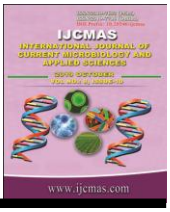
Int.J.Curr.Microbiol.App.Sci (2019) *8*(10): 150-168

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 8 Number 10 (2019)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2019.810.016

Influence of Zinc Applications on Photosynthesis, Transpiration and Stomatal Conductance in *Kharif* Rice (*Oryza sativa* L.) Genotypes

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ABSTRACT

A field experiment in rice investigation was conducted during *kharif* seasons of 2017 and 2018 at college farm, Navsari Agricultural University, Navsari to study the effect of variety and zinc application on rice. The experiment was laid out in a randomized block design with factorial concept and replicated thrice. The experiment consisted of three varieties *viz.*, IET-25450 (V_1), BPT-5204 (V_2) and IET-24766 (V_3), three soil base zinc application (S_1) control (00 kg $ZnSO_4$ ha^{-1}), (S_2) 10 kg $ZnSO_4$ ha^{-1} at the time of transplanting and (S_3) 20 kg $ZnSO_4$ ha^{-1} at the time of transplanting and two foliar Zn application (F_1) 0 % Zn EDTA and (F_2) 1 % Zn EDTA spray at tillering and grain filling stage. Varieties differed significantly in photosynthetic rate, stomatal conductance and transpiration rate. Among the varieties, IET-24766 gave significantly highest photosynthetic rate ($44.78 \mu Mm^{-2}s^{-1}$), stomatal conductance ($0.35 mMm^{-2}s^{-1}$) and transpiration rate ($5.66 mMm^{-2}s^{-1}$) followed by IET-25450and BPT-5204 and soil base zinc application of 20 kg $ZnSO_4$ ha^{-1} at the time of transplanting as well as foliar zinc application of 1 % Zn EDTA spray at tillering and grain filling stage resulted in highest values in photosynthetic rate (43.52 and 43.02 $\mu Mm^{-2}s^{-1}$, respectively), stomatal conductance (0.35 and 0.34 $mMm^{-2}s^{-1}$, respectively), transpiration rate (5.38 and 5.17 $mMm^{-2}s^{-1}$, respectively) during in pooled analysis.

Keywords

Rice, Photosynthesis, Stomatal Conductance, Transpiration, Zinc

Article Info

Accepted:
04 September 2019
Available Online:
10 October 2019

Introduction

Rice (*Oryza sativa* L.) is the world's most important food crop and a primary source of major food staple and energy of more than half the world population, being the major source of carbohydrate and even protein. However, rice is a poor source of essential micronutrients such as Zinc (Zn). Deficiency of Zn in rice has been reported in lowland rice in India (Mandal, *et al.*, 2000 and Qadar, 2002), in Brazil (Fageria, 2001; Fageria and

Stone, 2008) and in the Philippines (De Datta, 1981). Zn is a vital micronutrient for plants growth and it act as cofactor for many enzymes which are involved in various physiological and biochemical functions of crop plants. There are many enzymes which require zinc as cofactor to carry their functions *viz.,* Copper- zinc super oxide dismutase (Cu/Zinc SOD), peroxidase, carbonic anhydrase and catalase that plays an important role in protecting crop versus oxidative harm catalyzed by reactive oxygen species (Barak and Helmke, 1993). Zn is also involved with nitrogen metabolism (e.g., glutamate dehydrogenase) and anaerobic metabolism (e.g., alcohol dehydrogenase) (Fageria *et al.,* 2011). Zn deficient plants usually have reduced leaf chlorophyll (Chl) concentration and lower Chl a:b ratio, which indicates damage to intrinsic quantum efficiency of the photosystem-II (PS-II) units (Chen *et al.,* 2008). Such damage to photosynthetic centers, decreased leaf photosynthetic capacity due to a decreased number of PS-II units per unit leaf area, making them susceptible to photodamage (Chen *et al.,* 2008 and Rehman *et al.,* 2012).

A global study by the Food and Agriculture Organization (FAO) showed that about 30% of the cultivated soils of the world are Zn deficient (Sillanpaa, 1982). Zinc deficiency in crop plants reduces not only grain yield but also the nutritional quality of the grain. However, the frequency of soil Zn deficiency is greater in rice than other crops, with more than 50% of the crop worldwide prone to this nutritional disorder (Hacisalihoglu and Kochian, 2003 and Rehman*et al.,* 2012). Therefore, Zn deficiency is considered as one of the most important nutritional stresses limiting rice production in Asia (Ahmad *et al.,* 2012). Genotypic variation in rice grain Zn concentration might be due to the difference in physiological processes determining Zn accumulation in grains (Gao*et al.,* 2012). Application of $ZnSO_4$ is the general strategy to improve grain yield and grain Zn concentration in cereals grown on Zn deficient soils. Cakmak (2008) and Rehman*et al.,* (2012) have reviewed several studies where Zn application to Zn-deficient soils significantly and remarkably increased plant growth and grain yield, grain Zn concentration. Most common method of Zn fertilization is through soil application by broadcasting, banding in vicinity of seed, or via irrigation and commonly applied in rice under lowland condition before flooding or after transplanting to prevent Zn deficiency and for increased grain yield (Dobermann and Fairhurst 2000, Naik and Das 2007). Zn can be absorbed by leaf stomata when applied as foliar spray and then transported via the vascular system to where it is needed (Marschner, 1995). A number of Zn sources [$ZnSO_4$, $Zn(NO_3)_2$, Zn-EDTA] have been used as foliar fertilizers in a number of crops (Yoshida *et al.,* 1970). Foliar application of $ZnSO_4$ is effective in correcting Zn deficiency and improving grain Zn concentration (Yoshida *et al.,* 1970, Wilhelm *et al.,* 1988, Jiang *et al.,* 2008, Stomph *et al.,* 2011). Significant increases in grain yield, straw and grain Zn contents were observed with foliar application of Zn as Zn-EDTA and $ZnSO_4$, but the highest increase was observed with Zn-EDTA application (Karak and Das, 2006). Although foliar application is effective in increasing seed Zn content (Welch 2002, Yang *et al.,* 2007, Jiang *et al.,* 2008, Cakmak, 2009), time of foliar Zn application is an important factor in this regard (Jiang *et al.,* 2008; Stomph *et al.,* 2011). Generally, large increases in grain Zn occur when it is foliar applied at later stages of plant development. Higher translocation of Zn from flag leaves to grains occurred when Zn had been applied at booting or anthesis stage in a nutrient solution when genotypes with high or low grain Zn were used (Wu *et al.,* 2010). Foliar application of Zn at panicle initiation was effective in increasing whole grain Zn contents 2-fold

(Phattarakul *et al.*, 2011). The overall aim of the study is to understand the effect of soil base Zn application and foliar Zn application on effect of photosynthetic rate, stomatal conductance and transpiration rate of selected rice genotypes.

Materials and Methods

Planting materials and treatments

To study the effect of Zn application on physiological parameters, a field experiment was conducted during 2017 and 2018 in a randomized block design with factorial concept and replicated thrice at college farm, Navsari Agricultural University, Navsari, Gujarat, India.

The experiment consisted of three varieties *viz.*, IET-25450 (V_1), BPT-5204 (V_2) and IET-24766 (V_3), three soil base zinc application (S_1) control (00 kg $ZnSO_4$ ha^{-1}), (S_2) 10 kg $ZnSO_4$ ha^{-1} at the time of transplanting and (S_3) 20 kg $ZnSO_4$ ha^{-1} at the time of transplanting followed by two foliar Zn application (F_1) 0 % Zn EDTA and (F_2) 1 % Zn EDTA spray at tillering and grain filling stage. Other recommended agronomical practices in vogue were followed for reaping good crop.

The photosynthetic rate, stomatal conductance and transpiration rate were measured using portable photosynthetic meter (Model CI- 340, Handheld Photosynthetic System, CID-Bioscience).

Every observation was recorded with leaf covering full window of the system of five plants in each plot at 60, 90 DAT and at harvest. It was measured in upper leaf (2nd leaf from top), middle leaf (6th leaf from top) and lower leaf (2nd leaf from bottom). These measurements for each plot at each stage were worked out and recorded. The observations recorded during the course of investigation were tabulated and analyzed statistically to draw a valid conclusion. The data were analyzed as per the standard procedure of "Analysis of Variance" (ANOVA) as described by Gomez and Gomez (1984).

The significance of treatments was tested by 'F' test (Variance ratio). Standard error of mean (S.Em.±) was computed for various factors. Critical difference (CD) was used to know the differences exist between treatment mean at 5% level of significance where 'F' test showed significant differences among means by the following formula:

$$\text{C.D.} = \text{S.E. (d)} \times t_{0.05,\ edf}$$

Results and Discussion

Photosynthetic rate

Effect of different variety on photosynthetic rate at 60, 90 DAT and at harvest

An appraisal of data tabulated in Table 1, 2 and 3 represent the photosynthetic rate of rice significantly affected by different variety during both of the years and in pooled analysis.

Significantly highest mean photosynthetic rate was recorded with (V_3) IET-24766 during first and second year of experiment as well as in pooled data at 60 DAT (44.45, 45.51 and 44.98 $\mu Mm^{-2}s^{-1}$, respectively), 90 DAT (44.24, 44.83 and 44.53$\mu Mm^{-2}s^{-1}$, respectively) and at harvest (24.15, 24.36 and 24.25$\mu Mm^{-2}s^{-1}$, respectively) whereas lowest mean photosynthetic rate were recorded in (V_2) BPT-5204 during year of 2017, 2018 and in pooled analysis at 60 DAT (38.92, 39.99 and 39.46 $\mu Mm^{-2}s^{-1}$, respectively), 90 DAT (38.72, 39.38 and 39.05 $\mu Mm^{-2}s^{-1}$, respectively) and at harvest (17.71, 17.97 and 17.84 $\mu Mm^{-2}s^{-1}$, respectively).

Effect of soil base Zn application on photosynthetic rate at 60, 90 DAT and at harvest

The individual years of experimentation and in pooled analysis, the result in Table 1, 2 and3 revealed that the photosynthetic rate of rice at 60, 90 days after transplanting (DAT) and at harvest were influenced significantly due to soil base Zn application at the time of transplanting.

The mean photosynthetic rate showed that maximum photosynthetic rate during both the years as well as in pooled analysis at 60 DAT (42.81, 44.22 and 43.52 µMm^{-2}s^{-1}, respectively), 90 DAT (42.60, 43.46 and 43.03 µMm^{-2}s^{-1}, respectively) and at harvest (21.78, 22.06 and 21.92µMm^{-2}s^{-1}, respectively)were noted under application of 20 kg $ZnSO_4$ ha^{-1}(S_3) but it was being at par with (S_2) 10 kg $ZnSO_4$ ha^{-1} during individual years of study and in pooled analysis at 60 DAT (41.60, 42.67 and 42.13 µMm^{-2}s^{-1}, respectively), 90 DAT (41.40, 42.26 and 41.83 µMm^{-2}s^{-1}, respectively) and at harvest (20.75, 21.00 and 20.27 µMm^{-2}s^{-1}, respectively).

The lowest photosynthetic rate was recorded under the treatment (S_1) 00 kg $ZnSO_4$ ha^{-1} during 20117, 2018 and in pooled analysis at 60 DAT (40.34, 41.04 and 40.69 µMm^{-2}s^{-1}, respectively), 90 DAT (40.13, 40.28 and 40.20 µMm^{-2}s^{-1}, respectively) and at harvest (19.53, 19.75 and 19.64µMm^{-2}s^{-1}, respectively) except in pooled analysis at harvest.

Effect of foliar Zn application on photosynthetic rate at 60, 90 DAT and at harvest

It is evident from Table 1, 2 and 3 that the photosynthetic rate of rice was influenced significantly due to foliar Zn application at tillering stage during consecutive years of experiment and in pooled analysis

During individual years of experiment and in pooled analysis, significantly maximum mean photosynthetic rate at 60 DAT (42.37, 43.67 and 43.02 µMm^{-2}s^{-1}, respectively), 90 DAT (42.17, 42.79 and 42.48 µMm^{-2}s^{-1}, respectively) and at harvest (21.44, 21.70 and 21.57 µMm^{-2}s^{-1}, respectively) were recorded under application of 1 % Zn EDTA(F_2). Lowest photosynthetic rate was recorded to control (F_1) 0 % Zn EDTA during both the years of experimentation and in pooled analysis.

Interaction effect on photosynthetic rate at 60, 90 DAT and at harvest

The interaction effects in respect of photosynthetic rate between variety and soil base Zn application (V x S), variety and foliar Zn application (V x F), soil base Zn application and foliar Zn application (S x F) and variety, soil base Zn application and foliar Zn application (V x S x F) were did not exert any significant effect in individual years of study and in pooled analysis.

Stomatal conductance

Effect of different variety on stomatal conductance at 60, 90 DATand at harvest

Data presented in Table 4, 5 and 6 revealed that, varietal effect are significantly different for the stomatal conductance during both the years of experimentation and in pooled findings.

Variety (V_3) IET-24766 showed higher stomatal conductance during both years of experimentation and in pooled analysis at 60 DAT (0.345, 0.348 and 0.347mMm^{-2}s^{-1}, respectively), 90 DAT (0.333, 0.336 and 0.334 mMm^{-2}s^{-1}, respectively) and at harvest (0.135, 0.144 and 0.139 mMm^{-2}s^{-1}, respectively), but it was remained at par with variety (V_1) IET-25450 during consecutive years of experiment and in pooled analysis at 60 DAT (0.336, 0.349 and 0.338 mMm^{-2}s^{-1}, respectively) and 90 DAT (0.324, 0.327 and 0.326 mMm^{-2}s^{-1}, respectively).

Whereas, lowest mean stomatal conductance were recorded in variety (V$_2$) BPT-5204 during individual years of experiment and in pooled analysis at 60 DAT (0.318, 0.321 and 0.319mMm^{-2}s^{-1}, respectively), 90 DAT (0.307, 0.310 and 0.308 mMm^{-2}s^{-1}, respectively) and at harvest (0.110, 0.118 and 0.114 mMm^{-2}s^{-1}, respectively).

Effect of soil base Zn application on stomatal conductance at 60, 90 DAT and atharvest

The result in Table 4, 5 and 6 revealed that the stomatal conductance of rice was influenced significantly due to different level of soil base Zn application at the time of transplanting during consecutive years of experimentation and in pooled analysis.

Maximum mean stomatal conductance was recorded during first and second year of experiment as well as in pooled data at 60 DAT (0.346, 0.349 and 0.348 mMm^{-2}s^{-1}, respectively), 90 DAT (0.334, 0.337 and 0.336 mMm^{-2}s^{-1}, respectively) and at harvest (0.137, 0.147 and 0.142 mMm^{-2}s^{-1}, respectively)) under application of 20 kg ZnSO$_4$ ha^{-1}(S$_3$).

But, application of 20 kg ZnSO$_4$ ha^{-1}(S$_3$) remained at par with (S$_2$) 10 kg ZnSO$_4$ ha^{-1} during both the years as well as in pooled analysis at 60 DAT (0.334, 0.337 and 0.335 mMm^{-2}s^{-1}, respectively) and 90 DAT (0.322, 0.325 and 0.324 mMm^{-2}s^{-1}, respectively).

Application of (S$_1$) 00 kg ZnSO$_4$ ha^{-1} gave lowest stomatal conductance during individual years of study and in pooled analysis at 60 DAT (0.319, 0.322 and 0.320 mMm^{-2}s^{-1}, respectively), 90 DAT (0.308, 0.311 and 0.309 mMm^{-2}s^{-1}, respectively) and at harvest (0.110, 0.116 and 0.113 mMm^{-2}s^{-1}, respectively).

Effect of foliar Zn application on stomatal conductance at 60, 90 DAT and at harvest

It is evident from Table 4, 5 and 6 that the stomatal conductance of rice was influenced significantly due to foliar Zn application at tillering stage during individual years of experiment and in pooled finding.

During year of 2017, 2018 and in pooled analysis was recorded significantly maximum mean stomatal conductance at 60 DAT (0.343, 0.346 and 0.344 mMm^{-2}s^{-1}, respectively), 90 DAT (0.330, 0.334 and 0.332 mMm^{-2}s^{-1}, respectively) and at harvest (0.134, 0.144 and 0.139 mMm^{-2}s^{-1}, respectively) under application of 1 % Zn EDTA(F$_2$) and lowest was recorded to control (F$_1$) 0 % Zn EDTA during consecutive years of experiment and in pooled analysis at 60 DAT (0.323, 0.326 and 0.325 mMm^{-2}s^{-1}, respectively), 90 DAT (0.312, 0.315 and 0.313 mMm^{-2}s^{-1}, respectively) and at harvest (0.114, 0.121 and 0.117 mMm^{-2}s^{-1}, respectively).

Interaction effect on stomatal conductance at 60, 90 DAT and at harvest

The interaction effects in respect of stomatal conductance between variety and soil base Zn application (V x S), variety and foliar Zn application (V x F), soil base Zn application and foliar Zn application (S x F) and variety, soil base Zn application and foliar Zn application (V x S x F) were did not show any significant effect in both the years of study and in pooled analysis.

While at harvest during in pooled analysis, interaction effect of soil base Zn application and foliar Zn application (S x F) was significant while all other interaction were not significant.

Treatment receiving interaction effect of (S x F) soil Zn application and foliar Zn application produced significantly maximum mean stomatal conductance at harvest (0.149 mMm^{-2}s^{-1}) was recorded under application of 20 kg $ZnSO_4$ ha^{-1}+ 1 % Zn EDTA(S_3F_2) and lowest (0.101 mMm^{-2}s^{-1}) was recorded to control (S_1F_1) 00 kg $ZnSO_4$ ha^{-1} + 0 % Zn EDTA during in pooled analysis.

Transpiration rate

Effect of different variety on transpiration rate at 60, 90 DAT and at harvest

The results in Table 7, 8 and 9 revealed that varieties showed significant variation on the transpiration rate during both the years of study and in pooled findings.

During year of 2017, 2018 and in pooled analysis, variety IET-24766 (V_3) showed significantly higher transpiration rate at 60 DAT (5.65, 5.66 and 5.66 mMm^{-2}s^{-1}, respectively), 90 DAT (5.45, 5.47 and 5.46 mMm^{-2}s^{-1}, respectively) and at harvest (3.17, 3.36 and 3.27 mMm^{-2}s^{-1}, respectively) over rest of all other treatments.

While, significantly lowest mean transpiration rate were recorded in variety BPT-5204 (V_2) during both of the years of experiment and in pooled analysis at 60 DAT (3.58, 3.59 and 3.58 mMm^{-2}s^{-1}, respectively), 90 DAT (3.37, 3.39 and 3.38 mMm^{-2}s^{-1}, respectively) and at harvest (1.56, 1.68 and 1.62 mMm^{-2}s^{-1}, respectively).

Effect of soil base Zn application on transpiration rate at 60, 90 DAT and at harvest

The result in Table 7, 8 and 9 revealed that the transpiration rate of rice was influenced significantly due to different level of soil base Zn application at the time of transplanting during both the years of experimentation and in pooled analysis. Significantly maximum mean transpiration rate during both years of experimentation and in pooled analysis at 60 DAT (5.37, 5.38 and 5.38 mMm^{-2}s^{-1}, respectively), 90 DAT (5.18, 5.19 and 5.19 mMm^{-2}s^{-1}, respectively) and at harvest (2.71, 2.87 and 2.79 mMm^{-2}s^{-1}, respectively) was recorded under application of 20 kg $ZnSO_4$ ha^{-1}(S_3) over rest of all other treatments.

Treatments exhibit significant superiority over control (S_1) 00 kg $ZnSO_4$ ha^{-1} during consecutive years of experiment and in pooled analysis at 60 DAT (3.90, 3.92 and 3.91 mMm^{-2}s^{-1}, respectively), 90 DAT (3.69, 3.71 and 3.70 mMm^{-2}s^{-1}, respectively) and at harvest (2.14, 2.27 and 2.21 mMm^{-2}s^{-1}, respectively).

Effect of foliar Zn application on transpiration rate at 60, 90 DAT and at harvest

It is seen from the data in Table 7, 8 and 9 that the transpiration rate of rice was influenced significantly due to foliar Zn application at tillering stage and grain filling stage (except 60 DAT) during both the years of experimentation and in pooled analysis.

During first and second year of experiment as well as in pooled data, significantly maximum mean transpiration rate at 60 DAT (5.16, 5.18 and 5.17 mMm^{-2}s^{-1}, respectively), 90 DAT (4.98, 4.99 and 4.98 mMm^{-2}s^{-1}, respectively) and at harvest (2.61, 2.78 and 2.69 mMm^{-2}s^{-1}, respectively) were recorded under application of 1 % Zn EDTA(F_2) and lowest mean transpiration rate was recorded to control (F_1) 0 % Zn EDTA during consecutive years of experiment and in pooled analysis at 60 DAT (4.06, 4.08 and 4.07 mMm^{-2}s^{-1}, respectively), 90 DAT (3.85, 3.87 and 3.86 mMm^{-2}s^{-1}, respectively) and at harvest (2.25, 2.37 and 2.31 mMm^{-2}s^{-1}).

Table.1 Photosynthetic rate (μMm^{-2}s^{-1}) at 60 DAT

	V			S			F		Y
	V_1	V_2	V_3	S_1	S_2	S_3	F_1	F_2	
Y1	41.37	38.92	44.45	40.34	41.60	42.81	40.79	42.37	41.58
Y2	42.43	39.99	45.51	41.04	42.67	44.22	41.62	43.67	42.64
Pooled	41.90	39.46	44.98	40.69	42.13	43.52	41.20	43.02	42.11

		V	S	F	V x S	V x F	S x F	V x S x F	Y
Y1	**S.Em.±**	0.67	0.67	0.55	1.17	0.95	0.95	1.65	
	C.D.	1.94	1.94	1.58	NS	NS	NS	NS	
Y2	**S.Em.±**	0.87	0.87	0.71	1.51	1.24	1.24	2.14	
	C.D.	2.51	2.51	2.05	NS	NS	NS	NS	
Pooled	**S.Em.±**	0.54	0.54	0.45	0.93	0.77	0.77	1.31	0.450
	C.D.	1.54	1.54	1.26	NS	NS	NS	NS	NS
Interaction with year	**S.Em.±**	0.78	0.78	0.64	1.35	1.10	1.10	1.91	
	C.D.	NS	NS	NS	NS	NS	NS	NS	
C.V. %		7.86							

V_1- IET-25450	**S_1- 00 kg $ZnSO_4$ ha^{-1}**	**F_1- 0 % Zn EDTA**
V_2- BPT-5204	S_2- 10 kg $ZnSO_4$ ha^{-1}	F_2- 1 % Zn EDTA
V_3- IET-24766	S_3- 20 kg $ZnSO_4$ ha^{-1}	

Table.2 Photosynthetic rate (μMm^{-2}s^{-1}) at 90 DAT

	V			S			F		Y
	V_1	V_2	V_3	S_1	S_2	S_3	F_1	F_2	
Y1	41.17	38.72	44.24	40.13	41.40	42.60	40.58	42.17	41.38
Y2	41.79	39.38	44.83	40.28	42.26	43.46	41.21	42.79	42.00
Pooled	41.48	39.05	44.53	40.20	41.83	43.03	40.90	42.48	41.69

		V	S	F	V x S	V x F	S x F	V x S x F	Y
Y1	**S.Em.±**	0.67	0.67	0.54	1.15	0.94	0.94	1.63	
	C.D.	1.92	1.92	1.57	NS	NS	NS	NS	
Y2	**S.Em.±**	0.66	0.66	0.54	1.15	0.94	0.94	1.62	
	C.D.	1.91	1.91	1.56	NS	NS	NS	NS	
Pooled	**S.Em.±**	0.46	0.46	0.38	0.79	0.66	0.66	1.12	0.384
	C.D.	1.31	1.31	1.08	NS	NS	NS	NS	NS
Interaction with year	**S.Em.±**	0.66	0.66	0.54	1.15	0.94	0.94	1.63	
	C.D.	NS	NS	NS	NS	NS	NS	NS	
C.V. %		6.77							

Table.3 Photosynthetic rate (µMm^{-2}s^{-1}) at harvest

	V			S			F		Y
	V_1	V_2	V_3	S_1	S_2	S_3	F_1	F_2	
Y1	20.21	17.71	24.15	19.53	20.75	21.78	19.94	21.44	20.69
Y2	20.48	17.97	24.36	19.75	21.00	22.06	20.17	21.70	20.94
Pooled	20.34	17.84	24.25	19.64	20.87	21.92	20.05	21.57	20.81

		V	S	F	V x S	V x F	S x F	V x S x F	Y
Y1	**S.Em.±**	0.37	0.37	0.30	0.65	0.53	0.53	0.91	
	C.D.	1.07	1.07	0.88	NS	NS	NS	NS	
Y2	**S.Em.±**	0.38	0.38	0.31	0.66	0.54	0.54	0.94	
	C.D.	1.10	1.10	0.90	NS	NS	NS	NS	
Pooled	**S.Em.±**	0.26	0.26	0.22	0.45	0.37	0.37	0.64	0.218
	C.D.	0.74	0.74	0.61	NS	NS	NS	NS	NS
Interaction with year	**S.Em.±**	0.38	0.38	0.31	0.65	0.53	0.53	0.93	
	C.D.	NS	NS	NS	NS	NS	NS	NS	
C.V. %		7.71							

Table.4 Stomatal conductance (mMm^{-2}s^{-1}) at 60 DAT

	V			S			F		Y
	V_1	V_2	V_3	S_1	S_2	S_3	F_1	F_2	
Y1	0.336	0.318	0.345	0.319	0.334	0.346	0.323	0.343	0.333
Y2	0.339	0.321	0.348	0.322	0.337	0.349	0.326	0.346	0.336
Pooled	0.338	0.319	0.347	0.320	0.335	0.348	0.325	0.344	0.334

		V	S	F	V x S	V x F	S x F	V x S x F	Y
Y1	**S.Em.±**	0.008	0.008	0.006	0.013	0.011	0.011	0.019	
	C.D.	0.022	0.022	0.018	NS	NS	NS	NS	
Y2	**S.Em.±**	0.008	0.008	0.006	0.013	0.011	0.011	0.019	
	C.D.	0.022	0.022	0.018	NS	NS	NS	NS	
Pooled	**S.Em.±**	0.05	0.005	0.004	0.009	0.008	0.008	0.013	0.004
	C.D.	0.015	0.015	0.012	NS	NS	NS	NS	NS
Interaction with year	**S.Em.±**	0.008	0.008	0.006	0.013	0.011	0.011	0.019	
	C.D.	NS	NS	NS	NS	NS	NS	NS	
C.V. %		9.66							

Table.5 Stomatal conductance (mMm^{-2}s^{-1}) at 90 DAT

	V			**S**			**F**		**Y**
	V_1	**V_2**	**V_3**	**S_1**	**S_2**	**S_3**	**F_1**	**F_2**	
Y1	0.324	0.307	0.333	0.308	0.322	0.334	0.312	0.330	0.321
Y2	0.327	0.310	0.336	0.311	0.325	0.337	0.315	0.334	0.324
Pooled	0.326	0.308	0.334	0.309	0.324	0.336	0.313	0.332	0.323

		V	**S**	**F**	**V x S**	**V x F**	**S x F**	**V x S x F**	**Y**
Y1	**S.Em.±**	0.007	0.007	0.006	0.013	0.010	0.010	0.018	
	C.D.	0.021	0.021	0.017	NS	NS	NS	NS	
Y2	**S.Em.±**	0.007	0.007	0.006	0.012	0.010	0.010	0.018	
	C.D.	0.021	0.021	0.017	NS	NS	NS	NS	
Pooled	**S.Em.±**	0.005	0.005	0.004	0.009	0.007	0.007	0.012	0.004
	C.D.	0.014	0.014	0.012	NS	NS	NS	NS	NS
Interaction with year	**S.Em.±**	0.007	0.007	0.006	0.012	0.010	0.010	0.018	
	C.D.	NS	NS	NS	NS	NS	NS	NS	
C.V. %		9.45							

Table.6 Stomatal conductance (mMm^{-2}s^{-1}) at harvest

	V			**S**			**F**		**Y**
	V_1	**V_2**	**V_3**	**S_1**	**S_2**	**S_3**	**F_1**	**F_2**	
Y1	0.128	0.110	0.135	0.110	0.126	0.137	0.114	0.134	0.124
Y2	0.136	0.118	0.144	0.116	0.135	0.147	0.121	0.144	0.133
Pooled	0.132	0.114	0.139	0.113	0.130	0.142	0.117	0.139	0.128

	F_1			**F_2**		
	S_1	**S_2**	**S_3**	**S_1**	**S_2**	**S_3**
Y1	0.098	0.113	0.130	0.121	0.138	0.143
Y2	0.104	0.120	0.140	0.129	0.149	0.155
Pooled	0.101	0.117	0.135	0.125	0.144	0.149

		V	**S**	**F**	**V x S**	**V x F**	**S x F**	**V x S x F**	**Y**
Y1	**S.Em.±**	0.002	0.002	0.002	0.004	0.003	0.003	0.006	
	C.D.	0.007	0.007	0.005	NS	NS	NS	NS	
Y2	**S.Em.±**	0.003	0.003	0.002	0.005	0.004	0.004	0.006	
	C.D.	0.007	0.007	0.006	NS	NS	NS	NS	
Pooled	**S.Em.±**	0.002	0.002	0.001	0.003	0.002	0.002	0.004	0.001
	C.D.	0.005	0.005	0.004	NS	NS	0.007	NS	0.004
Interaction with year	**S.Em.±**	0.002	0.002	0.002	0.004	0.004	0.004	0.006	
	C.D.	NS	NS	NS	NS	NS	NS	NS	
C.V. %		8.08							

Table.7 Transpiration rate ($mMm^{-2}s^{-1}$) at 60 DAT

	V			S			F		Y
	V_1	V_2	V_3	S_1	S_2	S_3	F_1	F_2	
Y1	4.61	3.58	5.65	3.90	4.56	5.37	4.06	5.16	4.61
Y2	4.63	3.59	5.66	3.92	4.59	5.38	4.08	5.18	4.63
Pooled	4.62	3.58	5.66	3.91	4.58	5.38	4.07	5.17	4.62

	V_1			V_2			V_3		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
Y1	3.59	4.72	5.53	3.00	3.49	4.24	5.12	5.49	6.34
Y2	3.60	4.76	5.55	3.02	3.51	4.26	5.14	5.50	6.35
Pooled	3.59	4.74	5.54	3.01	3.50	4.25	5.13	5.49	6.35

	V_1		V_2		V_3	
	F_1	F_2	F_1	F_2	F_1	F_2
Y1	3.89	5.33	3.10	4.05	5.19	6.11
Y2	3.91	5.36	3.12	4.07	5.20	6.12
Pooled	3.90	5.35	3.11	4.06	5.20	6.12

	F_1			F_2		
	S_1	S_2	S_3	S_1	S_2	S_3
Y1	3.56	3.86	4.76	4.24	5.27	5.98
Y2	3.57	3.88	4.77	4.26	5.29	6.00
Pooled	3.57	3.87	4.77	4.25	5.28	5.99

		V	S	F	V x S	V x F	S x F	V x S x F	Y
Y1	**S.Em.±**	0.076	0.076	0.062	0.132	0.108	0.108	0.186	
	C.D.	0.219	0.219	0.179	0.379	0.310	0.310	0.536	
Y2	**S.Em.±**	0.083	0.083	0.068	0.144	0.117	0.117	0.203	
	C.D.	0.238	0.238	0.195	0.413	0.337	0.337	0.584	
Pooled	**S.Em.±**	0.055	0.055	0.046	0.095	0.078	0.078	0.134	0.046
	C.D.	0.157	0.157	0.129	0.267	0.221	0.221	0.378	NS
Interaction with year	**S.Em.±**	0.080	0.080	0.065	0.138	0.113	0.113	0.195	
	C.D.	NS	NS	NS	NS	NS	NS	NS	
C.V. %		7.30							

Table.8 Transpiration rate (mMm^{-2}s^{-1}) at 90 DAT

	V			S			F		Y
	V_1	V_2	V_3	S_1	S_2	S_3	F_1	F_2	
Y1	4.42	3.37	5.45	3.69	4.38	5.18	3.85	4.98	4.42
Y2	4.43	3.39	5.47	3.71	4.39	5.19	3.87	4.99	4.43
Pooled	4.43	3.38	5.46	3.70	4.38	5.19	3.86	4.98	4.42

	V_1			V_2			V_3		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
Y1	3.35	4.56	5.35	2.82	3.29	4.02	4.91	5.29	6.17
Y2	3.37	4.57	5.36	2.83	3.30	4.03	4.92	5.30	6.19
Pooled	3.36	4.56	5.35	2.82	3.29	4.03	4.91	5.29	6.18

	V_1		V_2		V_3	
	F_1	F_2	F_1	F_2	F_1	F_2
Y1	3.70	5.14	2.88	3.87	4.98	5.93
Y2	3.71	5.15	2.90	3.88	5.00	5.93
Pooled	3.70	5.15	2.89	3.87	4.99	5.93

	F_1			F_2		
	S_1	S_2	S_3	S_1	S_2	S_3
Y1	3.34	3.65	4.57	4.05	5.10	5.79
Y2	3.35	3.67	4.59	4.06	5.11	5.80
Pooled	3.34	3.66	4.58	4.05	5.10	5.79

		V	S	F	V x S	V x F	S x F	V x S x F	Y
Y1	**S.Em.±**	0.08	0.08	0.06	0.13	0.11	0.11	0.19	
	C.D.	0.22	0.22	0.18	0.38	0.31	0.31	0.54	
Y2	**S.Em.±**	0.08	0.08	0.06	0.13	0.11	0.11	0.19	
	C.D.	0.22	0.22	0.18	0.38	0.31	0.31	0.54	
Pooled	**S.Em.±**	0.05	0.05	0.04	0.09	0.08	0.08	0.13	0.044
	C.D.	0.15	0.15	0.12	0.26	0.21	0.21	0.36	NS
Interaction with year	**S.Em.±**	0.08	0.08	0.06	0.13	0.11	0.11	0.19	
	C.D.	NS	NS	NS	NS	NS	NS	NS	
C.V. %		7.32							

Table.9 Transpiration rate ($mMm^{-2}s^{-1}$) at harvest

	V			S			F		Y
	V_1	V_2	V_3	S_1	S_2	S_3	F_1	F_2	
Y1	2.55	1.56	3.17	2.14	2.43	2.71	2.25	2.61	2.43
Y2	2.69	1.68	3.36	2.27	2.58	2.87	2.37	2.78	2.58
Pooled	2.62	1.62	3.27	2.21	2.51	2.79	2.31	2.69	2.50

	V_1			V_2			V_3		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
Y1	2.05	2.56	3.05	1.43	1.57	1.68	2.95	3.17	3.40
Y2	2.17	2.68	3.21	1.52	1.70	1.82	3.13	3.35	3.59
Pooled	2.11	2.62	3.13	1.48	1.64	1.75	3.04	3.26	3.50

	V_1		V_2		V_3	
	F_1	F_2	F_1	F_2	F_1	F_2
Y1	2.27	2.84	1.46	1.66	3.03	3.32
Y2	2.35	3.02	1.57	1.80	3.20	3.52
Pooled	2.31	2.93	1.51	1.73	3.11	3.42

	F_1			F_2		
	S_1	S_2	S_3	S_1	S_2	S_3
Y1	1.99	2.15	2.61	2.29	2.71	2.81
Y2	2.08	2.28	2.76	2.46	2.88	2.99
Pooled	2.04	2.22	2.68	2.38	2.80	2.90

		V	S	F	V x S	V x F	S x F	V x S x F	Y
Y1	**S.Em.±**	0.035	0.035	0.029	0.061	0.050	0.050	0.087	
	C.D.	0.102	0.102	0.083	0.177	0.144	0.144	0.250	
Y2	**S.Em.±**	0.039	0.039	0.032	0.068	0.056	0.056	0.097	
	C.D.	0.113	0.113	0.093	0.196	0.160	0.160	0.278	
Pooled	**S.Em.±**	0.026	0.026	0.022	0.045	0.037	0.037	0.063	0.022
	C.D.	0.074	0.074	0.061	0.126	0.105	0.104	0.178	0.061
Interaction with year	**S.Em.±**	0.038	0.038	0.031	0.065	0.053	0.053	0.092	
	C.D.	NS	NS	NS	NS	NS	NS	NS	
C.V. %		6.36							

Table.10 Interaction effect

	60 DAT			90 DAT			At harvest		
Treatments	**Year 1**	**Year 2**	**Year pooled**	**Year 1**	**Year 2**	**Year pooled**	**Year 1**	**Year 2**	**Year pooled**
$V_1S_1F_1$	3.18	3.19	3.18	2.94	2.96	2.95	1.86	1.92	1.89
$V_1S_1F_2$	4.00	4.01	4.00	3.77	3.78	3.77	2.23	2.42	2.33
$V_1S_2F_1$	3.60	3.61	3.61	3.40	3.41	3.40	2.04	2.10	2.07
$V_1S_2F_2$	5.84	5.91	5.87	5.72	5.73	5.72	3.08	3.27	3.17
$V_1S_3F_1$	4.90	4.92	4.91	4.75	4.77	4.76	2.89	3.04	2.97
$V_1S_3F_2$	6.16	6.18	6.17	5.94	5.95	5.94	3.21	3.37	3.29
$V_2S_1F_1$	2.79	2.81	2.80	2.57	2.58	2.58	1.34	1.42	1.38
$V_2S_1F_2$	3.21	3.22	3.21	3.06	3.07	3.07	1.52	1.63	1.57
$V_2S_2F_1$	2.98	3.02	3.00	2.78	2.80	2.79	1.43	1.55	1.49
$V_2S_2F_2$	3.99	4.00	3.99	3.79	3.80	3.79	1.71	1.86	1.79
$V_2S_3F_1$	3.52	3.53	3.52	3.29	3.31	3.30	1.61	1.73	1.67
$V_2S_3F_2$	4.95	4.98	4.97	4.75	4.76	4.75	1.75	1.90	1.83
$V_3S_1F_1$	4.71	4.72	4.71	4.50	4.52	4.51	2.77	2.91	2.84
$V_3S_1F_2$	5.52	5.56	5.54	5.31	5.32	5.31	3.13	3.34	3.23
$V_3S_2F_1$	5.00	5.03	5.01	4.78	4.80	4.79	2.99	3.18	3.09
$V_3S_2F_2$	5.97	5.98	5.97	5.79	5.80	5.79	3.35	3.53	3.44
$V_3S_3F_1$	5.85	5.87	5.86	5.66	5.68	5.67	3.33	3.50	3.41
$V_3S_3F_2$	6.83	6.84	6.83	6.68	6.69	6.68	3.47	3.69	3.58
S.Em.±	0.19	0.203	0.03	0.19	0.19	0.13	0.09	0.10	0.06
C.D.@ 5 %	0.54	0.584	0.38	0.54	0.54	0.36	0.25	0.28	0.18

Interaction effect on transpiration rate at 60, 90 DAT and at harvest

The interaction effects of transpiration rate (Table 10) between variety and soil base Zn application (V x S), variety and foliar Zn application (V x F), soil base Zn application and foliar Zn application (S x F) and variety, soil base Zn application and foliar Zn application (V x S x F) shows significantly in individual years of study and in pooled analysis.

The interaction effects (V x S) during 2017, 2018 and in pooled analysis were recorded significantly higher transpiration rate at 60 DAT (6.34, 6.35 and 6.35 $mMm^{-2}s^{-1}$, respectively), 90 DAT (6.17, 6.19 and 6.18 $mMm^{-2}s^{-1}$, respectively) and at harvest (3.40, 3.59 and 3.50 $mMm^{-2}s^{-1}$, respectively) in the interaction V_3S_3 (IET-24766 + 20 kg $ZnSO_4$ ha^{-1}) and the lowest mean transpiration rate at 60 DAT (3.00, 3.02 and 3.01 $mMm^{-2}s^{-1}$), 90 DAT (2.82, 2.83 and 2.82 $mMm^{-2}s^{-1}$, respectively) and at harvest (1.43, 1.52 and 1.48 $mMm^{-2}s^{-1}$, respectively) were recorded in the treatment combination V_2S_1 (BPT-5204 + 00 kg $ZnSO_4$ ha^{-1}) during both years of experimentation and in pooled data, respectively.

The interaction effects of variety and foliar application (V x F) during consecutive years of experiment and in pooled analysis were recorded significantly maximum mean transpiration rate at 60 DAT (6.11, 6.12 and 6.12 $mMm^{-2}s^{-1}$, respectively), 90 DAT (5.93, 5.93 and 5.93 $mMm^{-2}s^{-1}$, respectively) and at harvest (3.32, 3.52 and 3.42 $mMm^{-2}s^{-1}$, respectively) under interaction V_3F_2 (IET-24766 + 1.0 % Zn EDTA). Whereas, the lowest transpiration rate were recorded in the treatment combination V_2F_1 (BPT-5204 + 0.0 % Zn EDTA) during both year of experimentation and in pooled analysis at 60 DAT (3.10, 3.12 and 3.11 $mMm^{-2}s^{-1}$, respectively), 90 DAT (2.88, 2.90 and 2.89 $mMm^{-2}s^{-1}$, respectively) and at harvest (1.46, 1.57 and 1.51 $mMm^{-2}s^{-1}$, respectively).

Treatment receiving interaction effect of (S x F) soil Zn application (20 kg $ZnSO_4$ ha^{-1}) and foliar Zn application (1.0 % Zn EDTA) (S_3F_2) produced significantly higher transpiration rate during individual years of experiment and in pooled analysisat 60 DAT (5.98, 6.00 and 5.99 $mMm^{-2}s^{-1}$,respectively), 90 DAT (5.79, 5.80 and 5.79 $mMm^{-2}s^{-1}$, respectively) and at harvest (2.81, 2.99 and 2.90 $mMm^{-2}s^{-1}$, respectively). While, lowest mean transpiration rate were recorded in S_1F_1 Control (00 kg $ZnSO_4$ ha^{-1}+ 0.0 % Zn EDTA) in both years of experiment and in pooled data at 60 DAT (3.56, 3.57 and 3.57 $mMm^{-2}s^{-1}$, respectively), 90 DAT (3.34, 3.35 and 3.34 $mMm^{-2}s^{-1}$, respectively) and at harvest (1.99, 2.08 and 2.04 $mMm^{-2}s^{-1}$, respectively).

During consecutive years of experiment as well as in pooled analysis, the interaction effects (V x S x F) were recorded significantly. The data revealed the highest mean transpiration rate at 60 DAT (6.83, 6.84 and 6.83 $mMm^{-2}s^{-1}$, respectively), 90 DAT (6.68, 6.69 and 6.68 $mMm^{-2}s^{-1}$, respectively) and at harvest (3.47, 3.69 and 3.58 $mMm^{-2}s^{-1}$, respectively) was recorded significantly in the treatment combination $V_3S_3F_2$ (IET-24766 + 20 kg $ZnSO_4$ ha^{-1} + 1% Zn EDTA). Whereas, lowest mean transpiration rate at 60 DAT (2.79, 2.81 and 2.80 $mMm^{-2}s^{-1}$, respectively), 90 DAT (2.57, 2.58 and 2.58 $mMm^{-2}s^{-1}$, respectively) and at harvest (1.34, 1.42 and 1.38 $mMm^{-2}s^{-1}$, respectively) were recorded in the treatment combination $V_2S_1F_1$ (BPT-5204 + 00 kg $ZnSO_4$ ha^{-1} + 0 % Zn EDTA).

The photosynthetic rate, stomatal conductance and transpiration rate were highest at 60 DAT thereafter decreases. Significantly maximum photosynthetic rate were recorded with IET-24766 over IET-25450 and BPT-5204. The changes in the photosynthesis in the cultivars was due to changes in rubisco activity, protein content and chlorophyll per unit leaf area could have been caused by an alteration of rice leaf thickness or photosynthetic components per unit cell or per unit chloroplast. There was variation in the stomatal conductance might be due to genetic variation to having highest number of stomata. These findings are strongly supported by Dore *et al.,* (2018).

The application of soil base Zn application (20 kg $ZnSO_4$ ha^{-1}) and foliar Zn application (1 % Zn EDTA) were recorded significantly higher photosynthetic rate, stomatal conductance and transpiration rate over rest of other treatment. Zn deficiency depressed plant leaf photosynthetic capacity may be associated to decrease in intercellular CO_2 concentration and function of stomata (Sharma *et al.*, 1994). Stomatal closure allows plants to limit transpiration, but it also limits CO_2 absorption, which leads to a decreased photosynthetic activity (Sharma *et al.,* 1995). Reported a significant influence of Zinc in the regulation of the stomatal aperture, which is accounted for possible role of Zn in maintaining a high K content in guard cells. A decrease in carbonic anhydrase activity due to Zn deficiency may also contribute to the reduced net photosynthetic rate (Hacisalihoglu *et al.,* 2003). In addition, the accumulation of saccharides in leaves may be an important factor for the reducing photosynthesis activity under Zn-deficiency (Cakmak, 2000 and Dore *et al.,* 2018). Zinc is a constituent of carbonic anhydrase and is required for the activity of ribulose 1, 5-bisphosphate carboxylase/

oxygenase (Rubisco) (Shrivastava and Gupta, 1996 and Storey, 2007), the photosynthetic enzymes catalyzing the diffusion of CO_2 through the cell to the chloroplasts (Hatch and Slack, 1970). Zn-deficient plants usually have reduced leaf chlorophyll (Chl) concentration and lower Chl a:b ratio, which indicates damage to intrinsic quantum efficiency of the photosystem-II (PSII) units (Chen *et al.*, 2008). It can be attributed to reduced antioxidant enzyme activities and high oxidative stress damage in chloroplasts due to a blockage of energy spillover from PS-II to photosystem-I (PS-I) (Chen *et al.*, 2009). Such damage to photosynthetic centers, decreased leaf photosynthetic capacity due to a decreased number of PS-II units per unit leaf area, making them susceptible to photodamage (Chen 2008 and Rehman *et al.*, 2012). These findings are in agreement with those obtained by Dore *et al.,* (2018).

The causes of photoinhibition could be lower Chl content in Zn-deficient leaves. Leaf Chl concentration has a crucial role for the susceptibility to photoinhibition and leaves with less Chl are more susceptible to photoinhibition (Pätsikkä*et al.*, 2002). Moreover, Zn deficiency conditions likely cause an excess of reducing power (NADP + H^+) because of reduction in the CO_2 available at carboxylation sites i.e. stomatal closure (Harbinson 1994). In addition, simultaneous reduction of qP and qN indicates an over excitation of the photochemical system likely accompanied by accumulation of reduced electron acceptors.

Under such conditions the probability of generation of reactive radicals which further injure PSII components is very high (Barber and Anderson 1992). With more severe Zn deficiency or longer growth period under deficiency conditions serious damage to photosystems was expected to occur in Zn-deficient leaves.

Net CO_2 uptake per leaf surface area was depressed by low Zn supply. In addition, the remarkable reduction of total plant leaf area likely affected whole plant photosynthesis and contributed further to the low biomass production under Zn deficiency conditions. Stomatal conductance was significantly lower in Zn-deficient leaves, which caused in turn lower transpiration. Reduction of stomatal conductance due to low supply of Zn was reported for other plants such as maize (Wang and Jin 2005) and rice (Hajiboland and Beiramzadeh 2008). Involvement of Zn in stomatal opening was attributed to the structural role of Zn in carbonic anhydrase needed for maintaining adequate HCO_3^- in the guard cells and also to controlling effect of Zn on K^+ uptake by the guard cells (Sharma *et al.*, 1995). However, since stomatal aperture is affected also by deficiency of other nutrients such as Fe (Molassiotis *et al.*, 2006 and Hajiboland and Beiramzadeh 2008), it seems to be influenced rather indirectly by factors being common under deficiency of other nutrients, e.g. loss of membrane integrity and passive leakage of K^+ from guard cells.

The present study aimed to provide the most suitable zinc application in rice to overcome Zn deficiency problem in rice, by comparing the effects of different methods of Zn application on rice growth, It was observed that zinc application has significant positive effect on growth, among the varieties, IET-24766 recorded significant highest physiological parameters which was followed by IET-25450 and BPT-5204. Zinc application had also significant influence on physiological parameters. The soil base Zn treatment, 20 kg $ZnSO_4$ ha^{-1} at the time of transplanting recorded significantly higher physiological parameters of rice followed by 10 kg $ZnSO_4$ ha^{-1} at the time of transplanting and control 00 kg $ZnSO_4$ ha^{-1}. While, foliar Zn application recorded significantly maximum physiological parameters of rice under 1% Zn EDTA spray at tillering and grain filling stage followed by 0 % Zn EDTA. Further study should be needed to elaborate role of zinc at molecular level for photosynthesis mechanism to strength the knowledge of role of zinc in photosynthetic rate, stomatal conductance and transpiration rate.The obtained results of this study would be beneficial to mitigate Zn deficiency in rice and therefore, improve zinc use efficiency.

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How to cite this article:

Vivek N. Zinzala, Ajay V. Narwade, Nilima Karmakar and Patel, P.B. 2019. Influence of Zinc Applications on Photosynthesis, Transpiration and Stomatal Conductance in *Kharif* Rice (*Oryza sativa* L.) Genotypes. *Int.J.Curr.Microbiol.App.Sci.* 8(10): 150-168.
doi: https://doi.org/10.20546/ijcmas.2019.810.016