiments were laid in Randomised Block Design and replicated thrice.

B. **Nitrogenous aerosols and their application:** Pot plants were sprayed with solutions (1500 cm3 per stage) each of NH4Cl and NaNO2 @ 100 and 200 ppm respectively along with a control (distilled water) at four growth stages viz., maximum tillering, panicle initiatiation and reproductive development. In field, plants were treated with the same N aerosols (1000 cm3 stage wise) @ 200 ppm & 400 ppm respectively at the stages of growth. Care was taken to avoid drifting of solutions from one plant to another at the time spraying.

C. **Measurement of photosynthesis:** Plants were incubated in transparent, airtight acrylic assimilation chambers (60x60x60 cm3 for potted plants and 15x15x15cm3 for field samples). Temperature (27-31oC), Relative humidity (40-66%) and Light intensity (12-15 K lux) prevailing inside the assimilation chambers were recorded by Hygrometer and Light meter during measurement of net photosynthesis. The pot plants or fresh moistened leaf samples from field were incubated in presence of normal ambient carbon dioxide concentration (380 ppm) inside the chambers during mid day under sunlight for half an hour. Air samples (10 cm3) were collected by clinical syringe through the rubber port of the chambers, and injected into the Environmental Gas Monitor (EGM-4) for measuring the carbon dioxide concentration after incubation. The rate of net photosynthesis was expressed as ppm CO2 absorbed per gram plant dry weight per hour as suggested by Larson and Karsaw, 1975.

D. **Estimation of Nitrate reductase (NR) activity in plants.:** The NR estimation was based on conversion of nitrate to nitrite and inhibition of nitrite reduction to ammonia in anaerobic condition (Srivastava and Ormrod, 1984). Green leaf samples (300 mg) of 10-15 mm square were put into 2.5 ml each of solutions containing 200 mM phosphate buffer (pH 7.5), 30 mM KNO3, 5 % (v/v) propanol in assay tubes. The tubes were incubated in a water bath at 30oC for 30 minutes, incubated further for 2 minutes in 1000C and allowed them to cool to room temperature. To detect nitrite in the assay tubes, colour development reagent i.e. 1 ml each of 1% sulfanilamide in 1N HCl and 0.02% N-(1-naphthyl)-ethylenediaminedihydrochloride were added to the solution. Mixed thoroughly and placed it in the dark at room temperature for 15 min. To determine NR activity the absorbency readings obtained at 540 nm in spectrophotometer were plotted on a standard curve, which was prepared from a stock of 25 n mol nitrite per ml using KNO2 in water.

E. **Determination of peroxidase activity in plants:** The level of lipid peroxidation was measurd in terms of Malondialdehyde content (MDA), a product of lipid peroxidation by following the method of Heath and Packer (1968). The leaf sample of 0.5 g was homogenized in 10 ml of 0.1 per cent trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000g for 5 minutes. Two milliliters of aliquot of the supernatant and 4 ml of 0.5% thiobarbuteric acid (TBA) in 20 per cent of TCA were mixed. The mixture was heated at 95ºC for 30 minutes and cooled in ice bath. It was centrifuged at 10,000g for 5 minutes and the absorbance of supernatant was recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted from the value of 532 nm. The absorption coefficient of 155 n mol per cm was used to calculate MDA content as: MDA (n mol per g fresh weight) = (OD x 6)/0.155 x volume extract / (2 x weight of sample)

F. **Calculation of Nitrogen use efficiency (NUE) of the crop varieties:** The Kjeldhal method was used to determine total Nitrogen content, which is based on catalytic conversion of organic nitrogen into ammonia and its subsequent estimation by acid base titration (Yoshida et. al. 1971). NUE of rice varieties at different growth stages under treatment were calculated as the product of per cent Nitrogen in grain and total grain yield per plant or per pot.

G. **Cell membrane Stability (CMS) and estimation of cellular cations:** Twenty pieces of young leaves from the treated plants were cut into about one centimeter square and immersed them first into 20 cm3 distilled water taken in plastic bottles (60 ml capacity). The mouth of the bottles was closed tightly to avoid leaking of the solution and checked gently using magnetic stirrer. Thus, freely water soluble ions in intercellular spaces of leaf were removed by the three serial washes with distilled water (each 10 min, 20 cm3). Now, to extract the cations present in the exchangeable sites of the cellular locations, the same leaf discs were eluted by two treatments (each 1 h 20 cm3) with 25 mM Sr2Cl (Bharali and Bates 2002). The solutions were collected into other plastic bottles. The plant samples were oven dried at 60oC to a constant weight. The electrical conductivity readings of these solutions against the samples collected from the experimental treatments were used to compute CMS. CMS is a measure of changes in membrane permeability due to cellular injury caused by the external agents as suggested by Sullivan and Rose (1972). Cations (K+ & Ca2+) present in both in intercellular and exchangeable sites as extracted by using distilled water and 25mM Sr2Cl respectively as above were estimated using a Thermo Jarrel Ash S12 atomic absorption/emission spectrophotometer (Franklin, MA, USA) and air-acetylene mixture. Element contents of the leaves were expressed in terms of their oven dry weight.

H. **Economic yield:** Grains were threshed out from the plants under respective treatments at different growth stages of the crop and expressed as gram per plant (in case of potted plants) and quintal per hectare in field harvest.

I. **Statistical analysis:** Data were analysed following GLIM program of Royal Society of London (Crawley, 1993). Significant differences between two mean values due to treatments or varieties and their interaction at a crop growth stage were computed by comparing their significant levels at $P<0.05$.

III. RESULTS

A. **Net photosynthesis (Pn) rates of rice crop:** In the pot culture experiment (Table 1) both Ammonium chloride (30.07%) and Sodium nitrite (22.77%) as compared to the control decreased the Pn measured at germination cum tillering stage (i.e. 0-30DAS). On an average, variety Bishnuprasad showed the highest Pn followed by Joymati>Joytiprasad>Kanaklata>Mulagavaru. At the maximum tillering stage i.e. 30-60 DAS, varieties and treatments had significant effects on Pn. The variety Jyotiprasad had the highest Pn followed by Mulagavaru>Kanaklata>Bishnuprasad>and>Jaymati. The treatment Ammonium chloride and Sodium nitrite reduced Pn up to 54.97% and 35.74% as that of the control respectively. At the panicle initiation stage i.e. 60-90 DAS interaction between varieties and treatments was significant in respect of Pn. The variety Mulagavaru was followed by Jyotiprasad>Joymati>Kanaklata>and Bishnuprasad in this regard. There were 8.38% and 26.96% reductions of Pn by Ammonium chloride and Sodium nitrite respectively. At the reproductive development i.e. 90-120 DAS, the treatments exerted significant effects on Pn of the varieties. Reductions of Pn by 12.6% and 37.49% in Ammonium chloride and Sodium nitrite treated plants were found respectively. A variation of Pn rate in varieties was found as Joytiprasad >Bishnuprasad>Mulagavaru>>Jaymati> and Kanaklata.

TABLE 1:
RATE OF PHOTOSYNTHESIS OF THE RICE VARIETIES FOLLOWING TREATMENT AT DIFFERENT DAYS AFTER SOWING (DAS)

Treatments	Ppm CO_2 absorbed per gm dry wt of varieties following treatment at stage 1 (0-30 DAS)					Ppm CO_2 absorbed per gm dry wt of varieties following treatment at stage II (30-60 DAS)				
	Bishnuprasad	Kanaklata	Joymati	Jyotirprasad	Mulag-avoru	Bishnuprasad	Kanaklata	Joymati	Jyotirprasad	Mulag-avoru
DDW (control)	1753.7	1561.5	1627.5	2261.7	1488.3	1319.6	2054.6	572.5	2189.8	1933.0
NH_4Cl (100 ppm)	1244.1	1075.7	1666.8	1231.3	864.0	778.0	532.2	705.2	853.4	629.7
$NaNO_2$ (200 ppm)	2442.8	969.4	1362.0	1160.8	781.7	900.2	764.3	644.6	11945	1488.4
			SEDiff (±)	LSD (0.05)				SEDiff (±)	LSD (0.05)	
Variety			-	n.s.				516.599	1012.53	
Treatment			-	n.s.				426..64	836.21	
Variety x treatment			-	n.s.				-	n.s.	
Cont'ed.										

Treatments	Ppm CO_2 absorbed per gm dry wt of varieties following treatment at stage III (60-90 DAS)					ppm CO_2 absorbed per gm dry wt of varieties following treatment at stage IV (90-120 DAS)				
	Bishnuprasad	Kanaklata	Joymati	Jyotirprasad	Mulag-avoru	Bishnuprasad	Kanaklata	Joymati	Jyotirprasad	Mulag-avoru
DDW (control)	1819.2	2183.0	1954.7	1586.2	745.8	1329.9	1045.1	1359.4	2275.9	2046.6
NH_4Cl (100 ppm)	1189.6	1016.2	1537.6	1908.5	941.5	1494.6	1557.6	1046.5	1631.0	1309.8
$NaNO_2$ (200 ppm)	838.7	745.8	862.5	1076.2	2532.2	1182.9	822.9	1105.8	1314.6	606.4
			SEDiff (±)	LSD (0.05)				SEDiff (±)	LSD (0.05)	
Variety			-	n.s.				-	n.s.	
Treatment			-	n.s.				3077.60	6032.097	
Variety x treatment			319.182	625.60				-	n.s.	

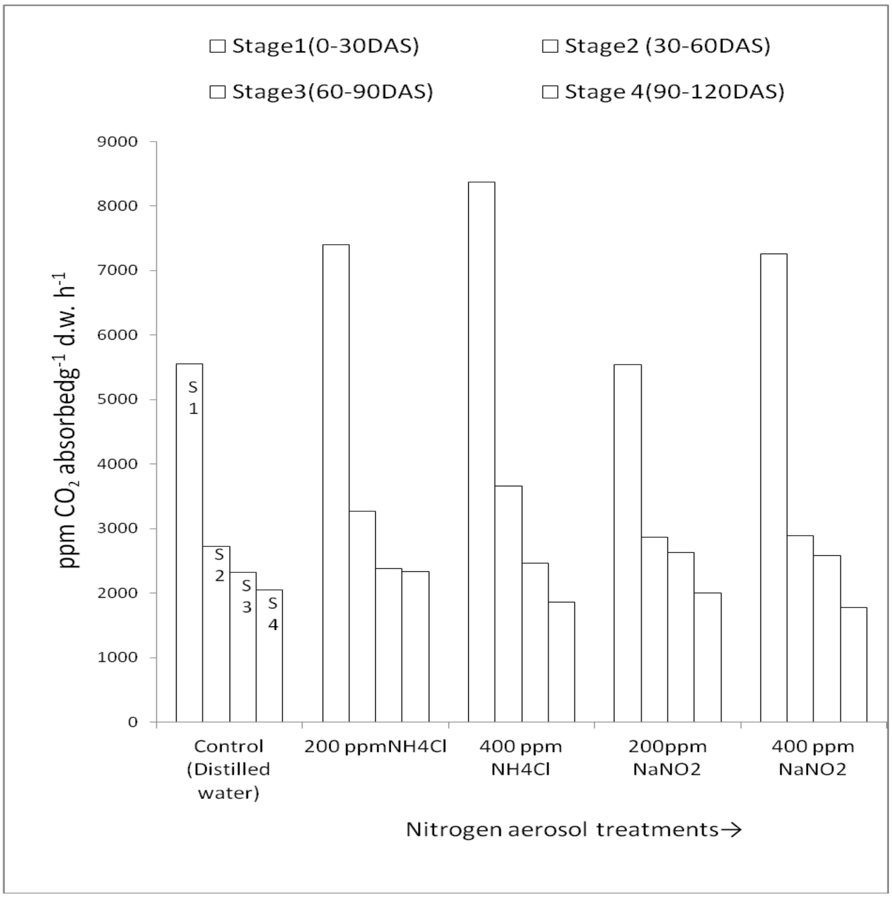

FIG. 1 NET PHOTOSYNTHESIS IN RICE

In the field experiment (Fig.1), the rate of Pn varied significantly due the aerosol treatments at different days after sowing of the crop. The aerosols influenced the carbon exchange rate but the values were lower due to NaNO2 (3.13%, 14.68%) than NH4Cl (21.67%, 29.42%) in 200ppm and 400ppm each of the aerosols as compared to the rate in the control. The rates of Pn were higher in the earlier stages than the later ones. So, the highest Pn rate was found at 0-30DAS followed by 30-60DAS and the lowest rate was found at 90-120DAS of the crop.

B. Nitrate reductase activity (NR): In the pot culture experiment (Table 2), the treatments and varieties had the significant effects on NR activity if the aerosols were sprayed at germination-tillering stage. The variety Mulagavaru had the highest NR activity followed by Jyotiprasad>Kanaklata>Jaymati>Bishnuprasad. Both Ammonium chloride and Sodium nitrite had reduced NR activity by 18.93% and 19.67% respectively as compared to the control. At maximum tillering stage the varieties had significant effects on NR activity only. On an average, the variety Jyotiprasad had the highest NR activity followed by Mulagavaru>Jaymati>Bishnuprasad>Kanaklata. In general, ammonium chloride and sodium nitrite reduced NR activity only by 0.59% and 3.39% as compared to the control respectively. The varieties had significant effects on NR activity of rice crop, if the aerosols were deposited at panicle initiation stage. The variety Jaymati had the highest NR activity followed by Kanaklata>Bishnuprasad>Mulagavaru>and Jyotiprasad. Ammonium chloride and Sodium nitrite increased NR by 31.22% and 3.93% at the last stage. On an average, Bishnuprasad>Jaymati>Mulagavaru>Kanaklata>and Jyotiprasad maintained a decreasing order of NR activity in their leaves.

In the field experiment (Fig.2), Nitrate reductase activity differed significantly due to the aerosols and their time of application. On an average, a little higher (up to 6.62%) NR activity was shown by the reduced aerosol, whereas NR activity was lowered by the oxidised aerosols (up to 15.77%) as compared to the control. The NR activity was reduced by the later application of the aerosols as S1 (28.15 %)>S2(30.21 %)>S4(26.76 %)>S3.

TABLE 2:
NITRATE REDUCTASE ACTIVITY (NM NO3-1 REDUCED G-1 H-1) OF RICE VARIETIES FOLLOWING TREATMENT AT DIFFERENT DAYS AFTER SOWING (DAS)

Treatments	Nitrate reductase activity of varieties					Nitrate reductase activity of varieties at stage II (30-60 DAS)				
	Bishnuprasad	**Kanaklata**	**Joymati**	**Jyotirprasad**	**Mulag-**	**Bishnuprasad**	**Kanaklata**	**Joymati**	**Jyotirprasad**	**Mulag-avoru**
DDW	35.83	50.00	33.33	40.00	56.67	61.67	46.67	53.33	60.00	58.33
NH_4Cl	28.33	36.37	30.00	35.00	45.00	55.00	53.33	60.00	60.00	50.00
$NaNO_2$	30.00	25.00	23.33	46.67	48.33	48.33	38.33	53.33	60.00	70.50
			SEDiff (±)	LSD (0.05)				SEDiff (±)	LSD (0.05)	
Variety			5.180	10.153				4.712	9.236	
Treatment			4.006	7.851				-	n.s.	
Variety x treatment			-	n.s.				-	n.s.	

Concentration	Nitrate reductase activity of varieties at stage III (60-90 DAS)					Nitrate reductase activity of varieties at stage IV (90-120 DAS)				
	Bishnu prasad	**Kanaklata**	**Joymati**	**Jyotirprasad**	**Mulag-avoru**	**Bishnu prasad**	**Kanaklata**	**Joymati**	**Jyotirprasad**	**Mulag-avoru**
DDW	58.33	91.67	115.00	56.67	71.67	36.67	53.33	47.50	61.67	45.00
NH_4Cl	71.67	61.67	71.67	66.67	55.00	91.67	65.00	85.00	43.33	70.00
$NaNO_2$	57.50	75.00	76.67	53.33	54.17	62.50	45.00	50.00	43.33	53.33
			SEDiff (±)	LSD (0.05)				SEDiff (±)	LSD (0.05)	
Variety			9.98	19.567				-	NS	
Treatment			8.267	16.202				9.292	18.211	
Variety x treatment			-	n.s.				-	n.s.	

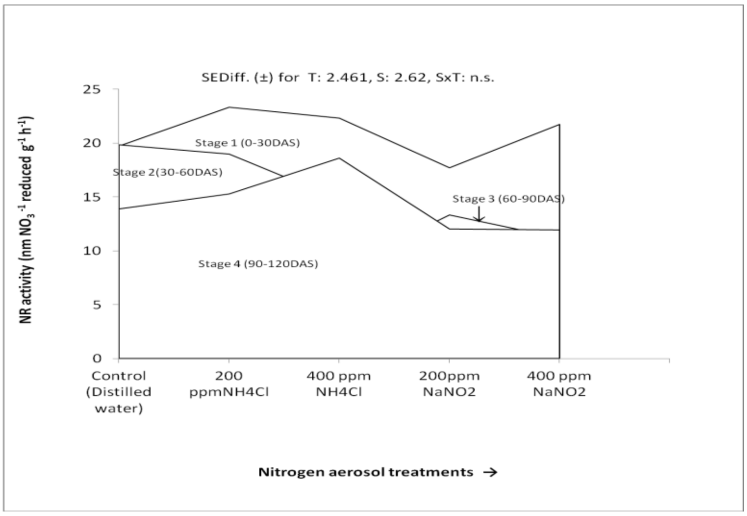

FIG.2 NR ACTIVITY IN RICE

C. Nitrogen use efficiency (NUE): Data on Nitrogen use efficiency in rice grains from the laboratory experiment are presented in Table 3. In the pot culture experiment, NUE varied significantly among the varieties (as Mulagavaru>Jyotiprasad>Kanaklata>Bishnuprasad> Jaymati) at the tillering stage of the crop. NUE in grains increased by 24.07% and 18.22% in Ammonium chloride and Sodium nitrite aerosol treatments respectively. The treatments ($F=25.64$, $p<0.05$) had significant effects on grain NUE while N-aerosols were sprayed at the maximum tillering stage. There were increases in NUE in grains of the rice varieties by 46.38% and 37.36% due to Ammonium chloride and Sodium nitrite treatments respectively. The varieties Bishnuprasad>Kanaklata>Jaymati>Mulagavaru>Jyotiprasad had significant differences in NUE. At panicle initiation stage of rice crop NUE also varied significantly due to interaction between varieties and treatments. There was 4.96% reduction of grain NUE by Ammonium chloride, while 7.09% of it was lowered by Sodium nitrite. A decreasing order of NUE was maintained in the varieties as Bishnuprasad> Jaymati>Mulagavaru>Kanaklata>>Jyotiprasad. Nitrogen use efficiency in rice grains of plants treated with the N-aerosols at reproductive development stage differed significantly due to varieties. The reductions of NUEs were 24.65% and 9.26% by Ammonium chloride and Sodium nitrite respectively. The varieties Kanaklata>Bishnuprasad>Mulagavaru>Jaymati>Jyotiprasad showed variations in NUE among themselves.

TABLE 3
NITROGEN USE EFFICIENCY (NUE) IN GRAIN OF VARIETIES FOLLOWING TREATMENT AT DIFFERENT GROWTH STAGES (DAYS AFTER SOWING:DAS)

Treatments	NUE (%) of varieties at 0-30 DAS					NUE (%) of varieties at 30-60 DAS				
	Bishnu-prasad	**Kanak-lata**	**Joy-mati**	**Jyotir-prasad**	**Mula-gavoru**	**Bishnu-prasad**	**Kana-klata**	**Joy-mati**	**Jyotir-prasad**	**Mulag-avoru**
DDW (control)	14.557	26.23	18.293	11.067	14.20	18.583	7.630	8.767	9.053	11.990
NH_4Cl (100 ppm)	13.70	11.823	18.597	29.483	29.53	18.267	22.6	19.143	13.707	15.670
$NaNO_2$ (200 ppm)	24.013	24.550	8.977	25.630	27.930	22.250	19.77	20.203	23.427	18.810
		SEdiff(±)		LSD(05)			SEdiff(±)		LSD(0.05)	
Variety		-		n.s.			-		-	
Treatment		-		n.s.			3.096		6.069	
Variety x treatment		5.108		10.0			2.321		4.549	
Treatments	**NUE (%) of varieties at 60-90 DAS**					**NUE (%) of varieties at 90-120 DAS**				
DDW (control)	14.950	13.09	9.617	10.507	22.310	12.113	17.570	10.187	11.700	8.620
NH_4Cl (100 ppm)	9.640	15.45	19.237	8.697	12.410	14.537	18.243	12.050	6.450	15.050
$NaNO_2$ (200 ppm)	20.320	12.39	15.363	12.153	6.710	23.770	16.873	13.023	13.043	13.170
		SEdiff(±)		LSD(05)			SEdiff(±)		LSD(0.05)	
Variety		-		n.s			3.325		6.935	
Treatment		-		n.s.			3.081		6.039	
Variety x treatment		1.609		3.155			2.505		4.909	

In the field experiment, significant differences in NUE% were found in between two treatment means, but not for application time (Fig.3). Higher is the dose of Ammonium chloride, 16.70% more is the NUE% , but it decreased at the 400ppm Sodium nitrite treatment by 6.68%. It might be due the negative influence of the later on NUE%.

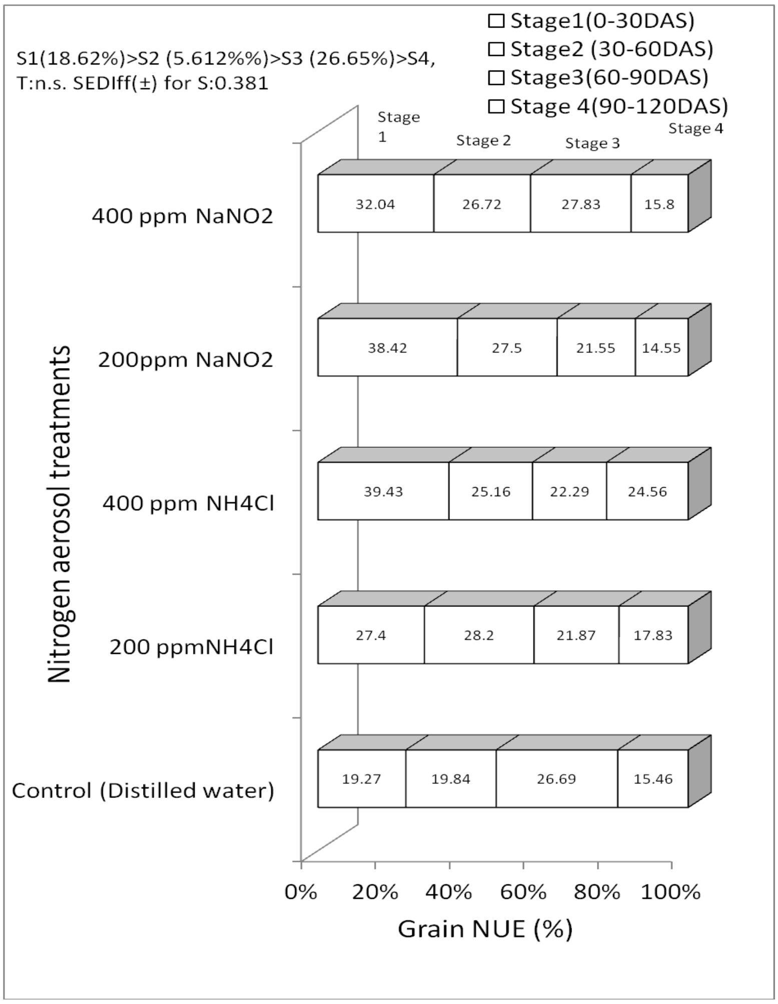

FIG.3 NUE IN RICE

D. Cell Membrane Stability (CMS): In the pot culture experiment (Table 4), both varieties and treatment had significant effects on CMS for foliar application of nitrogen aerosols at tillering stage of rice crop. The CMS decreased in the varieties treated with Ammonium chloride (by 58.83%) and Sodium nitrite (by 72.78%). The varieties Jaymati>Kanaklata>Mulagavaru>Jyotiprasad>Bishnuprasad differed in their CMS status. At maximum tillering stage, the treatments and its interaction with varieties had significant effects on CMS of the crop. Ammonium chloride lessened CMS (by 38.41%) and Sodium nitrite increased it (by 1.74% only) in the varieties. The varieties Jyotiprasad>Bishnuprasad>Mulagavaru>Jaymati>Kanaklata had strong differences in their CMS. Only varieties (Jaymati>Kanaklata>Mulagavaru>Jyotiprasad>Bishnuprasad) had significant effects on CMS of the crop treated at panicle initiation stage of the crop. The varieties Bishnuprasad>Jaymati>Kanaklata>Mulagavaru>Jyotiprasad exhibited significant differences in CMS at the reproductive stage. Generally, Ammonium chloride reduced CMS by 17.83% and Sodium nitrite increased it by 15.13%.

In the field experiment (Fig.4) Cell membrane stability varied significantly due to the aerosol treatments and their time of application. The CMS was reduced by both the reduced and the oxidised aerosols at 200ppm and 400ppm as (35.13%, 30.22%) and (40.81%, 95.85%) respectively as compared to the control. So, the oxidized aerosol caused more injury to cell membrane than the reduced nitrogen. Higher depletion of CMS as S1(28.34%)>S2(46.66%)>S3(76.91)>S4 was also found following their application at days after sowing. Therefore, it’s best to apply the aerosols at the earlier stages rather than the latest one.

TABLE 4
CELL MEMBRANE STABILITY (CMS) OF RICE VARIETIES FOLLOWING TREATMENT AT DIFFERENT GROWTH STAGES

Treatments (T)↓	CMS of varieties at stage I (0-30DAS) (DAS)					CMS of varieties at stage II(30-60DAS)				
Varieties (V)→	(V1)	(V2)	(V3)	(V4)	(V5)	(V1)	(V2)	(V3)	(V4)	(V5)
DDW (control)	5.82	18.05	94.41	15.23	15.27	2.32	2.26	3.25	5.95	2.53
NH_4Cl (100 ppm)	38.18	1.9	0.51	-1.06	0.96	2.55	2.08	2.64	3.82	5.51
$NaNO_2$ (200 ppm)	17.09	12.13	17.45	3.43	11.89	5.63	0.67	1.457	0.86	1.43
				SEdiff(±)	LSD (0.05)				SEdiff (±)	LSD (0.05)
Variety (V)				17.23	35.94				-	n.s.
Treatment (T)				15.59	30.56				1.188	2.328
V xT				-	n.s.				0.144	0.218
(Cont'ed)										
Treatments	**CMS at stage III (60-90DAS)**					**CMS at stage IV (90-120 DAS)**				
DDW (0ppm)	2.68	5.35	19.59	7.58	8.01	7.010	7.450	10.220	4.410	1.420
NH_4Cl (100 ppm)	5.92	9.88	10.41	4.71	6.8	19.270	2.680	7.410	3.10	3.49
$NaNO_2$ (200 ppm)	3.47	14.46	11.98	1.65	5.02	3.920	7.04	2.67	2.06	9.380
				SE diff(±)	LSD (0.05)				SE diff(±)	LSD (0.05)
Variety				2.424	5.057				0.326	0.678
Treatment				-	n.s.				-	n.s.
V xT				-	n.s.				-	n.s.

n.s.: non significant

Varieties (V) : V1: Bishnuprasad, V2:Kanaklata, V3:Joymati, V4:Jyotirprasad, V5:Mulagavaru

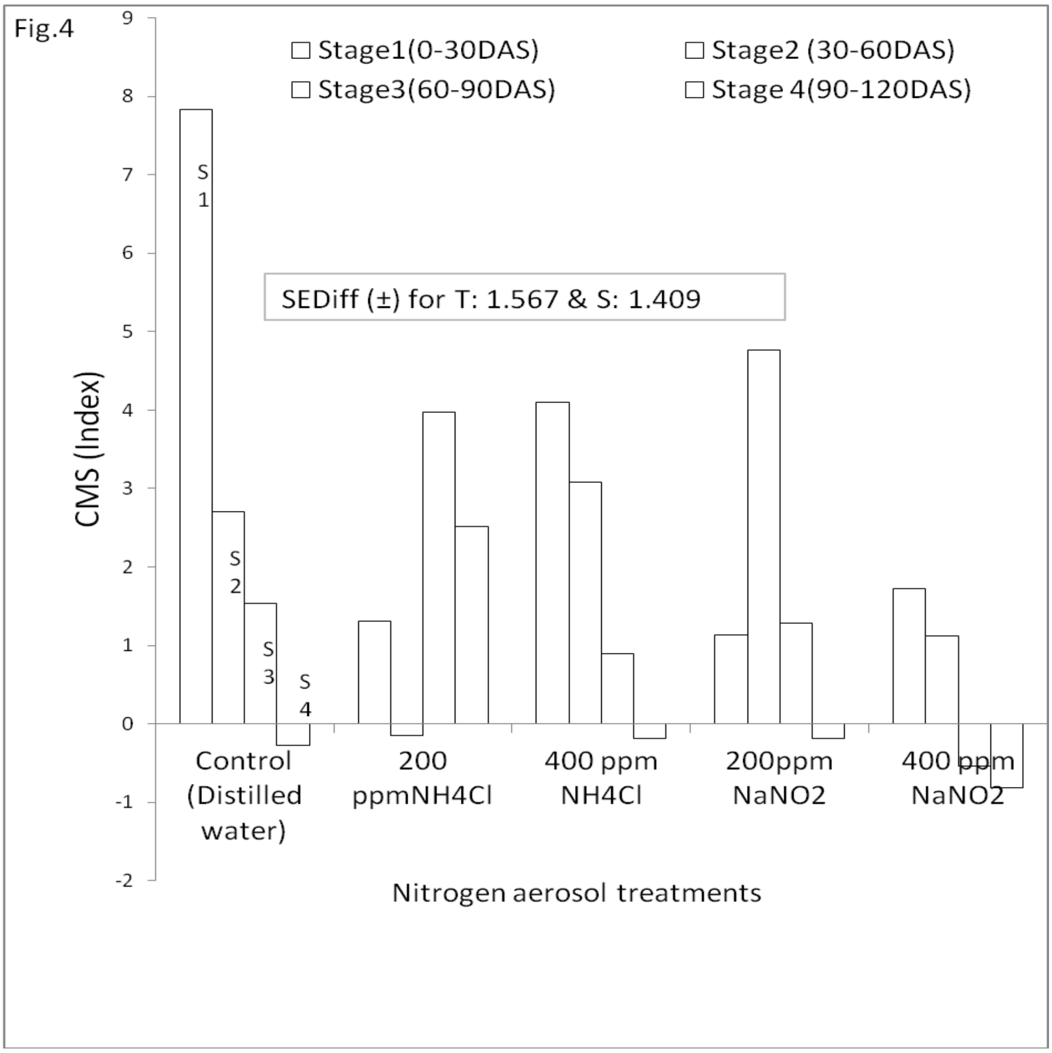

FIG.4 CMS IN RICE

E. **Economic yield:** In the pot culture experiment, economic yields of rice varieties corresponding to the treatments are presented in Table 5. Economic yield varied significantly among the rice varieties due to treatments at the tillering stage of the crop. Ammonium chloride and Sodium nitrite increased crop yield by 22.63% and 16.64% respectively. The Varieties had differences in their economic yield as Jyotiprasad>Kamnaklata>Bishnuprasad>Mulagavaru>>Jaymati. The treatments at the maximum tillering stage had significant effects on grain yield. Here, Ammonium chloride and Sodium nitrite decreased grain yield by 18.27% and 5.47% respectively. The varieties Jaymati>Bishnuprasad>Kanaklata>Jyotiprasad>Mulagavaru also showed variations in grain yield production under the treatments. The varieties (Kanaklata>Bishnuprasad>Jyotiprasad>Jaymati>>Mulagavaru) had significant effects on grain yield in treatment with N-aerosols at panicle initiation stage. Ammonium chloride and Sodium nitrite increased grain yield up to 6.07% and 20.31% respectively. The treatments had significant effects on economic yield of rice varieties (Bishnuprasad>Jyotiprasad>Kanaklata>Jaymati>Mulagavaru) treated with N-aerosols at thei reproductive development stage. Overall, there were increases in grain yield in Ammonium chloride (by 19.26%) and Sodium nitrite (by 25.63%) treated varieties.

In field trial, economic yield did not differ significantly due to the treatments or the time of application of the aerosols. However, a few significant interaction effects of the aerosols and the time of applications were observed (Fig. 5). The highest and the lowest economic yields were 3.06 t/ha and 2.09 t/ha in 400 ppm Ammonium chloride and 200 ppm Sodium nitrite treated plants at the reproductive development stage respectively.

Distribution of cations in the cellular locations: Cations (Ca2+ and K+) present in intercellular and exchangeable sites varied significantly among the rice varieties due to the aerosol treatments (Table 6). Ammonium chloride depleted cations from the water free spaces and exchangeable sites more than the oxidized nitrogen. Also, appreciable quantities of the cations were estimated in the aliquots of distilled water and SrCl2 used to incubate the cells.

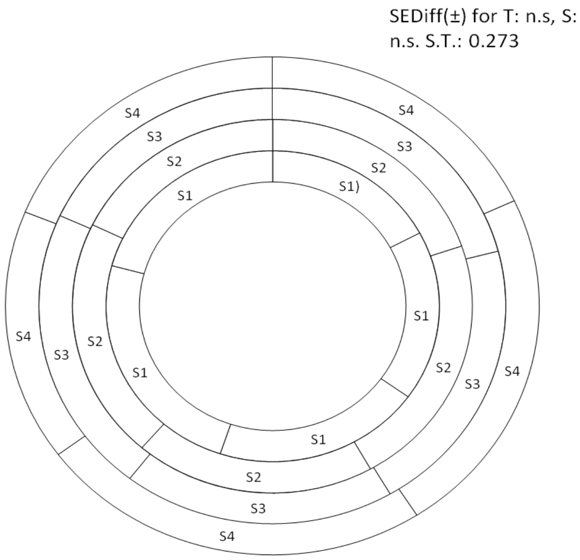

FIG. 5 ECONOMIC YIELD IN RICE

TABLE 6

EFFECTS OF OXIDISED (NANO2) REDUCED (NH4CL) NITROGEN AEROSOLS ON DISTRIBUTION OF CALCIUM AND POTASSIUM IONS IN INTERCELLULAR AND EXCHANGEABLE SITES OF RICE (ORYZA SATIVA L.) CROP TREATED AT DIFFERENT DAYS AFTER SOWING (DAS).

Varieties	Bishnuprasad			Kanaklata			Joymati		
Treatments (T:1,2,3) →	**Distilled H_2O (0ppm)**	**$NH_4Cl1($ 100 ppm)**	**$NaNO_2$ (200 ppm)**	**Distilled H_2O (0ppm)**	**NH_4Cl (100 ppm)**	**$NaNO_2$ (200 ppm)**	**Distilled H_2O (0ppm)**	**NH_4Cl (100**	**$NaNO_2$ (200 ppm)**
0-30 DAS									
Intercellular	46.06	15.26	40.75	19.64	9.80	19.48	43.56	18.78	30.86
Exchangeable	62.68	51.76	81.50	44.47	21.39	43.37	73.27	55.67	65.00
Intercellular K^+	2.01	3.67	2.22	1.54	0.95	2.56	5.12	2.06	1.02
Excahgeable K^+	4.34	4.90	4.78	3.74	1.42	4.07	6.50	5.64	3.34
30-60 DAS									
Intercellular	32.30	30.24	20.06	16.16	17.73	29.70	33.23	11.34	19.45
Exchangeable	44.55	42.34	22.36	31.19	28.74	42.18	49.55	30.72	37.56
Intercellular K^+	2.63	3.23	1.80	1.26	2.13	1.71	1.48	0.81	1.05
Excahgeable K^+	4.23	3.95	3.38	1.66	2.76	3.53	1.82	1.36	1.93
60-90 DAS									
Intercellular	10.28	22.46	38.74	28.70	41.37	45.05	64.85	15.09	32.87
Exchangeable	49.35	42.42	45.70	35.77	71.06	52.95	74.76	30.18	66.59
Intercellular K^+	2.73	2.70	2.56	1.92	6.15	2.02	1.69	4.46	5.01
Excahgeable K^+	5.94	4.02	3.83	5.06	3.31	4.45	4.37	5.41	3.74
90-120 DAS									
Intercellular	21.73	23.90	31.87	25.04	22.79	30.63	31.82	34.02	24.26
Exchangeable	65.18	68.79	62.38	33.82	38.66	41.21	150.21	60.76	543.66
Intercellular K^+	2.16	2.03	4.31	2.20	2.60	2.33	2.61	1.16	1.14
Excahgeable K^+	4.74	1.70	5.65	2.74	3.60	4.00	4.60	1.93	2.33

Cont……

Varieties (V:4, 5)→	**Jyotiprasad ($mMg^{-1}d.w.$)**			**Mulagavaru (mM $g^{-1}d.w.$)**		
Treatments (T:1,2,3) →	**Distilled H_2O (0ppm)**	**NH_4Cl (100**	**$NaNO_2$**	**Distilled H_2O**	**NH_4Cl**	**$NaNO_2$**
0-30 DAS						
Intercellular Ca^{2+}	28.01	5.11	22.63	42.68	37.67	4.7
at P(0.05) T2<T1 & V2<V1						
Exchangeable Ca^{2+}	51.05	5.88	57.28	55.15	66.70	5.74
Intercellular K^+	3.00	0.48	2.96	4.31	4.43	0.34
Excahgeable K^+	4.31	0.40	5.51	5.25	4.81	0.51
30-60 DAS						
Intercellular Ca^{2+}	28.48	36.69	36.11	43.95	43.66	35.12
at P(0.05) V2, V3, V4<V5, & V2,V3<V4 only						
Exchangeable Ca^{2+}	52.21	46.13	47.60	56.00	72.51	48.57
at P(0.05) V1, V2, V3 <V5 & V1, V2<V4 only						
Intercellular K^+	0.78	2.80	3.03	4.25	2.40	2.61
at P (0.05) V3<V2 only						
Excahgeable K^+	2.76	10.31	5.44	4.32	6.86	4.06
at P(0.05) V1,V2,V4 V5< V3& V2,V3, V4<V5 only						
60-90 DAS						
Intercellular Ca^{2+}	28.14	39.52	22.83	37.04	13.47	41.58
Exchangeable Ca^{2+}	22.01	108.89	39.39	63.74	35.15	32.38
Intercellular K^+	4.21	2.84	1.26	1.52	3.05	4.20
at P (0.05) T1<T2 only						
Excahgeable K^+	0.97	3.38	4.26	5.15	3.58	4.97
90-120 DAS						
Intercellular Ca^{2+}	32.86	31.72	22.98	39.86	21.15	19.09
Exchangeable Ca^{2+}	51.73	42.37	44.67	48.73	43.24	49.07
at P(0.05) V1,V2,V4, V5<V3 &V2,V4,V5<V1 only						
Intercellular K^+	3.14	2.39	2.86	4.99	3.12	2.45
at P(0.05) V4<V3 only						
Excahgeable K^+	2.70	8.20	3.16	4.77	4.12	3.99

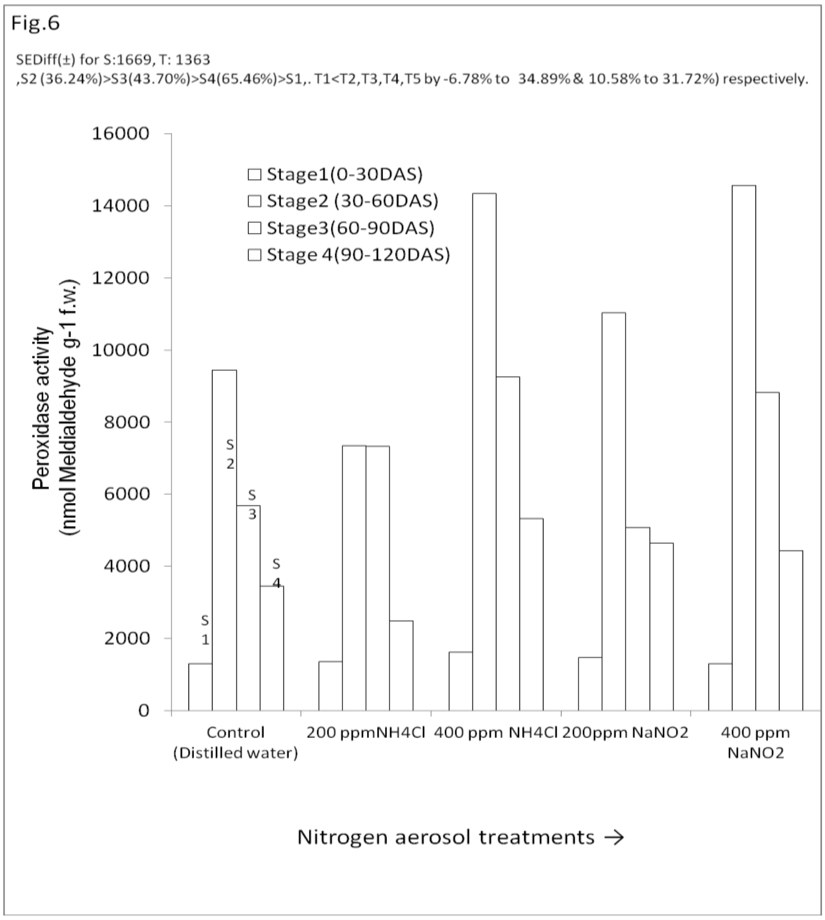

FIG. 6 PEROXIDASE ACTIVITY IN RICE

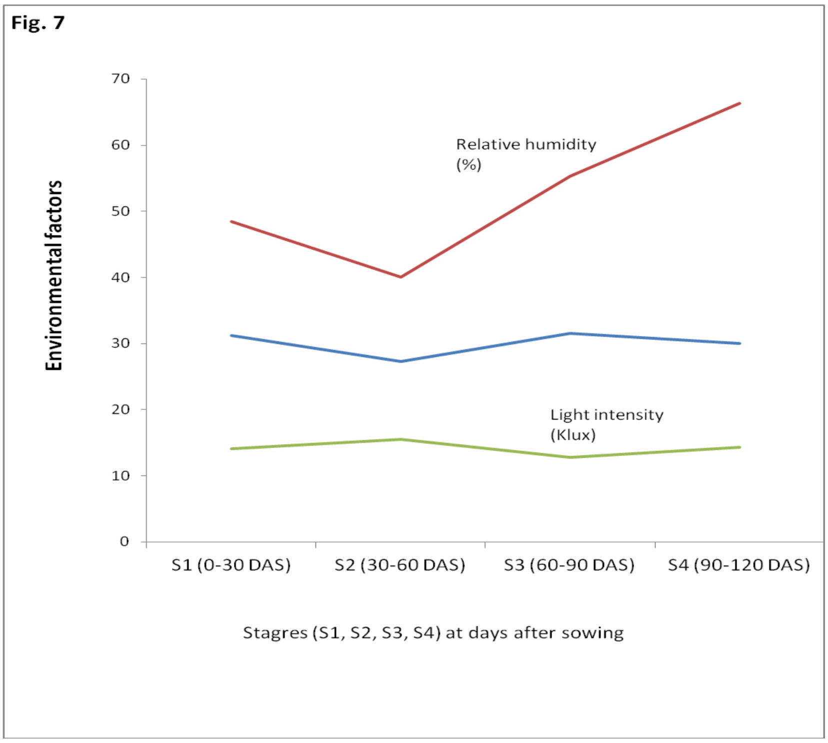

FIG. 7 ENVIRONMENTAL FACTORS FOR RICE

IV. DISCUSSION

The present investigation reveals the impacts of oxidized and reduced nitrogen aerosols at different growth stages of rice (Oryza sativa L.) crop. We also sorted out the most suitable variety and its stage (s) responding positively to the N-aerosols. The mechanism(s) of injury to the rice varieties by the aerosols are also discussed.

In laboratory pot culture, the nitrogenous aerosols were simulated and applied as Sodium nitrite and Ammonium chloride @ 200ppm (20kgN/ha/yr) and 100ppm (10kgN/ha/yr) respectively at different growth stages of rice crop. We preferred the N-aerosols for foliar feeding to plants due to lack of fumigation chambers. The rates of net photosynthesis (Pn) in rice varieties varied significantly due to concentrations of aerosols. The Pn in crop varieties also behaved differently due to aerosols at their growth stages. There are several previous reports on Pn depression in higher plants by nitrogenous pollutants. A concentration of NO2 at 0.6µll-1 may cause Pn inhibition of oat (Avena sativa L.) and alfalfa (Medicago sativa L.) during 90 min fumigation (Hill and Bennett, 1970). Similarly Srivastava et al. (1975) demonstrated that decrease in Pn of bean (Phaseolus vulgaris) is related to NO2 (1-7 µll-1 over a period of 5h). Chloroplastic pH increases when the number of protons in the chloroplast exceeds the proton required (six) for a NO2 reduction. The key enzyme of carbon assimilation (ribulose-1-5-bisphosphate carboxylase/oxygenase) is pH dependent. So such changes in pH are likely to be harmful (Heldt et. al. 1986). Photosynthetic depression has also been related to ultrastructural changes in plants by NO2. The changes include protrusion from the chloroplast (Lopata and Ulrich, 1975), and swelling of thylakoids (Wellburn et al. 1972). Sodium and chloride ions might enter into cells, but they were neglected as non-physiological at relatively alkaline pH (>5.0) of the aerosols in our work.

Nitrate reductase activity in rice was lowered in shoots by Ammonium chloride and Sodium nitrite at almost all stages of the crops. The crop varieties also differed in respect of NR in their leaves. The NR which catalyses the reduction of nitrate to nitrite, is substrate induced and hence, its levels of activity are determined by the supply of nitrate (Beevers and Hegeman 1969). Murray and Wellburn (1985) reported a significant increase in NR activity in tomato cultivar (Ailsa Craig), but not in pepper varieties (Capsicum annum L. cvs. Rowland et al. (1989) exposed barley mutants to a concentration of 0.3µll-1 for 9d in a nutrient culture experiment and found decreases in NR. Squash cotyledons when exposed to high levels of NO2, induction of NR may be abolished. The inhibition is caused by accumulating larger amount of ammonium ions and certain amino acids in squash cotyledons during fumigation (Hisamatsu et al., 1988).

In the present study, nitrogen use efficiencies in rice crops increased mostly with treatments of Ammonium chloride and Sodium nitrite invariably at their growth stages except a few. On an average, varieties also showed remarkable differences in their NUE in grains. However, NUE in grains decreased with the higher concentration of nitrogen aerosols applied to plants in both pot culture and in field experiments. Vergeer et al. (2008) studied the adaptive responses of Arabidopsis lyrata petraea accessions to nitrogen depositions reflecting the rates at the different locations. They found differences between accessions in the response to N for physiological and phonological variables. It was pointed out that plant from low deposition areas had higher NUE than plants from high nitrogen deposition grown at low deposition rates. The NUE decreased in all accessions at higher experimental deposition rates. Also, plants from high deposition areas showed a limited capacity to increase their NUE at lower deposition. Therefore, in the present study, the varieties having high NUE with lower deposition of Nitrogen (e.g. Ammonium chloride) might have faster growth and higher turnover rates.

In the current work, nitrogen nutrition enhanced grain yield of rice varieties irrespective of their growth stages under treatments usually. The enhancement is more prominent in Sodium nitrite than Ammonium chloride fed plants. Higher nitrogen use efficiency with lower quantity of nitrogen from the source might improve nutritive quality of the crop varieties (Prasad and Rao, 1980). In rice, Bishnuprasad followed by Jyotiprasad>Kanaklata>Jaymati>Mulagavaru) emerged as commendable varieties in the current study. In these potential varieties, CMS was found to be higher irrespective of treatments. Cell membrane permeability increased (with lower CMS) by both Ammonium chloride and Sodium nitrite as compared to control. As Ammonium chloride treated plants had higher leakage of ions from the cells, they possessed higher quantum of the cations in the intercellular and exchangeable sites. Similarly, Sodium nitrite treated plants had higher CMS and lower membrane leakage than Ammonium chloride treated ones, a lower amount of the cations were recovered from the cellular locations. In general, the rate of PO activity of rice crop treated with the oxidized aerosol was higher than the rate shown by the reduced aerosol treatment as compared to the control. Therefore, the membrane damages caused by Ammonium and nitrite aerosols were brought by two different mechanisms. The former depleted the cations from the membrane directly and the later caused peroxidation of lipids present in the membrane. Hence, the membrane became leaky for the cations, and their quantum was higher in the intercellular and exchangeable sites irrespective of varieties, which were detected in the extraction processes with water and SrCl2 solutions respectively. Although, nitrite causes swelling of thylakoids (Wellburn et al., 1972), and changes membrane stability, direct interference of free radicals with critical enzymes (Wellburn, 1990), may be responsible for reduction in growth and yield of crops. The oxides of nitrogen following the lipid breakdown in membrane cause cellular plasmolysis (Pryor and Lightsey, 1981). Apart from uncoupling electron transport chain in chloroplast (Lilley et al., 1975), ammonia reduces cations viz., Calcium, magnesium, and potassium (Boxman et al., 1991). In plant cells, calcium is one of the integral components of plasma membrane, where it helps maintain stability (Legge et al., 1982, Bharali and Bates 2006). Calcium ions binds with modulator proteins e.g. calmodulin (Dieter, 1984) and serves as chemical signaling that in some cases equips the plant to resist external stresses (Bharali and Bates, 2004). These possibilities have not been explored meticulously in the present studies.

In the study, data were reproduced from pot and fields experiments in natural environmental conditions. The plants faced with varying in light intensity, temperature, relative humidity during their growth periods, and particularly during incubation period for measurement of net photosynthesis. It was clear from presentation of environmental data that none but the relative humidity varied mostly (Fig.7).. This might have some roles on physiological variations and productivity of the crop stage-wise. Pierson and Elliott (1988) reported that there are differences between species imparting carbon dioxide fixation, and reduction of nitrite only at low light levels and high nitrite concentration. So, the effects of irradiance and desiccation on the pollution responses of the selected crop varieties and role of oxidative damage in these responses are being carried out in field trial presently. All these largely indicate that the changes of the physiological parameters and productivity of the winter rice crop may have quite different facets in context with the impacts of nitrogenous pollutants. However, scrubbing of the nitrogenous pollutants in the form of aerosols for foliar feeding to plants at lower doses mostly at earlier growth stage is important in addition to basal use of recommended fertilizers. It might also compensate leaching losses of nitrogen and boost up the productivity of the cereal crop in the subtropics.

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