Effect of seed priming with plant growth regulators on growth parameters, biochemical changes of rice (Oryza sativa L.)

ABSTRACT

The investigation entitled **"Effect of seed priming with plant growth regulator on growth parameters, biochemical changes of rice (*Oryza sativa* L.)"** was conducted during *kharif* season, 2018 at Student Instructional Farm (SIF) of Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya-224 229 (U.P.) in randomized block design with eight treatments and three replications on rice variety Sarju-52.The treatments were comprised of priming with plant growth regulators of different concentrations *viz* salicylic acid (50ppm, 100ppm and 150ppm), KNO_3 (1%, 1.5% and 2%) along with unprimed control and hydro-priming. Seed priming was done for 12 hours in different treatments and nursery was raised. Twenty one days old seedlings were transplanted. Observations were recorded on various growth parameters at 30, 60, 90 DAT such as leaf area index (LAI), relative water content (RWC), biochemical parameters like chlorophyll content (SPAD Value), catalase activity (EU mg^{-1} protein min^{-1}), total soluble sugar (mg g^{-1} dry weight), at harvest stages. All the plant growth regulators *viz* salicylic acid (50ppm, 100ppm and 150ppm) and KNO_3 (1%, 1.5% and 2%) influenced positively on the growth characters, chlorophyll content, & yield content in rice crop. Seed priming of KNO_3 @ 2% was found superior followed by salicylic acid 150ppm over rest of the treatments.

Keywords: Rice, seed priming, KNO_3 solution, ppm,

1. Introduction

Rice (*Oryza sativa* L.) is the most important cereal *kharif* season crop as it is a staple food of more than 70 percent of world population. That is why the rice production always holds a key role in the overall food situation of the whole world. Rice is a chief source of carbohydrate. Rice belongs to genus *Oryza* of Gramineae family. The genus *Oryza* includes 24 species, of which 22 wild and two are namely *Oryza sativa* and *Oryza glaberrima* are cultivated. All the varieties found in West Africa belong to species *O. glaberrima. Oryza sativa* is a diploid species having 24 chromosomes. The *Oryza* species of the world are commonly grouped into three sub-species namely *indica*, *japonica* and *javanica.* According to advance estimate, rice is cultivated in 43.57 million hectare which is with a production of 104.32 million tonnes and productivity is 2550kg/ha of our country and state production of rice is 12.95 million tonnes and area is 5.65 million hectare **(Anonymous, 2017-18).** Seed priming was proposed by Heydecker in 1973. Seed priming is an effective technology to enhance rapid and uniformed emergence and to achieve high vigour, leading to better stand establishment and yield **(Harris *et al*., 2007) .** Seed priming by using different chemical solutions (Osmo-priming) has made some considerable effect on plant growth. Seed priming has been successfully demonstrated to improve germination, seed emergence, uniform and vigorous crop stand in seeds of many field crops, such as vegetables and small seeded grasses, wheat, sugar beet, maize, soybean and sunflower **(Parera & Cantliffe, 1994).** Salicylic acid is a natural compound that plays a central role in certain physiological processes and defense responses in plants **(Shi and Zhu, 2008).** Potassium nitrate has a chemical formula of KNO_3. It is an ionic salt of potassium ions K^+ and nitrate ions

NO^{3-} and is, therefore, an alkali metal nitrate. It is a natural source of nitrate and has been used as a constituent for several different purposes, including food preservatives, fertilizers etc. Priming changes the interior condition of the embryo also. In here it has reported that KNO_3 priming increased the embryo length in tetraploid watermelon seeds **(Nerson *et al*., 1985).**

2. Material and methods

This work was conducted at Student Instructional farm (SIF) of Achraya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya during kharif season 2018. The work was laid down in randomized block design with three replications. The treatments were comprised of priming with plant growth regulators of different concentrations *viz* salicylic acid (50ppm, 100ppm and 150ppm), KNO_3 (1%, 1.5% and 2%) along with unprimed control and hydro-priming. Seed priming was done for 12 hours in different treatments and nursery was raised. Twenty one days old seedlings were transplanted.

2.1 Plant leaf area

The total number of leaves of two hills was divided into three groups and measured the length and broadest width of each group. The leaf area was calculated by multiplying this area to number of leaves in each group and a factor. This factor is determined on the basis of actual area measured by leaf area meter and recorded by above method. After calculating total leaf area it was divided by ground area in order to get leaf area index. The LAI was recorded three times during the course of investigation of crop at 30, 60 and 90 DAT and then averaged.

2.2 Relative water content (%)

The relative water content (RWC) was determined by the method described by **Turner and beg (1981)**. Leaf discs were cut from the leaves, weighed and saturated by floating on distilled water in petridish for six hours. The discs were surface dried and weighed. After that discs were kept in oven at 75 ± 5 0 C for 24 hours. After drying, weight of discs was calculated with the help of electronic balance.

2.3 Chlorophyll content (SPAD value)

Chlorophyll content of leaf was directly measured from intact leaves microprocessor based plant efficiency analyzer model: **X55/M-PEA.** The values were expressed as SPAD Value.Plant efficiency analyzer can measure three parameters (chlorophyll, nitrogen and leaf temperature) at the same time.

2.4 Total soluble sugar content (mg g^{-1} dry weight)

The total soluble sugar content in shoot was estimated by the method of **Yemm and Wills (1954).**

Reagents:

(1) Ethanol (80%)

(2) Anthrone (0.2%)

(3) Glucose

Anthrone reagent: 200 mg Anthrone in 100 ml concentrated H_2SO_4

100 mg dried plant sample was homogenized in 10 ml of 80% ethanol centrifuged at 4000 rpm for 20 minutes. Supernatant was collected and residue was re-extracted with 10 ml of 80% ethanol and again centrifuged at 4000 rpm for 20 minutes. Supernatant was collected and both the supernatants were combined and made up to final volume of 20 ml. 0.1-0.2 ml supernatant was dried in a test tube on water bath and cooled to room temperature. One ml of distilled water was added to each test tube and mixed thoroughly. Four ml of anthrone reagent was added along the wall of the test tube and mixed gently and heated on water a bath at 100 0 C for 10 minutes and cooled rapidly under running cold water. Absorbance was measured at 620 nm against the reagent blank. The amount of total carbohydrate present in the extract was calculated using a standard curve prepared from graded concentration of glucose.

2.5 Catalase activity (unit g^{-1} fresh weight min^{-1})

Catalase activity was assayed calorimetrically according to method given in analytical biochemistry (**Sinha, 1972**). Catalase facilitates the dismutation of H_2O_2 to water and O_2 according to the reaction. The enzyme plays an important role in association with SOD as well in photorespiration and glycolate pathway.

Reagents:

I. Phosphate buffer, 0.1 M, pH 7.0
II. II. Potassium dichromate acetic acid (5% potassium dichromate glacial acetic acid in 1:3 ratio)
III. III. H_2O_2 0.2 M

200 mg of fresh leaves material was homogenized with 10 ml of phosphate buffer 0.1 M (pH 7.0) and centrifuged at 10000 rpm for 30 minutes at 4 0 C.

Reaction mixture:

Test	Blank	Reagents
1.25 ml	-	H_2O_2
0.50 ml	0.50 ml	Enzyme extract
3.25 ml	4.50 ml	Phosphate buffer
5.00 ml	5.00 ml	

The reaction mixture was taken in Erlenmeyer flask and mixed rapidly at 37 0 C. At 3 minutes interval, 2.0 ml of reaction mixture was withdrawn in test tube and to it, 2.0 ml potassium dichromate acetic acid solution was added and kept on water bath for 10 minutes and colour intensity was measured after cooling at 570 nm on spectronic . Results were expressed as enzyme unit's $gram^{-1}$ fresh weight min^{-1}

3. Result and discussion

3.1 Leaf area index:

Data with respect to leaf area index as affected by different treatment of seed priming was recorded at different growth stages have been presented in table -1 and Fig 1. The effect of KNO_3 @ 2% and salicylic acid @150ppm was found more pronounced and both the treatments registered significant increase in leaf area index as compared to untreated control at all the stages of observation. The maximum increased in leaf area index was recorded with KNO_3 @ 2% (4.44, 5.28, 5.15 at 30, 60, 90 DAT respectively), followed by salicylic acid @150ppm treatment. However, the minimum leaf area index was (2.23, 2.39, 2.31 at 30, 60, and 90 DAT respectively) was recorded with hydro Priming against control. **Sujatha (2001)** reported that foliar application of salicylic acid (100ppm) on green gram at 75 DAS increased the plant height (50.4 cm), root length (16.9 cm), number of leaves (18.4) and LAI (1.30).These results are in accordance with the findings of **Pour *et al.*** (2012**)**, **Anisa *et al.*** *(*2017), in rice.

3.2 Relative water content (RWC) %

The examination of the data presented in table 2 and Fig- 2 revealed that all the treatments significantly increased in relative water content in leaves at all the stages of the observation with respect to control except hydro-priming. The maximum relative water content (%) was analyzed with KNO_3 @ 2%, seed priming, (85.48, 82.56, 76.12 at 30, 60, 90, DAT respectively) followed by salicylic acid @150ppm over rest of the treatments. At 30, 90 DAT salicylic acid @ 150ppm was found at par with KNO_3 @ 2% treatment. However, minimum relative water content was recorded with hydro Priming over control. The value of RWC decreasing trends were observed with increasing the crop age. **Yildirim *et al*. (2008)** reported that ameliorative effect of salicylic acid might be linked to the observable increase in WC, RWC and photosynthetic pigments as well as, leaf area consequently the efficiency of the photosynthetic apparatus was increased due to salicylic acid treatments. Similar finding have been reported by **Pour *et al.* (2012)** in fenugreek.

3.3 Total chlorophyll content in leaves (SPAD Value)

The data presented in Table-3 and Fig-3 clearly indicate that in general, chlorophyll content increased up to 60 DAT after that decline trend was recorded in primed as well as unprimed (control). All the seed priming treatments improved chlorophyll content in leaf at each stage of observation in comparison to unprimed (control). However, the effect of KNO_3 @ 2% and salicylic acid @ 150 ppm was found most promising and both the treatments registered significant increase in chlorophyll content in leaf as compared to unprimed control at all the stages of observation. The higher chlorophyll content was recorded with seed priming of KNO_3 @ 2% (7.11, 11.38 and 9.78 SPAD value at 30, 60 and 90 DAT, respectively) followed by salicylic acid @ 150ppm (6.48, 10.34, 8.66 SPAD value) over rest of the treatments. The minimum chlorophyll content was recorded with hydro-Priming (4.54, 8.58, 6.82 at 30, 60, and 90 DAT respectively) against control. These results are in accordance with the findings of **EI-Tayed (2005)** in barley, **Afzal (2011)** in tomato.

3.4 Total soluble sugar (mg g^{-1} dry weight)

It is evident from the data presented in Table 4 and Fig- 4 that all the seed priming statistically significant improved of total soluble sugar content in dry leaf up to 60 DAT and after that decline trend was recorded. Maximum increase in total soluble sugar content was recorded in

case of KNO_3 @ 2% seed priming at 30, 60, 90 DAT and harvest stages (136.81, 280.31, 250.51, and 239.36 mg g^{-1} respectively) followed by salicylic acid @ 150ppm over other treatments. At 60 and harvest stages DAT salicylic acid @150ppm was found at par with KNO_3 @ 2% treatment. While, minimum total soluble sugar was recorded with hydro priming as compare to control. Increase in total soluble sugar by salicylic acid have been reported by **Bharti** and **Malik (2013)**, in mustard, **Azooz (2004)**, in barley, Dashtmain *et al*.,(2014), in rice.

3.5 Catalase activity (EU mg^{-1} protein min^{-1})

The data given in Table-5 and Fig- 5 clearly indicate that in general, leaf catalase content increased up to 90 DAT was recorded in primed as well as control. All the levels and primed seed significantly improved the catalase activity content in leaf at all the stages of observation as compared to control. The maximum increase catalase activity in leaf was registered with @ 2% KNO_3 seed priming at 30, 60, 90 DAT (247.16, 382.58, 413.61 catalase activity, respectively) followed by salicylic acid @ 150ppm over rest of treatments. However, minimum value was recorded with hydro priming against control. Similar findings have also been reported **Chen *et. al.* (1993, 1997)** in rice, **Tulio S. Lara *et al,*** (2014) in Tomato.

Table-1: Effect of seed priming on leaf area index of rice at different growth duration stages

Treatments	**Leaf area index(LAI)**		
	30 DAT	**60 DAT**	**90 DAT**
T_1 Control	1.78	2.21	2.17
T_2 Hydro Priming	2.23	2.39	2.31
T_3 SA-50ppm	2.69	2.91	2.85
T_4 SA-100ppm	2.89	3.23	3.11
T_5 SA- 150ppm	4.08	4.78	4.63
T_6 KNO_3- 1%	3.38	3.48	3.42
T_7 KNO_3- 1.5%	3.48	3.90	3.85
T_8 KNO_3 -2 %	4.44	5.28	5.15
SEm+_	**0.11**	**0.12**	**0.12**
CD at (5%)	**0.34**	**0.35**	**0.34**

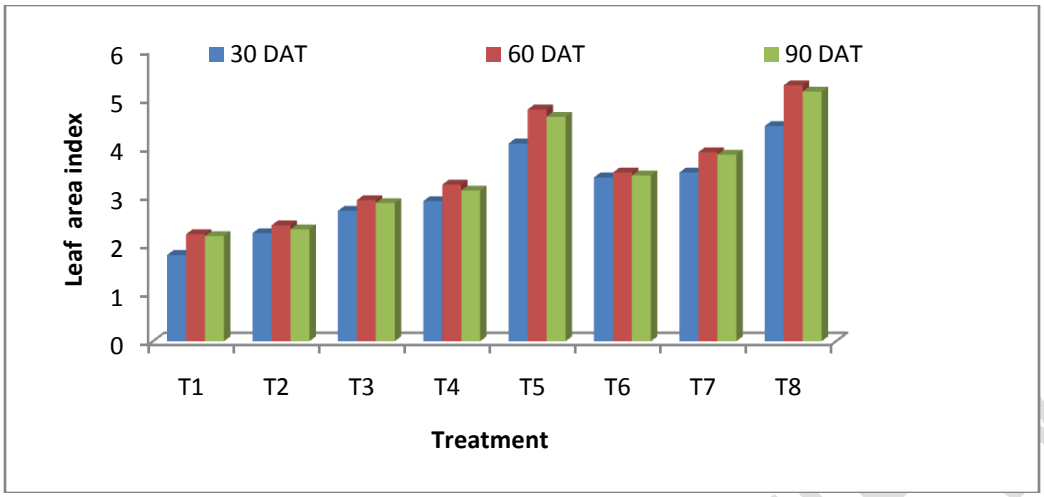

Fig-1: Effect of seed priming on leaf area index of rice at different growth duration stages

Table- 2: Effect of seed priming on relative water content of rice at different growth duration stages

Treatments	Relative water content (%)		
	30 DAT	**60 DAT**	**90 DAT**
T_1 Control	79.81	75.88	69.90
T_2 Hydro priming	80.12	76.66	70.46
T_3 SA-50ppm	80.32	77.54	71.22
T_4 SA-100ppm	81.83	78.91	72.45
T_5 SA-150ppm	85.02	81.60	75.35
T_6 KNO_3-1%	82.43	79.31	74.35
T_7 KNO_3-1.5%	83.45	80.42	73.43
T_8 KNO_3-2%	85.48	82.56	76.12
SEm+_	**0.15**	**0.29**	**0.25**
CD at (5%)	**0.46**	**0.89**	**0.77**

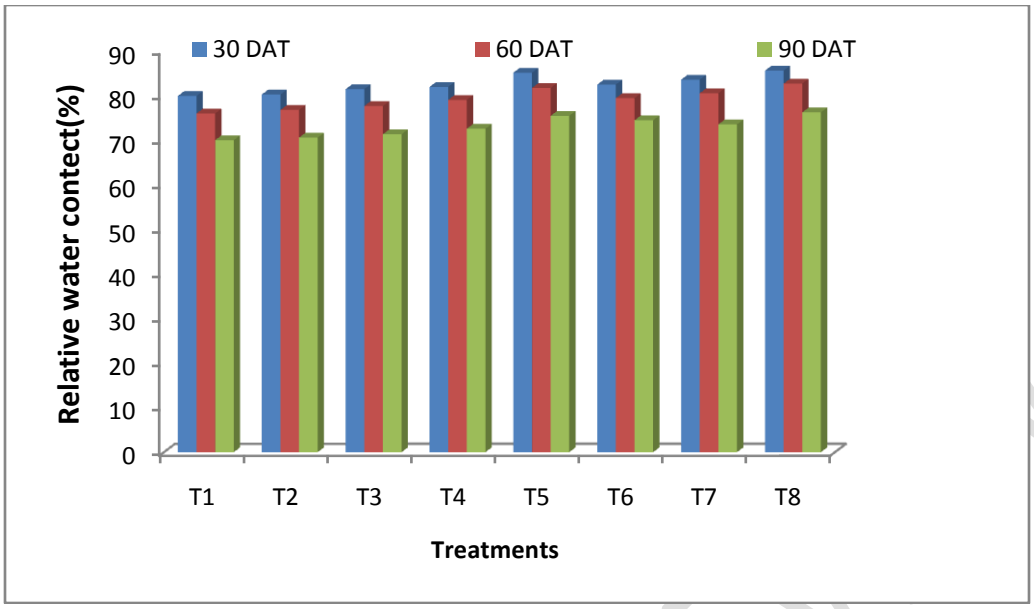

Fig.- 2: Effect of seed priming on relative water content of rice at different growth duration stages

Table 3: Effect of seed priming on chlorophyll content (SPAD Value) of rice at different growth duration stages

Treatments	Chlorophyll content (SPAD Value)		
	30 DAT	**60 DAT**	**90 DAT**
T_1 Control	4.39	8.12	6.69
T_2 Hydro Priming	4.54	8.58	6.82
T_3 SA-50ppm	5.18	9.73	7.91
T_4 SA-100ppm	5.37	9.85	8.15
T_5 SA-150ppm	6.48	10.34	8.66
T_6 KNO_3-1%	5.24	9.96	7.73
T_7 KNO_3 -1.5%	5.97	10.14	8.27
T_8 KNO_3- 2%	7.11	11.38	9.78
Sem+_	**0.21**	**0.21**	**0.33**
CD at (5%)	**0.64**	**0.63**	**1.01**

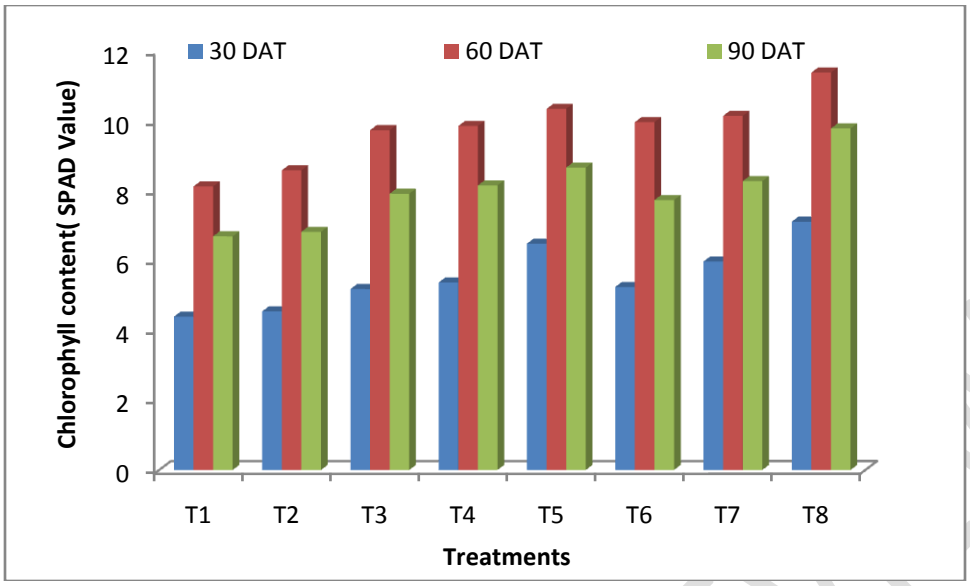

Fig. 3. Effect of seed priming on chlorophyll content (SPAD Value) of rice at different growth duration stages

Table-4: Effect of seed priming on total soluble sugar content of rice at different growth duration stages

Treatments	**Total soluble sugar content (mg g^{-1} dry weight)**			
	30 DAT	**60 DAT**	**90 DAT**	**At harvest**
T_1 Control	122.81	265.11	236.32	224.73
T_2 Hydro Priming	123.71	267.81	238.82	225.84
T_3 SA-50ppm	125.60	270.12	241.79	229.15
T_4 SA-100ppm	130.80	274.32	245.13	235.74
T_5 SA-150ppm	136.33	279.80	249.43	238.99
T_6 KNO_3-1%	133.80	275.15	246.01	236.28
T_7 KNO_3-1.5%	134.70	277.12	248.37	237.66
T_8 KNO_3-2%	136.81	280.31	250.51	239.36
SEm+_	**0.36**	**0.33**	**0.33**	**0.26**
CD at (5%)	**1.09**	**1.02**	**1.00**	**0.80**

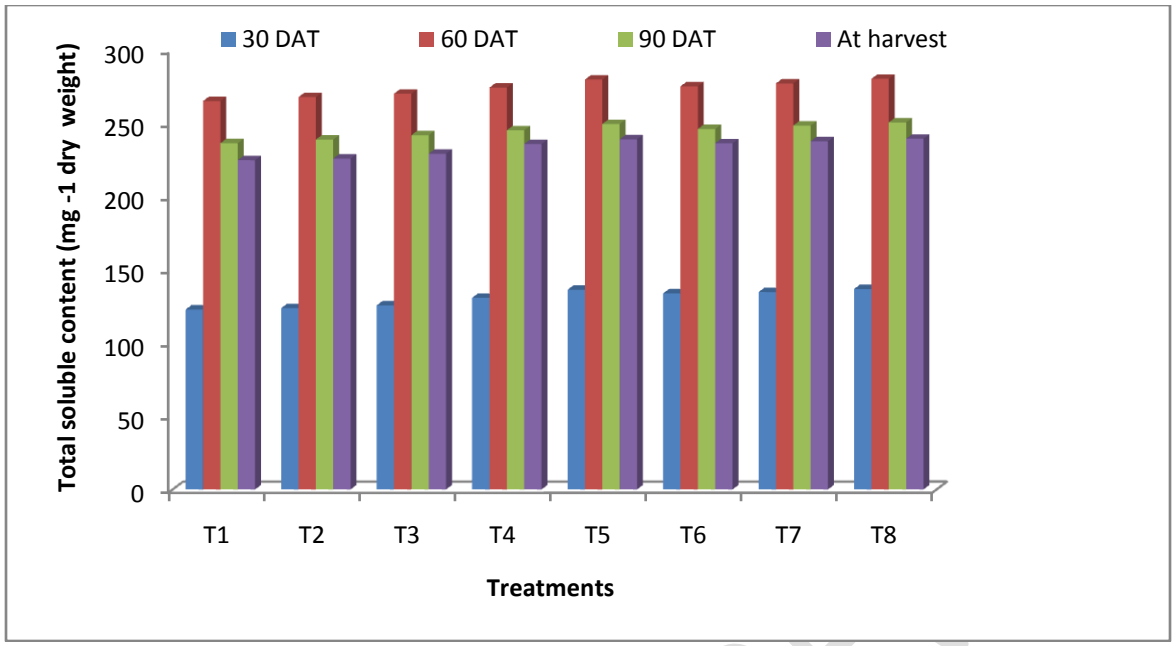

Fig-4: Effect of seed priming on total soluble sugar content of rice at different growth duration stages

Table-5: Effect of seed priming on catalase activity (EU mg^{-1} protein min^{-1}) of rice at different growth duration stages

Treatments	Catalase activity (EU mg^{-1} protein min^{-1})		
	30 DAT	**60 DAT**	**90 DAT**
T_1 Control	159.80	298.50	389.67
T_2 Hydro Priming	161.66	311.54	398.50
T_3 SA-50ppm	192.57	321.07	399.64
T_4 SA-100ppm	212.74	332.34	401.29
T_5 SA-150ppm	245.47	381.53	412.78
T_6 KNO3-1%	222.29	352.72	404.34
T_7 KNO3-1.5%	233.58	361.59	405.69
T_8 KNO3-2%	247.16	382.58	413.61
SEm+_	**0.76**	**0.44**	**0.27**
CD at (5%)	**2.30**	**1.35**	**0.81**

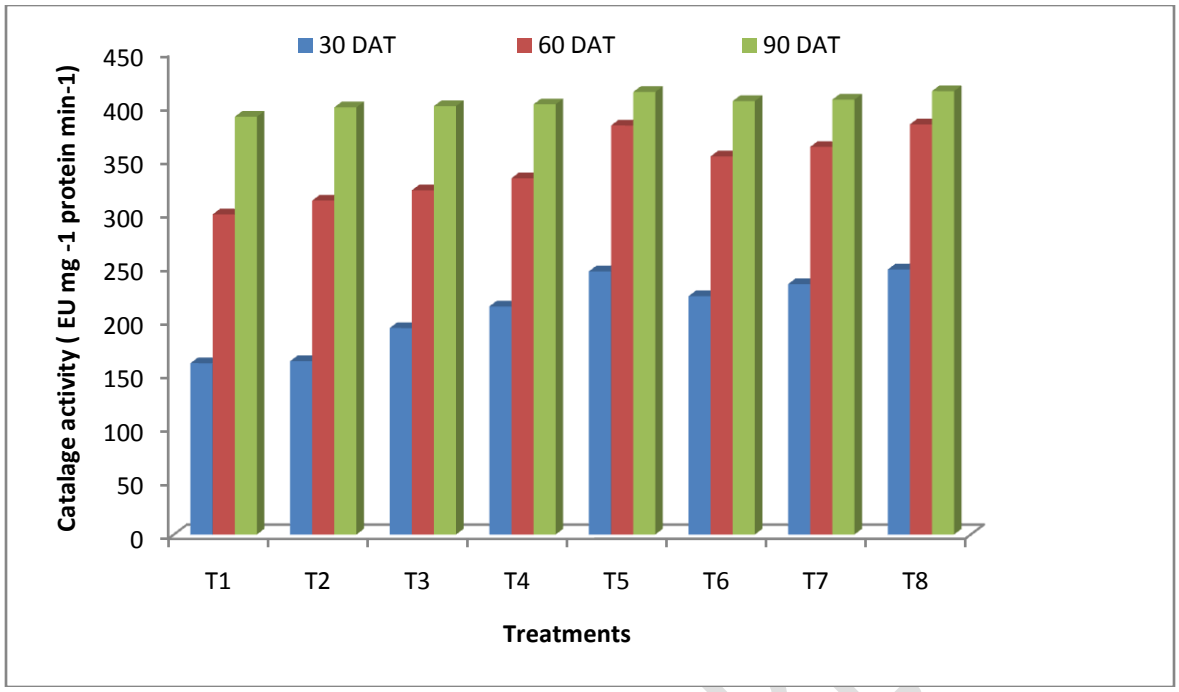

Fig-5: Effect of seed priming on catalase activity (EU mg^{-1} protein min^{-1}) of rice at different growth duration stages

4. Conclusion

The seed priming with KNO_3 and salicylic @150ppm was found most promising and both the treatment registered significant increase in chlorophyll content, total soluble sugar content and activity of antioxidant enzyme.The various seed priming sources (KNO_3 and salicylic acid) improved the physiological efficiency of crop which resulted in better growth and yield of rice. Seed priming in salicylic acid @ 150ppm and KNO_3 @ 2% may also be used as a potential tool to enhance 12-16 % yield of rice.

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