STUDY ON IMPROVING SEED GERMINATION AND ASSOCIATED TRAITS OF RICE UNDER SALINITY THROUGH PLANT GROWTH REGULATORS

Salinity stress mitigation in rice variety CO 51 through plant growth regulators

ABSTRACT

Salinity is one of the major abiotic stresses that adversely affect crop quality and productivity. About 21.5 million ha of land area is ~~thought to~~ be salt-affected in Asia; with India having 8.6 million ha , which is a major soil problem ~~constitutes a major part of problem soils~~. Rice has been identified as salt susceptible especially during seedling stage. Salinity is detrimental to seed germination, seedling growth and vigor leading to reduction in yield. To overcome this, various plant growth regulators were tried for improving seed germination and seedling vigour in rice under salinity. The major outcome of this study was reduced seed germination percentage and seedling vigour under different level s of salinity stress compared to control. In case of gibberellic acid treatment, the higher germination percentage of 91 was observed in 50 ppm (T_3) while higher seedling root length of 3.30 cm was found in 100 ppm (T_4) and shoot length 6.30 cm was found in 300 ppm (T_6) at 12^{th} DAS. Higher vigour index was found in 200 ppm (T_5). In case of salicylic acid treatment, the higher germination percentage of 88 was observed in ~~was~~ 50ppm (T_7). However, higher seedling root length of 3.45 cm was noted in 200ppm (T_{10}) and shoot length of 4.80 cm was found in 50ppm (T_7) at 12^{th} DAS. Highest vigour index was found in 50 ppm (T_7). In case of kinetin, the higher germination percentage of 84 was found in 100ppm (T_{13}), while the higher seedling root length of 1.96 cm was found in 10 ppm (T_{12}), seedling shoot length of 3.30 cm (T_{12}),and higher vigour index was found in 10 ppm (T_{12}). Among all the hormones, the higher germination percentage of 91 was noted in 50 ppm (T_3) of gibberellic acid (GA) as compared to control 97.00 spsce percent. , whileseedling root length of 3.45 cm was found at 200 ppm (T_{10}) in salicylic acid, higher mean shoot length of 6.30 cm was recorded in gibberellic acid. Similarly, seedling height maximum of 9.36 cm was recorded in 300 ppm (T_6) in gibberellic acid and maximum vigour index of 785 was found in 200 ppm (T_5) of gibberellic acid.

INTRODUCTION

Rice is the world's most important food crop and a primary source of food for more than half the world's population. It is one of the important cereal crops ranks second in consumption, which supports more than three billion people and represents 50 to 80 percent their daily calorie intake (Khush, 2005). In Asia, More than 90% rice is grown and consumed in world population. Rice is one of the top five major carbohydrate crops for the world's population, particularly in Asia. Rice accounts for 35–75% of the calories consumed by more than 3 billion Asians. It is cultivated around 154 million hectares annually or on about 11% of the world's cultivated land (Rajakumar, 2013).

Soil salinity is major problem in arid and semi-arid regions, where rainfall is insufficient to leach salt and excess of sodium ion down and out of root zone. In Asia, around 21.5million ha of cultivable land was affected by salinity. India is the largest rice growing country accounting for about one third of the world acreage under the crop. But this crop has been identified as salt susceptible. India had 8.6 million ha of salinity affected cultivable land (Misra and Niragidave 2013). Whereas, In Tamil Nadu, the area which affected by salinity is around 6 lakh ha. Salinity affected areas are Chengalpattu, Salem, Thanjavur, Trichy, Tirunelveli, Dharmapuri and Ramanathapuram (Vadivel *et al*., 2001). These areas are major rice belt in Tamilnadu. Salinity is one of the most serious environmental problems in the world and Salinity affects virtually all aspects of rice growth in varying degree at all stages from germination to maturity especially in seedling and flowering stages leading to yield reduction reported by Rao*et al* (2002) and Zeng *et al.* (2003). Salinity stress was imposed in reduction division stage leads to poor translocation of assimilates from the leaves to grain. The seedling and flowering stages are critically affected by the salinity through irrigation water observed by Rad *et al.* (2012). Chourey *et al*. (2003) revealed that Salinity stress triggers the expression of several osmo responsive genes and proteins in rice tissues. There has been a variation in the response of rice plant to salinity with its growth stages according to quantity and period of exposure to salt. Among the most commonly cultivated rice, young seedlings were more susceptible to salt stress (Flowers and Yeo 1981 and Lutts *et al*., 1995). Seed treatments not only improved the germination rate and time but also enhanced seedling vigor as indicated by higher leaf score, root length and seedling fresh and dry weights (Farooq *et al*., 2007). Plant hormones play an important role in alleviating salt stress and helps in creating the adaption mechanism (Harris *et al*., 2002). Foliar application of plant hormones generally stimulated the accumulation of carbohydrates leads to increase the germination percentage and related characters under salt stress reported by Noushina *et al*. (2011). Tania and Shukla (2011) reported application of Brassinosteroids help to increase the tolerant in rice under salinity stress at the germination, vegetative and reproductive growth stages. Plant endogenous cytokinin content induced the germination and growth in the rice. GA increases the germination under salinity conditions (Li and Tang, 2002). In rice (Basra *et al*., 2005) salicylic acid pre-treatment improved growth and resulted in higher resistance of plants to salinity, so that it increased germination percentage, seedling vigor index and growth parameters of the seedlings (Anwar *et al*., 2013). With this background, this research project was carried out with the objectives (i) To study the effect of salt stress on seed germination and associated traits in popular rice variety- CO 51 (ii) To improve the seed germination and related traits of popular rice variety CO51 under salinity using plant growth regulators.

MATERIALS AND METHODS

The present study was carried out with popular rice variety of rice CO 51 with the aim of improving the germination related traits under salinity stress using different plant growth regulators with varied concentrations. The research trial was carried out under controlled growth chamber conditions. The laboratory screening was done by incubating the rice variety CO 51 under salt stress along with different plant growth regulators *viz.,* gibberellic acid, salicylic acid, and kinetin. Rice variety CO-51 was obtained from the Agricultural College And Research Institute, Eachangkottai. CO-51 is a popular rice variety with medium duration cultivated all over Tamil Nadu and also sensitive to salinity. Thus, enhancing the germination associated traits of this variety would have practical utility from the farmers of point of view. Therefore, this study has been conducted. The rice variety CO-51 was used in the screening for salinity stress and application of plant growth regulators (gibberellic acid, kinetin and salicylic acid) for mitigation of salt stress. The initial germination, root length, shoot length, seedling length, vigour index and dry matter production were carried out in the laboratory in petri plates with salt solutions (120mM NaCl) used for screening the salt tolerance and application of plant growth regulators for mitigation of salt stress.

Seed Germination studies

Well filled, undamaged and uniform sized fifty CO 51 seeds were placed in petri plates lined with filter paper of 12cm diameter. The filter papers were moistened with 15ml of salt solution (120mM NaCl) and respective concentrations of different hormones along with control. Seeds of each group were then soaked for 24h. The petri plates were placed in racks to ensure optimum light and aeration to all the treatments for twelve days, after which the observations were recorded. How were the plates aerated?

Table 1. Details of different concentrations of plant hormones used in this experiment

Treatment s	Name and Concentration of different PGR
T_1	Absolute control (without stress)
T_2	Control (stress 120mM NaCl)
T_3	GA 50ppm
T_4	GA 100ppm
T_5	GA 200ppm
T_6	GA 300ppm
T_7	SA 50ppm
T_8	SA 100ppm
T_9	SA 150ppm
T_{10}	SA 200ppm
T_{11}	Kinetin 1ppm
T_{12}	Kinetin 10ppm
T_{13}	Kinetin100ppm
T_{14}	Kinetin200ppm

put the date in a table

K= kinetin
K 1 ppm

Germination percentage (%)

Already mentioned

~~Fifty seeds of CO-51 for each treatment was placed in petri plates and moistened with salt solutions and different hormones for twelve days~~. The germination was recorded on the 12th day after sowing. Number of seeds germinated was expressed as percentage under each treatment. incubation.

$$\text{Germination percentage} = \frac{\text{Number of normal germinated seedlings}}{\text{Number of seeds kept for germination}} \times 100$$

Seedling root length (cm)

CO 51

The root length of 6th and 12th day old seedling was measured from fifty seedlings in the CO51 variety from each replication and the calculated mean was expressed in cm.

Seeding shoot length (cm)

The shoot length of 6thand12th day old seedling was measured from fifty randomly seedlings from each replication and the mean was calculated and expressed in cm.

Seedling length (cm)

The seedling length was measured from tip of the primary leaf to tip of the root and the mean length was calculated and expressed as seedling length in centimeter.

Vigour Index

Vigour Index of twelve days old seedling was worked out by using the following formula given by Abdul-Baki and Anderson (1973).

Vigour Index = Germination percentage x (Root length + Shoot length)

EXPERIMENTAL RESULTS & DISCUSSIONS

Rice is the most important food crop in the world, which accounts for more than 21 % of the calories needs of the world population and up to 76 per cent of the calorific intake of the population of south East Asia. Out of 329 million hectares of cultivable land in the country, around 175 million hectares (53 per cent) is suffering from degradation in some form or the other. There are 7.61 M ha of salt affected soils in India as per the Ministry of Agriculture, GOI. In India the salt affected areas are Haryana, parts of Punjab, Rajasthan, Uttar Pradesh, Gujarat, Maharashtra, Madhya Pradesh, Karnataka, Andhra Pradesh and Tamil Nadu.

Optimization of salinity:

The rice variety CO-51 was subjected to different stress condition of salinity of 30mM, 60 mM, 90mM, 120mM, 150mM and 180mM respectively. Under these stress conditions, the seed germination and seedling characters like root length, shoot length, seedling length and dry matter production was observed on 6th and 12th day after sowing. Various parameters associated with germination also been taken and the data generated from the study are furnished in tables and the results are elaborated.

Table 2: Effect of salinity on seed germination and seedling characters under different concentrations.

Treatments	Germination percentage		Root length(cm)		Shoot Length(cm)		Fresh weight (g)	Dry weight (g)
	6 DAS	12 DAS	6DAS	12 DAS	6DAS	12 DAS	12 DAS	12DAS
Control (T_1)	90.0	94.0	3.10	4.24	2.60	5.96	2.70	0.83
30mM (T_2)	84.0	87.5	2.50	3.95	1.20	4.63	2.44	0.60
60mM (T_3)	87.0	80.0	2.60	4.28	0.86	4.33	2.46	0.59
90mM (T_4)	58.6	62.0	1.50	3.23	0.40	3.55	2.13	0.60
120mM (T_5)	50.6	54.5	1.18	2.67	0.72	3.20	2.00	0.56
150mM (T_6)	34.3	37.2	0.70	2.22	0.87	2.75	1.76	0.57
180mM (T_7)	24.0	28.0	0.20	0.98	0.20	0.81	0.70	0.26
Mean	**61.2**	**63.3**	**1.68**	**3.08**	**0.97**	**3.60**	**2.02**	**0.57**
SEd	**0.261**	**0.284**	**0.036**	**0.044**	**0.028**	**0.051**	**0.027**	**0.001**
CD @5%	**0.531**	**0.509**	**0.071**	**0.089**	**0.054**	**0.101**	**0.049**	**0.002**

it is evident that there is delayed germination by 12 DAS, and 50% germinated at 120 mM salinity (Table-2).

From the observations, ~~the optimized concentration of salinity was observed that 120Mm due to its low germination percentage. Furthermore, the experiment was carried out with most popular, recently released variety CO 51 using different concentration of various hormones for improving the germination under salinity condition. Because of the fact that salinity causes heavy reduction in germination, intervention through Plant Growth regulators would certainly improve the germination, thereby bring about practical implication in terms of farmers applications.~~

LD50 (lethal dose 50) is important which has practical implication that helps rice breeders to grow rice variety CO 50.

Various parameters associated with germination also been taken after the application of plant growth regulators under salinity (Table-2) and the data generated from the study are furnished in tables and the results are elaborated under appropriate headings.

Germination percentage (%)

Under salt stress irrespective of concentration (Table-2), the germination percentage at 6th and 12th days after sowing was reduced. In gibberellic acid, the germination percentage ranged from 70.00 and 78.20 in T_6 to 84.00 and 91.00 percent in T_3 at 6th and 12th day after sowing respectively. In salicylic acid germination percentage ranged from72.00 and 77.00 in T_{10} to 86.00 and 88.00 percent in T_7 at 6th and 12th day after sowing. However, in kinetin germination percentage ranged from 63.00 and 65.00 in $T_{11}$to 86.00 and 87.00 percent in T_{14} at 6th and 12th day after sowing respectively.

Seedling root length (cm)

Among the treatments, salicylic acid (2.70cm) in T_7recorded the higher mean seedling root length followed by gibberellic acid (2.60cm) in $T_4$and the lower mean seedling root length wasobservedinkinetin(1.78cm)inT_{12}at6thdaysaftersowing.While,in12thdaysaftersowing registered higher value (3.45cm) in salicylic acid of $T_7$and the lower value (3.30cm) in $T_4$of gibberellic acid.

Seedling shoot length (cm)

The shoot length showed differential responses to plant hormones under salinity treatments. Among the plant hormones the higher shoot length recorded in gibberellic acid (6.30cminT_6) followed salicylic acid(4.76cminT_7) and in kinetin (3.30 in T_4) at 12th days after sowing when compare to stress (3.02 cm)

Seedling length (cm)

In all treatment under salt stress the seedling length at 6th and 12thdays after sowing was reduced. The higher seedling length of 9.36cm was found in GA (T_6) a scompare to stress, while the higher seedling length of 8.00cm was found in SA (T_7) and the higher seedling length of 5.26cm in kinetin (T_{11}).

Vigour Index

Vigour Index was the product of germination percentage and seedling height. The analysis on the effect of salinity stress on vigour index with different concentrations of different hormones levels at 6th and 12th days after sowing. Vigour index at 6th and 12thdays after sowing was high in absolute control (576.6 and 1008.8) but in stress was very low (196.35 and 315.0).

The vigour index ranges from 627.9 to 785.8 in GA, while the GA shows lower vigour index of 627.90 (T_3) and the higher vigour index of 785.5 (T5). For SA, the vigour index ranges from 611.38 to 704.00, while SA shows the lower vigour index of 611.38 (T10) and the higher vigour index of 704.00 (T_7). For kinetin, the vigour index ranges from 287.82 to 415.54, while the lower vigour index of 287.82 (T14) and the higher vigour index of 415.54 (T_{12}).(Table-2)

Table 3. Effect of gibberellic acid, salicylic acid and kinetin on Germination (%) and Seedling root length (cm) under 120Mm of NaCl

S.No	Treatments	Germination percentage (%)	Seedling root length(cm)

		6 DAS	12 DAS	Mean	6 DAS	12 DAS	Mean
1.	Absolute control (without stress) (T_1)	95.00	97.00	**96.0**	2.98	3.86	**3.42**
2.	Control (120mM NaCl stress) (T_2)	51.00	63.00	**57.0**	1.50	1.98	**1.74**
3.	GA 50ppm (T_3)	84.00	91.00	**87.5**	2.30	3.18	**2.74**
4.	GA 100ppm (T_4)	82.30	90.20	**86.0**	2.60	3.30	**2.95**
5.	GA 150ppm (T_5)	72.40	89.30	**80.8**	2.05	2.52	**2.28**
6.	GA 200ppm (T_6)	70.00	78.20	**74.1**	2.03	3.06	**2.54**
7.	SA 50ppm (T_7)	86.00	88.00	**87.0**	3.20	3.20	**3.20**
8.	SA 100ppm (T_8)	72.00	83.00	**77.5**	2.77	2.77	**2.77**
9.	SA 150ppm (T_9)	83.00	87.00	**85.0**	2.92	2.92	**2.92**
10.	SA 200ppm (T_{10})	72.00	77.00	**74.5**	3.45	3.45	**3.45**
11.	Kinetin 1ppm (T_{11})	63.00	69.00	**66.0**	1.56	1.69	**1.62**
12.	Kinetin 10 ppm (T_{12})	72.00	79.00	**75.5**	1.78	1.96	**1.87**
13.	Kinetin 100 ppm (T_{13})	79.00	84.00	**81.5**	0.70	0.94	**0.82**
14.	Kinetin 200ppm (T_{14})	76.00	82.00	**79.0**	0.52	0.86	**0.69**
	SEd	**0.186**	**0.269**		**0.086**	**0.091**	
	CD@5%	**0.507**	**0.762**		**0.124**	**0.135**	

Table 4. Effect of gibberellic acid, salicylic acid and kinetin on Seedling shoot length (cm) and Seedling length (cm) under 120mM of NaCl

S.NO	Treatments	Seedling shoot length(cm)			Seedling length(cm)		
		6 DAS	12 DAS	Mean	6 DAS	12 DAS	Mean
1.	Absolute control (without stress) (T_1)	3.09	6.54	**4.81**	6.07	10.40	**8.23**
2.	Control (120mM NaCl stress) (T_2)	2.35	3.02	**2.68**	3.85	5.00	**4.45**
3.	GA 50ppm (T_3)	2.46	3.72	**3.09**	4.76	6.90	**5.83**
4.	GA 100ppm (T_4)	2.12	5.14	**3.63**	4.72	8.44	**6.58**
5.	GA 150ppm (T_5)	2.23	6.28	**4.25**	4.28	8.80	**6.54**
6.	GA 200ppm (T_6)	2.87	6.30	**4.58**	4.90	9.36	**7.13**
7.	SA 50ppm (T_7)	1.12	4.80	**2.96**	3.82	8.00	**5.91**
8.	SA 100ppm (T_8)	1.10	4.76	**2.93**	3.51	7.53	**5.52**
9.	SA 150ppm (T_9)	0.87	4.67	**2.77**	3.37	7.59	**5.48**
10.	SA 200ppm (T_{10})	0.96	4.49	**2.72**	3.50	7.94	**5.72**
11.	Kinetin 1ppm (T_{11})	1.62	2.96	**2.29**	3.18	5.26	**4.22**
12.	Kinetin 10 ppm (T_{12})	0.76	3.30	**2.03**	2.54	4.65	**3.59**
13.	Kinetin 100 ppm (T_{13})	1.08	3.18	**4.81**	1.78	4.12	**2.95**
14.	Kinetin 200ppm (T_{14})	1.60	2.65	**2.68**	2.12	3.51	**2.81**
	SEd	**0.097**	**0.106**		**0.101**	**0.129**	
	CD@5%	**0.213**	**0.241**		**0.225**	**0.282**	

Table 5. Effect of gibberellic acid, salicylic acid and kinetin on Seedling vigour and dry matter production (g) under salinity 120 mM of NaCl.

S.NO.	Treatments	Seedling vigour		Mean	Dry matter production (g)
		6 DAS	12 DAS		12 DAS
1.	Absolute control (without stress)(T_1)	576.60	1008.8	**792.7**	0.86
2.	Control (120mM NaCl stress) (T_2)	196.30	315.00	**255.6**	0.38
3.	GA 50ppm (T_3)	399.80	627.90	**513.8**	0.57
4.	GA100ppm (T_4)	388.40	761.20	**574.8**	0.51
5.	GA 150ppm (T5)	309.80	785.80	**547.8**	0.29
6.	GA 200ppm (T6)	343.00	731.90	**537.4**	0.46
7.	SA 50ppm (T7)	328.50	704.00	**516.2**	0.30
8.	SA 100ppm (T8)	252.72	624.90	**438.8**	0.45
9.	SA 150ppm (T9)	279.71	660.33	**470.0**	0.32
10.	SA 200ppm (T10)	226.80	611.38	**419.0**	0.42
11.	Kinetin1ppm (T11)	200.34	320.85	**260.5**	0.32
12.	Kinetin10 ppm (T12)	182.88	415.54	**299.2**	0.35
13.	Kinetin100 ppm (T13)	140.62	346.08	**243.3**	0.33
14.	Kinetin 200ppm (T14)	161.12	287.82	**224.4**	0.24
	SEd	**4.978**	**6.734**		**0.024**
	CD@5%	**11.312**	**13.412**		**0.031**

CO 51

In the laboratory experiment, the performance of popular rice variety CO51was evaluated by germination studies at room temperature using saline solution with four different plant hormones of different concentrations for mitigation of salinity. Various physiological traits associated with germination were used to assess the performance of treatments stress and the outcome of the findings has been discussed in this chapter.

Germination percentage (%)

The germination percentage was studied in salt susceptible popular rice variety CO 51. It was grown under salt stress with different concentration of plant hormones in the growing medium. Considering the performance of the different hormones,T_3 of gibberellic acid (GA) recorded maximum values for germination percentage under salinity, it is considered as best hormone under salinity. Plant hormones are considered as key regulators to seed germination and development (Davies, 1987). Gibberellic acid (GA) is well known to induce the synthesis of A- amylase and hydrolysis of starch in rice seeds (Palmiano and Juliano, 1972). These observations support the results obtained in the present study as the increasing in germination percentage under salt stress with plant hormones could be a representation to select a genotype for better mitigation for salinity stress. Salinity stress had three fold effects which reduced water potential, causing ion imbalance and disturbances in ion homeostasis and toxicity as reported by Hajer *et al*. (2006). The reason for the decrease in germination percentage may be attributed to decreased water imbibitions by seeds caused by lowered water potential of the growing medium, as pointed out by Bliss *et al*. (1986) and Poljak off - Mayber *et al*. (1994).

Seedling root length (cm)

The adverse effects of salt stresses on germination per cent also reflected on shoot and root growth of rice seedling. Application of plant hormones under salt stress condition the seedling root length was higher in salicylic acid of (T_{10}) at 12th day after sowing. Some of the similar findings observed by Gini *et al*. (2017).

Seedling shoot length (cm)

Salinity decreased germination percent, root length, callus size, coleoptile length, seedling growth, plant height, and dry weight decreased with increasing levels of salinity in rice reported by Tania and Shukla 2011. In this current study, higher shoot length was recorded in gibberellic acid treatment (T_{10}) at 12th day after sowing.

Seedling length (cm)

The shoot length and root length of the rice seedlings grown in salt solutions declined indicating that the salinity stress not only affected germination but also the seedling height. Maximum seedling height was observed in treatment T_6 of gibberellic acid. Some of the similar findings observed by Ruan *et al*. (2002); Mathew and Mohanasarida, 2005 and Anwar *et al.* (2013) in rice.

Vigour index

Previous work done by Poljakoff-Mayber *et al*. (1994) in the herb, Seashore Mallow reported that the selection of genotypes merely based on the seedling germination and height may be deceptive. Hence, the parameters *viz*., Vigour Index under salt stress was taken in to consideration for categorizing the best plant hormones and best concentration of each hormone treatment under salt tolerant in rice. In the present study was T_5 of gibberellic acid in 12th day after sowing was higher in vigour index.

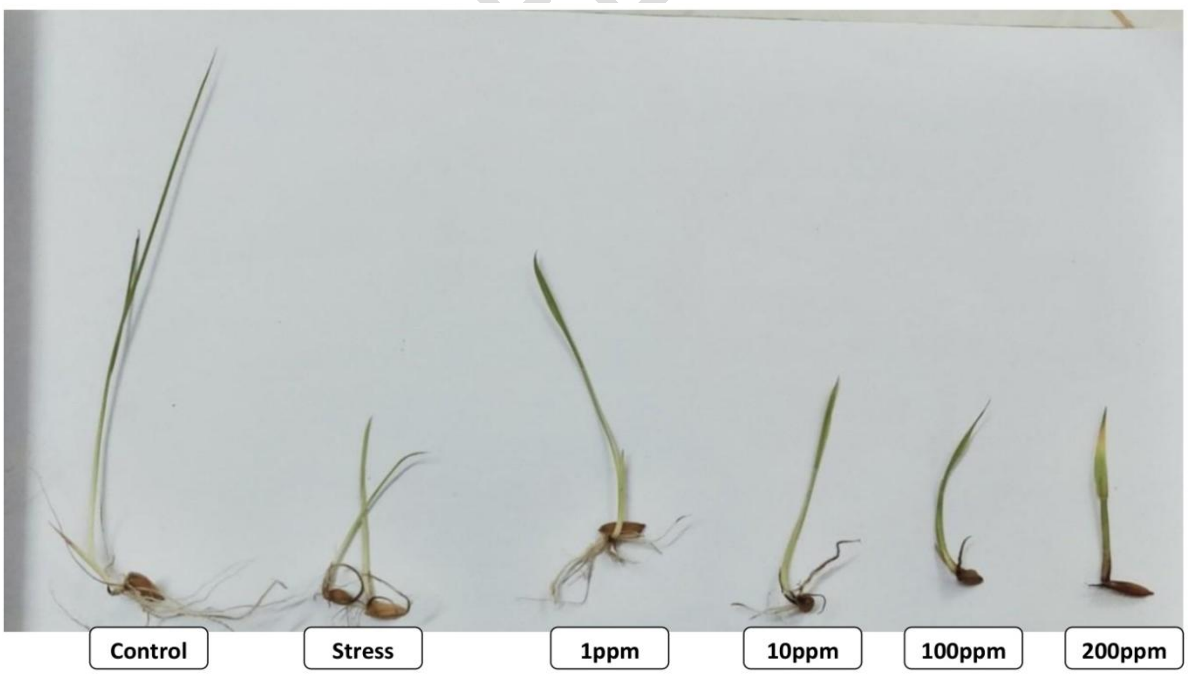
Figure 1. Observation on 12th day under control and different concentration of salinity on

variety CO-51

Figure 2. Observation on 12thday under salinity with treatment of different concentrations of Gibberellic acid on variety CO-51

Figure 3. Observation on 12thday under salinity with treatment of different concentrations of Salicylic acid on variety CO-51

Figure 4. Observation on 12th day under salinity with treatment of different concentrations of Kinetin on variety CO-51



COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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