

PHENOLOGICAL RESPONSE OF RICE PLANTS TO DIFFERENT MICRONUTRIENTS APPLICATION UNDER WATER SAVING PADDY FIELDS ON CALCAREOUS SOIL

¹Naeem Sarwar*, ¹Hakoomat Ali, ²Muhammad Maqsood, ²Ehsan Ullah , ²Muhammad Shahzad, ³Khuram Mubeen, ¹Ahmad Naeem Shahzad, ⁴Muhammad Asghar Shahid and ¹Shakeel Ahmad

> ¹Department of Agronomy, Bahauddin Zakariya University Multan, PAKISTAN ²Department of Agronomy, University of Agriculture Faisalabad, PAKISTAN ³Department of Agronomy, University of Poonch Rawalakot, PAKISTAN ⁴Department of Pest Warning and Quality of Pesticides Punjab, PAKISTAN *Corresponding author E-mail: bajwa834@gmail.com

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ABSTRACT

Due to a reduction of world water resources, rice cultivation has shifted from flooded to aerobic condition. This shift may alter soil physico-chemical conditions and cause reduced availability and uptake of nutrients, especially boron and zinc in calcareous soils, and may ultimately reduce yield. Thus a field experiment in a calcareous soil was conducted in which basal application of boron & zinc alone, or in combination, was undertaken in rice grown aerobically (T_1) , flooded for the entire growth period (T_2) or flooded for two weeks after transplanting, given supplemental irrigation up to panicle initiation and then flooded from panicle initiation to physiological maturity (T_3) . The study showed that rice crop was significantly reduced growth in term of Leaf Area Index (LAI), Leaf Area Duration (LAD), Crop Growth Rate (CGR), Total Dry Matter accumulation (TDM) and Net Assimilation Rate (NAR) when it was grown in aerobic condition. However, the crop was not affected significantly different from normal flooded rice (T_2) when it was grown in modified rice culture (T_3) . Application of zinc alone or in combination with boron enhanced chlorophyll contents in T_2 similar to T_3 while the crop grown in aerobic condition (T_1) had significantly lower chlorophyll content.

Key words: Rice, growth, boron, zinc and rice cultures

INTRODUCTION

Water shortage raised another question for rice growers all over the world and most of the farmers has shifted cultivation from conventional flooded method to new system of aerobic rice. Lot of water saving technologies are under study like aerobic rice, alternative wetting drying and system of rice intensification (Bouman et al., 2005; Stoop et al, 2002; Tabbal et al., 2002; Borell et al., 1997) to save the water input. In Pakistan, basmati rice is grown which have good national and international demand because of its specific fragrance. This system of aerobic rice and other water saving techniques are also under consideration in Pakistan without having specific aerobic cultivars. This cultivation shifting for water saving may broader the deficiencies of micronutrients such as boron and zinc in rice growing areas due to which high yield could not be realized. In Pakistan, soil is calcareous in nature with low organic matter and due to intensive cultivation deficiency of nutrients is increasing day by day especially zinc and boron (Rafique et al., 2006, 2008). Plant grown in aerobic field takes less zinc than plants

grown in flooded fields (Gao et al., 2006). In 1969, zinc deficiency was first time detected when scientists explored its role for "hadda" disease of rice (Yoshida and Tanaka, 1969). Zinc deficiency is found to be major constraint for annual crops as well as in rice area in developing as well as in developed countries like India, China, USA and Australia (Cakmak, 2002; Karak et al., 2006; Gao et al., 2006). Almost 30 percent soil is zinc deficient which cover 49 developed and developing countries of the world (Alloway, 2004). Crop production and quality is desperately affecting with emerging deficient micronutrients (B & Zn) all over the world (Rashid and Ryan, 2004; Rafique et al., 2008). Numerous studies have indicated that zinc deficiency is a serious nutritional problem for upland crops. Zinc is essential for several biochemical processes in the rice plant, such as cytochrome and nucleotide synthesis, auxin metabolism, chlorophyll content, enzyme activation and membrane integrity (IRRI, 2000). Zinc is not only imperative for rice crop but it also plays a significant role in other crops. Application of zinc resulted in improved yield as well

grain zinc contents not only in rice but also in another crops like wheat, maize, sorghum and alfalfa (Sahrawat et al., 2008; Khan et al., 2009; Ceylan et al., 2009). Boron is emerging deficient nutrient which is affecting crop impressively in rice tract. Rashid et al. (2004) perceived the effect of boron on rice cultivars Super Basmati, Basmati-385 and KS-282 and reported 14-25% increase in paddy yield as compared with control. They further explained that rice crop was affected positively with optimum boron dose at 0.75 kg/ha (Rashid et al., 2004). Studies on boron fertilization showed that paddy yield consistently increased with boron application (Rakshit et al., 2002; PARC, 2002; Tandon, 1999; Ali et al., 1996). Stimulating effects of boron may be linked with greater availability of sugars, increased enzymatic activity and respiration which favored the pollen viability and leads to improved yield or number of rice grains (Garge et al., 1979). In the case of severe boron deficiency, the root cap, quiescent centre and protoderm of root tips disappear and root growth ceases, leading to the death of root tips. Inhibited leaf expansion by low boron also indirectly decreases the photosynthetic ability of plants (Bernie and Longbin, 1997). Paddy yield was significantly higher with the application of micronutrients (Zn, B and Mo) alone or in combination with each other (Hossain et al., 2001). Keeping in view the shift of rice cultivation on micronutrients deficient soil, a field experiment was designed to see the uptake of different micronutrients (B & Zn) under various rice cultures and its resultantly effect on rice plant growth.

MATERIALS AND METHODS

The proposed field study was conducted at Agronomic Research Farm, Department of Agronomy, University of Agriculture, Faisalabad (310-25'N, 730-09'E) during 2008 and 2009. Experiment was laid out in randomized complete block design with split plot arrangement using three replications, having a net plot size of 2.20 m \times 4 m. Rice cultures were kept in main plots while micronutrients were in subplots of experimental layout. The soil texture was sandy clay loam (50.16% sand, 21.75% Silt, 25.40% Clay) with an average pH of 7.9, total soluble salts 0.21%, organic matter 0.74, 0.65ppm Zn and 0.38ppm B. Nursery was raised in 3rd week of June with recommended seed rate (2 kg/marla) and aerobic rice sowing was done at the same date using seed rate 75 kg per hectare with 20 cm apart rows by using hand drill. Basal application of boron (F1) in the form of borax (7.5 kg ha-1 and zinc (F2) in the form of zinc sulphate (12.5 kg ha-1 alone and in combination (F3) along with control treatment (F0) was done in aerobic rice (T1), flooded rice (T2) and "Flooding for two weeks after transplanting and will be maintained at field capacity up to panicle initiation and again will be kept flooded starting from panicle initiation up to physiological maturity" (T3). Manual transplanting was done with 20 cm apart rows. _ Nitrogen was applied at the rate of 140 kg N ha-1, 80 kg P ha-1 and 60 kg K ha-1 and 10 kg Zn ha-1 were applied in the form of urea, diammonium phosphate, sulfate of potash and zinc sulfate (ZnSO4 35%) respectively. Whole of P and K and 1/3 of N was incorporated into the soil at the time of seed bed preparation while remaining N was top dressed in 2 equal splits at the time of booting and panicle initiations. Water depth was maintained at 3-4 cm during transplanting. Measured amount of water was applied as per treatments for different rice cultures by using cut throat flume (90 cm x 20cm). Following formulae was used for calculating the time of application for required amount of water.

$$T = \frac{AD}{Q}$$

Where T represents application time (hours), A for field area (m-2), D shows the depth of irrigation (m) and Q signed for flow rate (m3/sec). Before one week of harvesting, irrigation was stopped when the sign of physiological maturity was appeared. For weed control Butachlor 60 % EC @ 800 mL ha-1 was applied after 7-d of transplanting in standing water (Reddy, 2004). Preemergence herbicide (Penoxolan @75ml/ha) was used in aerobic rice for controlling weeds. Carbofuran 10% GR was broadcasted @ 25 kg ha-1 at 55-d after transplanting to protect the crop from insects attack. Harvesting was done manually at harvest maturity and threshing was done separately for each plot.

Plant samples were collected fortnightly for observing growth behavior of the crop. Four plants were cut out from each treatment and then weighed for its fresh weight. In aerobic rice, row of 30cm was cut out from the base of the plants and fresh weight was calculated. Leaves were separated from each sample and then weighed separately for recording leaf and stem weight. Leaf sample (5g) from each treatment was used for recording leaf area. Growth parameters LAI, LAD and CGR were calculated by following procedure of Hunt, 1978.

$$LAI = \frac{\text{Leaf Area}}{\text{Land area}}$$
$$LAD_{1} = \frac{(\text{LAI}_{1} + \text{LAI}_{1}) \times (\text{T}_{2} - \text{T}_{1})}{2}$$
$$CGR = \frac{(W_{2} - W_{1})}{(\text{T}_{2} - \text{T}_{1})}$$
$$NAR = \frac{\text{TDM}}{\text{LAD}}$$

Sample (10g) was air dried and then placed into oven at 70 C0 for recording dry weight. Data of leaf area index (LAI) was used for further calculation of leaf area duration (LAD). Similarly the dry weight from different harvest was used for recording total dry matter production (TDM) which was further used for recording crop growth rate (CGR). Final yield was calculated at harvest following the standard procedure. Chlorophyll content was measured at different stages of the rice crop (seedling, tillering, panicle initiation and maturity) by using the procedure of Yoshida et al. (1976). Leaf samples were collected at respective stage and were weighed separately. Leaf sample of 0.05 g was measured on digital scale balance and was grinded in mortar by using 80% acetone. After dissolving all green portion of leaf final volume of 5ml was made with acetone (80%). The absorbance of chlorophyll solution was recorded at 645 and 663 wavelengths with a spectrophotometer. Chlorophyll content (expressed as mg/g fw of a sample) was estimated as follows:

Total chlorophyll

 $= (20.2 \times D645 + 8.02 \times D663) \times \frac{5}{1000 \times 0.05} mg/gfw$ Chlorophyll a = (12.7 × D663 - 2.69 × D645) $\times \frac{5}{1000 \times 0.05} mg/gfw$ Chlorophyll b = (22.9 × D645 - 4.68 × D663) $\times \frac{5}{1000 \times 0.05} mg/gfw$

The meteorological data for the growing period of the crop were provided from the Department of Crop Physiology, Agronomic Research Institute Faisalabad, Pakistan. Meteorological data showed (Table 3) that the year 2008 was cooler and rainy while the year 2009 was hot and dry. All the collected data was analyzed by using the Fisher's analysis of variance function of MSTAT statistical computer package and LSD at 5% probability was used to compare the treatment's means (Steel et al., 1997).

RESULTS

Growth Performance

Leaf area Index (LAI) progressively increased in all treatments during the growth period and was highest at 3rd harvest in both years (Figure 2, 3).

Combined effect of rice culture and nutrient application was significant on leaf area index in both the years (2008-09). Highest leaf area index (LAI) was calculated in T_2 & T_3 (8.06 & 7.86) rice cultures with the combined application of boron and zinc, while the minimum value was found in T_1F_0 where these nutrients were missed. Flooded rice (T_2) with combined application of boron and zinc produced maximum LAD (205.4) which was similar with T_3 (196.83) while the minimum value was recorded in T_1F_0 (73.92) which was similar with T_1F_1 (83.72) during 2008. Almost similar trend was observed for leaf area duration in second year of experimentation. Maximum average crop growth rate in 2008 occurred in T_2 (29.0) and T_3 (27.4) rice cultures with the combined application of boron and zinc while minimum value was observed in T_1F_0 (6.5). Maximum TDM was recorded in treatment combination of T_2F_3 (1642.57) & T_3F_3 (1564.90) while the minimum value (398.67) was found in T₁F₀ treatment combination. Similar trend was observed in second year of experimentation. Net assimilation rate is the physiological potential for converting the total dry matter into yield. In 2008, maximum value for NAR was noted in T_2F_3 , T_3F_3 and T_2F_2 treatment combinations while the minimum value was found in T_1F_0 combination. Similar trend was observed in second year (2009) (Table 1). All treatments progressively increased chlorophyll contents up to tillering stage in both of the years (2008-09). The maximum chlorophyll contents were recorded in T_2 (1.55) and T_3 (1.55) respectively, while crop grown in T₁ rice culture had lower chlorophyll contents (1.46). As regarding nutrients application, comparison of mean values of two years shows that the maximum chlorophyll contents were produced with $F_3 \& F_2$ (1.63 & 1.62) respectively, while the minimum contents were recorded in F_0 (1.39) which was similar with F_1 (1.43) (Table 2).

Table 1. Growth parameters as affected by boron and zinc in different rice cultures during 2008 and 2009

Treat	LAI (m^{-2})		LAD (days)		$\mathbf{CGR} \ (\mathbf{g} \ \mathbf{m}^{-2} \mathbf{d}^{-1})$		$TDM (g m^{-2})$		$NAR (g m^{-2} d^{-1})$	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
T_1F_0	3.7e	3.3f	73.9f	64.1e	6.5f	6.08f	398g	338f	5.3e	5.2e
T_1F_1	4.2cd	4.4de	83.7ef	77.2d	9.3e	8.9e	541f	481e	6.4d	6.2d
T_1F_2	5.0c	4.7d	122.4d	114.1c	15.3d	15.1c	854d	794c	7.0cd	7.0cd
T_1F_3	6.0b	4.7d	146.3c	146.7b	18.2c	19.0b	1018c	1033b	7.0cd	7.1bcd
T_2F_0	4.23cd	4.1de	91.4e	85.3d	11.0e	10.7de	655e	595d	7.2bcd	7.0cd
T_2F_1	4.6cd	4.4de	96.3e	90.1d	11.0e	10.8de	674e	614d	7.0cd	6.8cd
T_2F_2	6.4b	6.0b	160.5b	146.3b	21.0b	20.9b	1183b	1123b	7.4abc	7.7abc
T_2F_3	8.1a	7.7a	205.4a	196.4a	29.0a	27.4a	1642a	1582a	8.0a	8.1a
T_3F_0	4.3d	4.1e	89.3e	83.4d	10.3e	10.0de	622ef	562de	7.0cd	6.7d
T_3F_1	4.6cd	4.3de	95.3e	86.8d	11.1e	10.9d	673e	613d	7.1cd	7.1bcd
T_3F_2	6.2b	5.3c	153.3bc	145.2b	18.8c	19.1b	1077bc	1031b	7.0cd	7.1bcd
T_3F_3	7.9a	7.5a	196.8a	187.3a	27.4a	25.7a	1564a	1486a	7.9ab	8.0ab
LSD	0.58	0.61	13.32	12.96	2.2	1.99	113	97.97	0.79	0.93

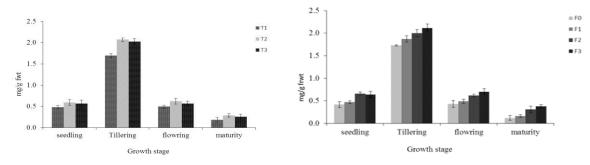
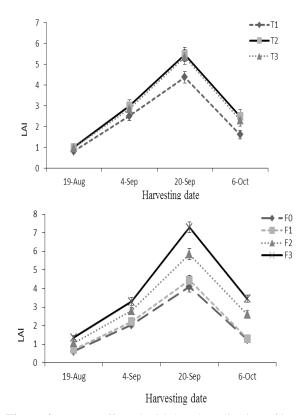


Figure 1. Total chlorophyll (mg/g fw) contents as affected by micronutrients application in different rice cultures



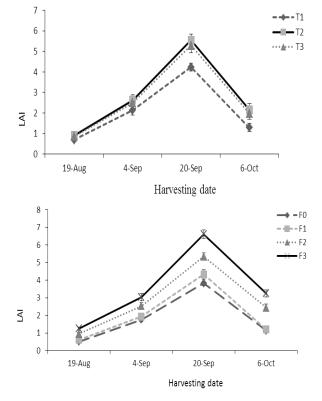


Figure 2. LAI as affected with basal application of boron and zinc (b) in different rice cultures (a) during 2008

Figure 3. LAI as affected with basal application of boron and zinc (b) in different rice cultures (a) during 2009

Table 2. Maximum chlorophyll contents (mg/g fw) as affected by boron and zinc in different rice cultures during 2008 and 2009

Treatments	Chlorophyll-a (mg/g fw)	Chlorophyll-b (mg/g fw)	Total chlorophyll a+b (mg/g fw)		
Rice cultures					
T ₁	0.94b	0.52b	1.46b		
T ₂	0.99a	0.56a	1.55a		
T ₃	0.98a	0.56a	1.55a		
LSD _{0.05}	0.014	0.013	0.027		
Fertilizers					
F ₀	0.90b	0.49b	1.39b		
F ₁	0.92b	0.51b	1.43b		
F ₂	1.02a	0.60a	1.62a		
$\overline{F_3}$	1.03a	0.60a	1.63a		
LSD _{0.05}	0.022	0.016	0.036		

 (T_1) Aerobic rice, (T_2) Flooded rice, (T_3) Flooding for two weeks after transplanting and then maintained at field capacity up to panicle initiation and again kept flooded starting from panicle initiation up to physiological maturity, (F_0) Control, (F_1) Boron, (F_2) Zinc and (F_3) Boron + Zinc

DISCUSSION

This study showed that aerobic cultivation for fine rice caused significant reductions in growth rate and other growth parameters like LAI, LAD, CGR, TDM and NAR. However, chlorophyll contents were similar between flooded rice and rice that was grown under partial flooding with supplemental irrigation indicating that flooded condition may not be necessary for whole crop growth period. The significant decline in growth rate for the crop grown in aerobic condition might be due to the higher moisture and nutrients stress at different growth stages of plant. Chlorophyll contents is an imperative indicator of crop growth which were higher in flooded rice although it was similar with modified rice culture (T_3) indicating that flooded condition is also not necessary for whole crop growth period. Almost similar results were reported by Viraktamath (2006) who found similar rice plant yield while grown in flooded and alternate wetting and drying conditions. Water use efficiency was decreased in full irrigation treatment as compared to deficit irrigation in some other crops like wheat, safflower (Erekul et al., 2012; Omidi et al., 2012). Chlorophyll contents were highest at tillering stage while the minimum chlorophyll contents were recorded at maturity due to the leaf senescence. Chlorophyll concentration showed that significant decline in growth rate was recorded when crop was grown in aerobic condition. This might be due to the higher moisture and nutrients stress at different growth stages of plant. Previous studies support this argument (Peng et al., 2006; Zulkarnain et al., 2009) and also show that above ground biomass significantly increases in

flooded soil. Many factors are expected to change in aerobic condition like redox potential (Gao et al., 2002) which effect the availability of nutrients. Soil pH may increase or decrease according to soil nature which will ultimately effect the availability of nutrients (Liu, 1996). Reduction of water contents in soil solution can also restrict the movement of the nutrient toward the root of the plant (Yoshida, 1981) and organic matter will be oxidized which will also cause the boron & zinc availability. As the availability of the zinc in aerobic rice declined which exhibited a suppressing effect on rice growth. All growth parameters were improved in flooded as well as in intermittent flooding and drying condition with the combined application of both micronutrients. This might be due to the higher availability of nutrients in these rice cultures as compared to aerobic rice. Rajesh and Thanunathan (2003) found that alternate wetting and drying uptake more nutrients as compared to aerobic rice culture due to improved root activity. Furthermore our results are also supported by Gao et al. (2006) who recorded inferior zinc contents in rice crop grown under aerobic condition. Crop performance was higher in year 2008 as compared to year 2009 which might be due the variation in weather condition. In the growing season of year 2008, crop received more rainfall with low temperature which favoured the rice growth and yield as compared to next growing season (Table-3). Aerobic rice cultivation is a best option for water saving rice production but a successful transition requires special rice genotypes. Thus plant breeder should play a role in developing aerobic varieties which can adopt that environment without harming growth and yield.

Month	Mean Temperature (C ⁰)		Relative H	umidity (%)	Rainfall (mm)	
	2008	2009	2008	2009	2008	2009
June	32.805	33.85	32	33.6	41.7	9.6
July	32.755	32.95	53	59	81.6	43.5
August	30.95	32.1	65	65.8	204.5	116
Sept	29.05	30.35	59.3	61	28.8	20.6
October	26.65	24.9	57.6	57.9	0	17.5
November	19.75	18.25	58.9	64.7	0	0.7

 Table 3. Meteorological data recorded during rice growing seasons

Due to the intense cropping and continuous removal of nutrients resulted in decreased availability of essential micronutrients like boron and zinc. Farmers are just concentrating on macronutrients as they are well aware about these nutrients but they are not conscious about micronutrients. It is the need of the time to grow crops with timely and balanced use of these nutrients. Deficiency of these nutrients can become a bigger problem which will affect the crop severely as farmers are shifting cultivation from flooded to aerobic condition. In this study, application of micronutrients in $T_2 \& T_3$ recorded similar and maximum yield as compared with T_1 rice culture indicating that decreasing moisture contents decreased the availability of nutrients to plants.

CONCLUSIONS

Results of the present study showed that cultivation of rice under fully aerobic conditions significantly reduced the crop growth as compared to that of fully-flooded rice. However partial flooding did not significantly affect the growth attributes. It is therefore concluded that a shift in rice cultivation from fully flooded to fully aerobic is likely to change the growth behavior of rice crop.

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