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Irrigation Regimes and N Levels Influence Chlorophyll, Leaf Area Index, Proline and Soluble Protein Content of Aerobic Rice (*Oryza sativa* L.)

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Abstract: Field experiment has been conducted at wetlands (*Vertic ustochrept*), Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during *Kharif* (June to October) season of 2005, to ascertain the optimum irrigation method and nitrogen dose to enhance aerobic rice (*Oryza sativa* L.) productivity with PMK 3 cultivar. Four irrigation regimes viz., irrigation at IW/CPE ratio of 0.8, 1.0, 1.2 and micro sprinkler irrigation once in three days, four N levels viz., 100, 125, 150 and 175 kg ha^{-1} were tested in strip-plot design with three replications. Irrigation regimes and N levels increased the Leaf Area Index (LAI) and soluble protein content in a dose response manner. Irrigation treatments did not affect the chlorophyll content. However, N levels increased the chlorophyll content. Irrigation at 1.2 IW/CPE ratio recorded significantly minor proline accumulation, crop growth rate and yield with less moisture stress. N levels (150 and 175 kg N ha^{-1}) produced on par growth and yield. Hence, irrigation at 1.2 IW/CPE ratio with 150 kg N ha^{-1} will be optimum to realise the maximum productivity under aerobic rice cultivation.

Key words: Aerobic rice, IW/CPE ratio, proline, leaf area index, soluble protein content, specific leaf weight

INTRODUCTION

Rice (*Oryza sativa* L.) consumes about 90% of the fresh water resources in Asia used for agriculture (Barker *et al*., 1999; Gorantla *et al*., 2005). The estimated world demand for rice in 2025 will be 140 million tones (Singh, 2004). This projected demand can only be met by maintaining steady increase in production over years, but the per capita availability of fresh water is declining continuously and could reach alarming levels in most Asian countries by the year 2025. To match with ever increasing food grain demand with less water, the term Aerobic rice was coined by IRRI, which means growing high yielding rice in non-puddled and non-flooded aerobic soil with the support of external inputs like supplementary irrigation and fertilizers (Bouman *et al*., 2002). Aerobic rice has its own advantages and disadvantages, as water use seems to be 60% less than that of flooded rice, requires less labour (55%) and can be highly mechanized than low land rice (Wang *et al*., 2002). But yields of aerobic rice said to be 20-30% lower than that flooded rice (Belder *et al*., 2005). However, only few researchers aimed to tap the maximum potential of aerobic rice through suitable management strategies.

Nitrogen nutrition is critical in yield realization of irrigated rice ecosystems. Available N as NH_4^+ ion thought to be more beneficial to plants as a source of nitrogen because NH_4^+ requires less energy to metabolize than does NO_3^-. In flooded rice soil, NH_4^+ ions forms the major source of N whereas in aerated soil NO_3^- ion is more common. Kronzuker *et al*. (1999) and Duan *et al*. (2007) reported

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ratio that of 50/50 NH_4^+-N/NO_3^--N increased the average biomass of rice shoots and roots by 20% when compared with that of 100/0 NH_4^+-N/NO_3^--N. Thus growing aerobic rice with alternating wetting and drying will supply 50/50 NH_4^+-N/NO_3^--N and seems to be more beneficial than continuous flooding (Cessay and Uphoff, 2002).

In rice, proline accumulation seems to be a symptom of injury induced by water stress (Pandey *et al*., 2004; Gowri, 2005). Hence, observing free proline content is a reliable measure of moisture stress and can be useful in appraising optimum irrigation regime. With these perspectives, here we present the results how irrigation regimes and nitrogen levels influence chlorophyll, leaf area index, proline and soluble protein content of aerobic rice.

MATERIALS AND METHODS

Experimental Site and Initial Soil Characteristics

Field experiment has been conducted at wetlands (*Vertic ustochrept*), Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during *Kharif* (June to October) season of 2005, to ascertain the possible irrigation and nitrogen management practices to enhance aerobic rice productivity. Soil of the experimental site was clay textured (68.7% clay, 18.1% silt, 7.3% coarse sand, 5.9% fine sand) belonging to Noyyal series, classified as *Vertic ustochrept*. The soil analysed 246, 22.2 and 523.6 kg ha^{-1} of $KMnO_4$-N, Olsen-P and NH_4OAc-K, respectively with organic carbon content of 0.53%, EC of 0.65 dS m^{-1} and pH of 6.7. The irrigation water used in this study was neutral in reaction (pH = 7.76) with medium level of soluble salts (EC = 1.21 dS m^{-1}).

Selection of Cultivar, Experimental Design, Sowing and Fertilizer Schedule

Based on promising performance under previous irrigation and fertilizer response screening experiment (data not sown) cultivar PMK 3 (UPLRI X CO 43) was selected for this particular study. This variety is characterised by semi-dwarf, non-lodging, drought tolerant, early maturing (110-115 days) and fertiliser responsive.

Experiment was conducted in strip-plot design with 3 replications. Main plot treatments include four irrigation regimes viz., irrigation at IW/CPE ratio of 0.8, 1.0, 1.2 and micro sprinkler irrigation once in three days with the discharge rate of 120 L h^{-1}. These main plots were superimposed with 4 levels of N viz., 100, 125, 150 and 175 kg ha^{-1} as subplots (6×3 m^2).

Rice cultivar PMK 3 was direct-dry seeded in non-puddled soil with the spacing of 20×10 cm by adopting the seed rate of 60 kg ha^{-1}. Two weeks later the plots were thinned to maintain uniform population. The entire dose (50 kg ha^{-1}) of P_2O_5 as single super phosphate, zinc sulphate and $FeSO_4$ at the rate of 25 kg ha^{-1} each and gypsum at the rate of 500 kg ha^{-1} were applied basally to all the plots. Later foliar spray of 2% $FeSO_4$ was given at tillering and Panicle Initiation (PI) stages as the crop showed iron deficiency symptom. K_2O (50 kg ha^{-1}) as muriate of potash were applied in four equal splits at 20 DAS, tillering, panicle initiation and heading stages.

Irrigation Scheduling and N Fertiliser Application

As neutron probing, gravimetric moisture determination methods found to be laborious, time consuming and expensive, the relatively simple, inexpensive, practicable climatological approach (Prihar *et al*., 1976) has been followed to schedule the irrigation. Thirty millimeter depth of Irrigation Water (IW) was given when the Cumulative Pan Evaporation (CPE) reached the level of 37.5, 30 and 25 mm in order to get IW/CPE ratio of 0.8, 1.0 and 1.2, respectively. By multiplying the depth of irrigation (30 mm) and area of the plot (6×3 m^2), the volume of water required for each plot was arrived. Calculated volume of water was irrigated to each plot by measuring through Parshall flume set up at the experimental field. Micro sprinkler discharge time also adjusted based on the volume of water required and irrigation was given once in three days. Total water consumed by each irrigation treatment was shown in Table 4.

Nitrogenous fertiliser treatments were given as urea in four equal splits at 20 DAS, tillering, panicle initiation and heading stages.

Sampling, Biometrics and Yield Observations

Sampling and biometric observations were done at important physiological crop growth stages viz., Panicle Initiation (PI), flowering and maturity stages. Root volume was measured by placing the oven dried roots into a measuring cylinder containing known volume of water. By measuring the increase in the water column, root volume was assessed and expressed in CC per plant. Productive tillers per m^2 were recorded during the maturity stage. During the harvest, fresh grain and straw yield were recorded then they were expressed on oven dry (70°C for 4 days) basis.

Physiological Observations

Leaf area index was calculated by employing the formula of Williams (1946).

$$\text{LAI} = \frac{\text{Leaf area per plant}}{\text{Ground area occupied per plant}}$$

The Crop Growth Rate (CGR) was calculated by using the formula of Watson (1947) and expressed in g m^{-2} day^{-1}.

$$\text{CGR} = \frac{W_2 - W_1}{P\,(t_2 - t_1)}$$

Where:

W_1 and W_2 = Whole plant dry weight at time t_1 and t_2 respectively

t_2 and t_1 = Time in days; P-Ground area occupied by plant (m^2)

Chlorophyll content in leaves was estimated by using the method described by Hiscox and Israelstam (1979) and expressed in mg g^{-1} fresh weight. One hundred milligram of fully expanded young leaf tissue was placed in a vial containing 7 mL of Dimethyl Sulphoxide (DMSO) and chlorophyll was extracted without grinding at 65°C by incubating overnight. The extract was transferred to a graduated tube and made upto 10 mL with DMSO and assayed immediately. The chlorophyll content was calculated using the formula described below.

$$\text{Total chlorophyll} = \frac{\text{OD at 652V}}{34.5 \times W} \times \text{Vmg g}^{-1}$$

Where:

W = Weight of the leaf sample (g)

V = Volume of supernatant made-up

OD = Optical density

The free proline concentration was determined at Panicle Initiation (PI), flowering and maturity stage by adopting rapid determination method described by Bates *et al.* (1973) and expressed in µmoles g^{-1} fresh weight.

Soluble protein content of leaves was estimated by using the method of Lowry *et al*. (1951) and expressed as mg g^{-1} fresh weight.

The Water Productivity (WP) was worked out from the yield of paddy and the amount of water used and expressed in kg ha mm^{-1}

$$WP = \frac{\text{Grain yield (kg ha}^{-1})}{\text{Total water requirement (ha mm)}}$$

The sterility coefficient was calculated by working out ratio of unfilled grains to the total number of grains in the panicle and expressed as percentage.

Statistical Analysis

Recorded data were analysed statistically as per the method suggested by Gomez and Gomez (1984). Wherever the treatmental differences were significant, critical differences were calculated at five per cent probability level and used for interpretations.

RESULTS AND DISCUSSION

Effects of Irrigation on Growth Parameters

Plant height, total dry matter production, leaf area index, root volume and crop growth rate increased significantly under irrigation at IW/CPE ratio 1.2 followed by 1.0 and 0.8 IW/CPE ratios and micro sprinkler irrigation (Table 1). The growth parameters decreased with severity of water stress (Adriano *et al*., 2005; Gowri, 2005). Water deficit manifests many anatomical changes in the plant which includes decrease in cell size, cell division, cell elongation, inter cellular space and thickening of cell wall thereby limits overall plant growth. Similar observations have been reported by Prasad *et al*. (1987) and Banga *et al*. (1994) in maize. The observed increase in dry matter production under 1.2 IW/CPE ratio due to possible reduction in transpiration rate and normal gas exchange resulting in increased production of photosynthates and translocation to sink which in turn increased Dry Matter Production (DMP) (Kalaiselvi, 1997). The reduced dry matter production under irrigation at IW/CPE of 0.8 might be due to water stress induced impaired tillering or due to accelerated leaf senescence (Simane *et al*., 1993). The higher Leaf Area Index (LAI) under 1.2 IW/CPE ratio could be due to the availability of adequate water throughout growth period (Muchow and Carbery, 1989).

Table 1: Effect of irrigation and nitrogen treatments on plant height, total dry matter production, LAI, root volume and CGR at maturity stage

Treatments	Plant height (cm)	Total dry matter production (t ha^{-1})	Leaf area index (LAI)	Root volume	Crop growth rate (50-80 DAS) (g m^2 day^{-1})
Irrigation levels					
IW/CPE ratio 0.8	88.48^{c}	11.90^{c}	3.87^{c}	24.79^{c}	1.33^{bc}
IW/CPE ratio 1.0	89.93^{b}	12.39^{b}	4.00^{b}	26.17^{b}	1.41^{b}
IW/CPE ratio 1.2	92.74^{a}	13.31^{a}	4.23^{a}	29.33^{a}	1.57^{a}
Micro sprinkler	83.61^{d}	9.86^{d}	3.80^{d}	22.09^{d}	1.29^{bc}
CD (p = 0.05)	0.39	0.10	0.02	0.88	0.09
N levels					
N_{100}	85.73^{d}	10.96^{d}	3.81^{d}	23.43^{c}	1.30^{b}
N_{125}	87.95^{c}	11.67^{c}	3.93^{c}	24.88^{b}	1.35^{b}
N_{150}	90.10^{b}	12.26^{b}	4.07^{b}	26.68^{a}	1.47^{a}
N_{175}	91.00^{a}	15.38^{a}	4.19^{a}	27.39^{a}	1.49^{a}
CD (p = 0.05)	0.34	0.20	0.01	0.73	0.05

Irrigation and nitrogen main treatments are significant at $p<0.001$ and interactions are non-significant. Within each column the treatments sharing common alphabet are not statistically significant at 5% level

Table 2: Effect of irrigation and nitrogen treatments on proline content, total chlorophyll and soluble protein

Treatments	Proline content (μmoles g^{-1} fw)	Total chlorophyll content (mg g^{-1} fw)	Soluble protein content (mg g^{-1} fw)
Irrigation levels			
IW/CPE ratio 0.8	4.99^{ab}	1.82^{a}	6.86^{c}
IW/CPE ratio 1.0	4.71^{b}	1.98^{a}	7.33^{b}
IW/CPE ratio 1.2	3.52^{c}	2.08^{a}	7.90^{a}
Micro sprinkler	5.26^{a}	1.77^{a}	6.67^{c}
CD (p = 0.05)	0.49	0.86^{NS}	0.29
N levels			
N100	3.40^{d}	1.58^{c}	6.05^{d}
N125	4.05^{c}	1.81^{bc}	6.92^{c}
N150	5.08^{b}	2.04^{ab}	7.59^{b}
N175	5.94^{a}	2.22^{a}	8.20^{a}
CD (p = 0.05)	0.54	0.27	0.32

Irrigation and nitrogen main treatments are significant at $p<0.001$ and interactions are non-significant. Within each column the treatments sharing common alphabet are not statistically significant at 5% level

Physiological and Biochemical Parameters

The observed increase in root volume under 1.2 IW/CPE ratio was due to root thickening and dense proliferation of root at top layer in response to lower water stress (Vijayalakshmi and Nagarajan, 1994). The higher Crop Growth Rate (CGR) under 1.2 IW/CPE ratio could be due to beneficial effect of water in maintaining normal cell integrity, cell elongation and functioning of biopolymers apart from enhancing nutrient uptake (Singh, 2004). Under 1.0 and 0.8 IW/CPE ratios, reduction in CGR might be due to the inhibition of physiological activities by water stress condition (Zhang *et al*., 1997; Gowri, 2005).

Physiological and biochemical parameters like chlorophyll content and soluble protein contents (Table 2) increased significantly under irrigation at IW/CPE ratio 1.2 followed by 1.0 and 0.8 IW/CPE ratios and micro sprinkler irrigation. However, increased proline content observed with increasing moisture stress (Table 2) might be due to the transcriptional activation of the NADPH dependent PSC 5 (Babiychuk *et al*., 1996) and the concomitant increase in protease activity, which induce the breakdown of proteins under water stress conditions (Agarwal *et al*., 1995; Jain *et al*., 1996). The reduction in chlorophyll content under micro sprinkler irrigation could be due to enhanced chlorophyll enzyme activity under water stress, which is deleterious to plant productivity (Jayabalan *et al*., 1995; Sheela and Alexander, 1996). The observed decrease in soluble protein under micro sprinkler irrigation might be due to slow down in the regulation of photo system II activity under water stress as results in an imbalance between the generation and utilization of electrons, apparently resulting changes in quantum yield (Peltzer *et al*., 2002).

Yield Parameters

The yield components viz., number of productive tillers meter^{-2}, number of grains panicle^{-1}, number of filled grains panicle^{-1}, sterility coefficient and 1000 grain weight were higher when irrigation was scheduled at 1.2 IW/CPE ratio followed by 1.0 and 0.8 IW/CPE ratios and micro sprinkler irrigation (Table 3). The enhanced values in yield components could be due to increase in leaf area; leading to higher photosynthates and accumulation of more assimilates which led to increased sink size. The reduction of yield components might be due to the water stress, which restricted the plant to put forth reproductive organs (Gowri, 2005). The limitation of adequate moisture 1.0 and 0.8 IW/CPE ratios and micro sprinkler irrigation might have reduced the translocation of assimilates to the sink (Bakelana *et al*., 1986; Ajula *et al*., 1987).

Yield and Water Productivity

Irrigation at 1.2 IW/CPE ratio produced taller plants. Dry matter production, leaf area index, root volume and crop growth rate, in turn yield found to increase (Table 4) as followed by 1.0 and 0.8 IW/CPE ratios and micro sprinkler irrigation. This is clearly because of moisture stress as explained

Table 3: Yield and yield components at different levels of irrigation and nitrogen in aerobic rice

Treatments	Productive tillers (No. m^{-2})	Grains $panicle^{-1}$ (No. $panicle^{-1}$)	Filled grains $panicle^{-1}$ (No. $panicle^{-1}$)	Sterility coefficient (%)	1000 grain weight (g)
Irrigation levels					
IW/CPE ratio 0.8	295.10[c]	127.80[b]	91.20[c]	28.70[b]	24.3
IW/CPE ratio 1.0	329.20[b]	142.60[a]	107.40[b]	24.80[c]	24.4
IW/CPE ratio 1.2	361.90[a]	146.00[a]	114.20[a]	21.80[d]	24.4
Micro sprinkler	284.30[d]	55.10[c]	37.60[d]	31.80[a]	24.3
CD (p = 0.05)	2.77	7.44	4.95	0.12	NS
N levels					
N100	301.50[d]	110.40[c]	80.40[c]	28.30[a]	24.2
N125	308.40[c]	115.60[bc]	85.30[b]	27.20[b]	24.3
N150	326.60[b]	120.50[ab]	90.00[ab]	26.30[c]	24.5
N175	333.90[a]	125.10[a]	94.70[a]	25.30[d]	24.5
CD (p = 0.05)	4.88	6.74	4.85	0.12	NS

Irrigation and nitrogen main treatments are significant at $p<0.001$ and interactions are non-significant. Within each column the treatments sharing common alphabet are not statistically significant at 5% level

Table 4: Summary of irrigation treatments and their effects on yield and water productivity

Irrigation treatments	No. of irrigations	Total water used (mm)	Yield (kg ha^{-1}) Grain	Straw	Water productivity (kg rice ha mm^{-1})
IW/CPE ratio 0.8	15	498	4289[c]	6823[b]	8.61
IW/CPE ratio 1.0	18	556	4776[b]	7624[a]	8.58
IW/CPE ratio 1.2	21	618	4916[a]	7804[a]	7.95
Micro sprinkler	25	659	1888[d]	2948[c]	2.86

Within each column the treatments sharing common alphabet are not statistically significant at 5% level

by low chlorophyll, soluble protein and relative water contents (Table 2). Yield at 0.8 IW/CPE ratio was 13% lower when compared to 1.2 IW/CPE ratio and the same treatment recorded highest water productivity (Table 4). So this results suggests that water productivity could not be an accurate measure for experiments aiming for maximum growth and yield. It is supported by Tuong *et al.* (2004) and Belder *et al.* (2005) as they noticed decreasing water productivity with increasing levels of irrigation.

Effect of Nitrogen Levels on Growth, Yield and Water Productivity

Application of 175 kg N ha^{-1} recorded the highest values in growth parameters namely, plant height, dry matter production, root volume, leaf area index and crop growth rate. Since, application of higher nitrogen levels resulted in taller plants and larger leaf area, which increased the photosythate production, in turn enhanced DMP (Valarmathi, 1994). The increased levels of nitrogen increases cell volume, meristematic activities, formation and functioning of protoplasm, which consequently increased the CGR (Ashok Kumar *et al*., 1994). However, in the present investigation the root volume, LAI and CGR recorded a significant increase with N levels upto 150 kg ha^{-1} (Table 1) and beyond this level, the magnitude of increase was only marginal following the Mitchelish's law of diminishing returns. Similar results were reported by Rajeswari (1990) in rice and Zhao *et al*. (2005) in sorghum. Total Dry Matter Production (TDMP), grain and straw yields exhibited nice quadrate response with high R2 values (>0.973) for N levels (Fig. 1). But all the above were in par with 150 kg N ha^{-1}. Hence, 150 kg N ha^{-1} might be economic optimum to realize maximum yield under aerobic rice cultivation.

Physiological and biochemical parameters viz., proline, chlorophyll and soluble protein contents increased significantly under application of 175 kg N ha^{-1} followed by 150, 125 and 100 kg N ha^{-1}. The observed increase in chlorophyll and soluble protein contents under application 175 kg N ha^{-1} is due to the enhanced chlorophyll synthesis on supply of nitrogen, which are a major component of chlorophyll (Stevens *et al*., 2001; Zhao *et al*., 2005). In the present study, with increasing nitrogen supply, proline content also increased. It might be due to N is a constituent of proline, an amino acid

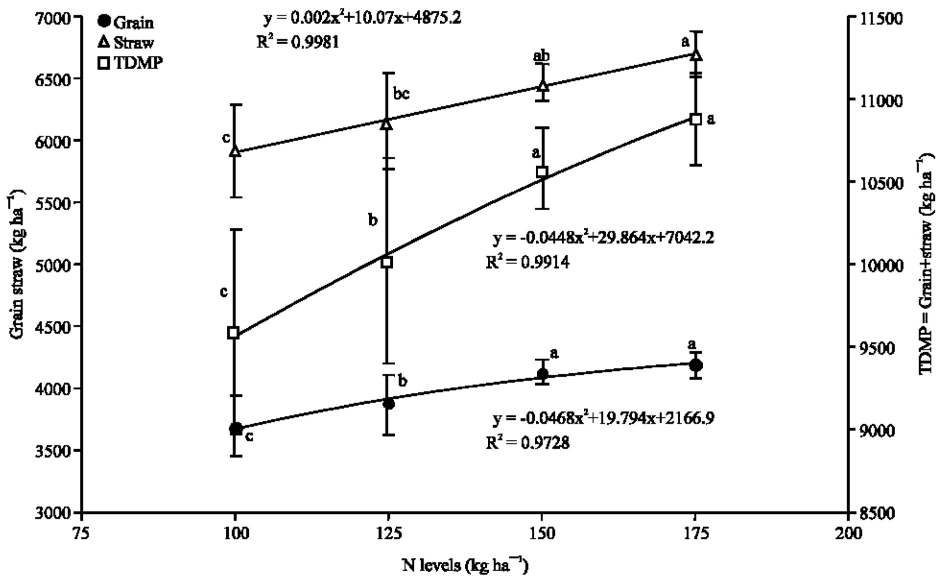

Fig. 1: Response of TDMP, grain and straw yield to N levels (the N levels sharing common alphabet are not statistically significant at 5% level)

and also due to the action on onithine-∂-aminotransferase under adequate N levels, suggesting the predominance of the ornithine pathway, which inturn to the inhibition of dehydrogenase activity (Sanchez *et al*., 2002).

Increase in nitrogen levels resulted in higher WUE in which is attributed to the increased yield under higher nitrogen levels with a constant rate of applied water. This is in agreement with that of Selvaraju (1990) and Nirmal Rajkumar (1998). Higher grain yield resulted from higher N supply together with less water consumption would have resulted in higher water productivity in the lower irrigation treatments (I_1 and I_2). Similar results ascertained by Belder *et al.* (2005). In the micro sprinkler treatment, water productivity was the least, resulted in lower grain yield in spite of higher water application rates.

CONCLUSIONS

Irrigation at 1.2 IW/CPE ratio significantly produced higher growth, chlorophyll and soluble protein contents, proline accumulation and sterility coefficient. N levels followed the quadratic response with 150 and 175 kg N ha^{-1} produced on par growth and yield. Thus, scheduling of irrigation at IW/CPE along with 150 kg N ha^{-1} is to realize maximum yield and water productivity with PMK 3 cultivar under aerobic condition. However, irrigation at IW/CPE ratio of 1.0 with 556 mm of irrigation water and application of 150 kg N ha^{-1} may also be adopted for aerobic rice cultivation at Coimbatore.

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