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Short Communication

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Effect of Various Concentrations of Kinetin on Seed Germination of Rice

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ABSTRACT

In the present investigation rice genotypes HUBR 10-9 and HUR 105 were selected to see the effect of hydro and hormonal seed priming on germination of rice. Seeds of selected genotypes were primed with distilled water (hydro) and six different concentrations of kinetin (hormonal). Different germination parameters, such as germination percentage, vigor index, α amylase activity, soluble and insoluble sugar content were measured at different study hours during germination. Among the various used concentrations 5 ppm of Kinetin was performed best except insoluble sugar in respect to other concentrations as well as hydro primed and non primed control sets in both the varieties.

Key words: *Seed priming; Germination percentage; α Amylase activity.*

INTRODUCTION

Biologically**,** seed is the mature ovule that consists of an embryo and stores food materials for germination and contains a protective covering (seed coat) or it can also be defined as a seed is a small embryonic plant enclosed with in a protecting covering called seed coat along with endosperm[10]. Seeds plays very important role in input technology in Agriculture sector due to its easy handling and transporting from one place to another place. Therefore, in agriculture seeds hold the key position. Further the quality of seeds is a determinant factor for the yield of particular crop. Seed has to cope up with number of environmental condition during germination which itself shows variability of soil and atmosphere both. The plant scientist on the basis of full knowledge are successful to develop a number of stress tolerance /resistant variety of important staple crops like rice, wheat, maize, legume and vegetable via using biotechnological tools. However in search of easily handable technology the scientists develop new technologies those are reliable and used towards increasing the productivity of various crops. In this connection the seed priming technology is an easy, acceptable one which can be used as climatic resilient technology. It is noted that often the rice grower failed to sowing of rice seed in time in nursery bed due to late monsoon or unavailability of proper field due to limited immigration facility.

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Good germination and emergence are important for achieving good crop establishment and maximum possible plant populations in the field, more so under adverse growing conditions. As such, speed of germination and emergence is important for successful establishment of seedling[14]. Seed priming is an age old practice, practiced eons ago by Greeks. Theophrastus (372-287 BC) had recommended presoaking of cucumber seeds in milk or water to make them to germinate earlier and vigorously. The word was coined by Heydecker in 1973 for the soaking drying seed treatments. Later, Heydecker[15] successfully used seed priming to improve germination and emergence under stressful conditions. This technique is a treatment applied prior to sowing in a specific environment wherein seeds are partially hydrated to a point the initiation process of germination without visible symptoms of radical emergence[7,9,17,12]. Its purpose is to partially hydrate the seeds to a point where germination processes are initiated but not completed[15]. Primed seeds exhibit rapid germination and emergence under field conditions[20,3]. Hence on the basis of above in present investigation effects of priming of seed, with distilled water(hydro), kinetin (PGR), were seen on rice varieties, namely HUBR 10-9 and HUR-105(both are selected one) by analyzing the germination related physiological and biochemical parameters.

MATERIAL AND METHODS

The seeds of rice varieties HUBR 10-9 and HUR 105 were procured from the department of Genetics and Plant Breeding. Seeds of uniform size were selected and surface sterilized before using for the experiment. They were first washed with tap water for 7 minutes and then sterilized with $HgCl_2$ (0.1%) for 5 minutes and then washed with sterile distilled water for 4 to 5 times. The sterilized seeds were primed by immersing the seeds in distilled water and different concentrations of (1.5, 2.5, 5, 10, 15 and 20 ppm represented C_1, C_2, C_3, C_4, C_5 and C_6) Kinetin for 18 h. After 18 h these seeds were air dried under fan to bring back to its original moisture content further the seeds were kept in paper packets and used within one month.

The germination study was carried out in the seed priming Laboratory of Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi Germination studies were carried out using Petri dish technique. Petri dishes of 3.0 inches diameter were used to conduct germination studies. They were washed well, first with water and then with alcohol, oven dried and autoclaved. Germination paper was fit in petri dishes with help of scissor and autoclaved. The lower side of the germination paper was provided with a thin layer of cotton and then placed in Petri dishes. The air dried seeds of hydro/kinetin primed were then placed equidistantly (20 seeds/Petri dish) in each petri dish. 5mL DW was poured in each petri dish containing the non primed seeds and primed seeds. Petri dishes were placed at room temperature in the laboratory. Germination percentage and vigor index were calculated by the using following formula.

$$\textbf{Germination \%} = \frac{\text{No.of seeds germinated}}{\text{No.of seeds present in petridish}} \times 100$$

Vigor index = germination % × Dry wt. of seedling

Dry weight of seedling: To take the seedling dry weight, samples were kept for an hour in an oven pre-set at 100-110°C for killing purpose. Thereafter, it was placed in an oven, preset at 60±2°C till to get constant weight.

Biochemical parameters

A - Amylase activity and soluble and insoluble sugar contents of germinating seeds were estimated at 72 an 96 hours in endosperm by the method of method of Bernfeld[5] and Dubios *et al*.[11] respectively.

Statistical Analysis

The data presenting to each of characters were analysed statically by applying the standard technique. Analysis of variance for Factorial Completely Randomized Design (CRD) was worked out and significance was tested by f test as described by Chandel[8]. Critical difference values were calculated at 5 percent level of significance in order to compare the treatment means.

RESULT

Germination Percentage

Table-1 represented the data of the percent germination recorded at 18, 24, 36, 48 and 60h, obtained from non primed(T_1), Hydro primed (T_2) and Kinetin(T_3) primed seeds having different concentrations [C_1,C_2,C_3,C_4,C_5 and C_6 represented 1.5, 2.5,5,10,15 and 20 ppm Kinetin respectively]. The data revealed that set primed with 5ppm concentration of kinetin (T_3C_3) presented highest germination percentage over other concentrations of kinetin. Whereas hydro primed set (T_2) showed better result in respect to control (T_1) only. Same trend was noted in both the varieties at all studied hours. However variety V_1 showed more germination % as compared to variety V_2. The data was found statistically significant for most of the studied factors. The interaction between V X T, V X C, TX C and V X T X C were also found statistically significant.

Vigor index

Table-2 represented vigor index of seedling, recorded at 24, 36, and 48 h, The results depicted that the kinetin treated set (T_3C_3) showed maximum vigor index at all studied hours over all used concentrations of the same followed by hydro primed set i.e. T_2 and non primed control set (T_1). However variety V_2 showed more vigor index as compared to variety V_1. The data was found statistically significant for factor T and C at each studied hours and factor V at 36 h. The interaction between V X T X C was also found statistically significant at 24h.

α- amylase activity (µg maltose h^{-1} g^{-1} FW)

Data regarding α-amylase activity has been presented in table: 3. The data were determined at 72 and 96 h in endosperm of seeds. The study depicted that at 72 h the α-amylase activity was more as compared to 96 h endosperm of germinating seeds. Maximum activity of α-amylase enzyme in endosperm was represented by kinetin (T_3C_3) followed by hydro primed set (T_2) over non primed control set (T_1). However variety V_1 showed more α-amylase activity in respect to variety V_2. The data was found statistically significant for factor T and C at each studied hours and factor V at 36 h.

Soluble and insoluble sugar contents

Table-4 represents soluble and insoluble sugar contents, recorded at 72 and 96 h in endosperm of seeds. Soluble sugar content was noted to increase with increasing time of germination but reduction was observed in the contents of total insoluble sugar. Data showed that primed sets have more soluble content over non primed sets at both the studied hours i.e. 72 and 96 and maximum soluble sugar content noted in kinetin (T_3C_3) primed set followed by hydro primed (T_2) sets respectively over all used concentrations of kinetin and non primed control set. Same trend was found in both varieties and both studied hours i.e. 72 and 96. The insoluble sugar content was found to decrease with the increasing hours of germination in both i.e. primed and non primed sets. However use of primed seeds mainly kinetin (T_3C_3) was significantly found to improve the rate of degradation of insoluble sugar by decreasing its amount in the endosperm of germinating rice seeds followed by hydro primed(T_2) set respectively for over all used concentrations of kinetin and non primed control (T_1) set. Variety V_1 showed less insoluble sugar content in comparison to V_2 variety. The similar trend was found in both varieties and both studied hours i.e. 72 and 96. The data was found statistically significant for most of the factors at each studied hours. The interaction between V X T was also found statistically significant.

DISCUSSION

During the study of germination percentage (Table 1.) it was recorded that hormonal (kinetin) primed sets treated with 5ppm concentration of kinetin have best results over non primed set at different studied hours. Data regarding vigor index also showed similar trends (Table- 2). This may occur due to improved mobilization of stored material from endosperm to embryo during germination in the primed seeds, which may be inducted or operative with induced activity of hydrolytic enzyme α-amylase. In the present case also the activity of α-amylase and soluble sugar contents (Table 3 and 4) in germinating rice seeds were found to improve in primed sets except insoluble sugar; a reduction in insoluble sugar content was noted in primed sets at different studied period for used chemicals, suggested the higher rate of hydrolysis of the stored carbohydrate. Secretion of α -amylase from the aleurone layer during germination plays an key role in hydrolysis of endosperm starch and change it in to metabolizable sugars and provide energy for the growth of roots and shoots[1,4]. The endosperm of cereal seeds laden with starch, as major reserve food material, hence starch hydrolysis in germinating cereal seeds provides essential soluble sugars which either act as substrate for respiration or convert in sucrose, remobilize towards growing embryo for the emerging seedling prior to the beginning of photosynthesis. Four enzymes required for the degradation of starch, in which α-amylase is considered to be the most important one in seeds as it is capable of hydrolysing intact starch grains[4]. Most of the studies on the possible link between α -amylase and seed dormancy (as distinct from germination) focus on the genetic defects of late-maturity, α –amylase activity and pre-harvest sprouting in cereal crops, where high levels of α -amylase synthesized late during grain development or retained in the pericarp cause premature starch degradation which is concomitant with the germination of seeds those are still attached to the ear[18]. Mondal and Bose[21] observed that wheat seeds while primed either with distilled water or with Ca $(NO_3)_2$ improve the germination due to the activation/ induction of the important hydrolyzing enzyme, α –amylase. Further a possible correlation was confirmed between α –amylase activity and soluble sugar by Anaytullah and Bose[2], using primed seeds of wheat, germinating under different temperature regime. A range of studies have shown that α-amylase activity and/or protein are absent in dry and mature cereal seeds and that expression is induced upon commencement of germination[19,23,6,13]. Seed priming with kinetin improved seed germination and seedling vigor of tomato via breakdown of dormancy[24]. Hussein[16] reported that seed priming with salicylic acid (100 mg\L) improved germination percentage, germination speed index (GSI), seedling vigour index (SVI) and RGR of okra. Hormone treatment with salicylic acid increased emergence percentage, emergence rate, chlorophyll band protein content by 82.0%, 130%, 7.9% and 1.9%, respectively, relative to the control in sweet sorghum[22].

Tomato seed priming with cytokinins was found to increase germination percentage parameters[21]. In the present study the kinetin primed seeds were found to have more nitrate redcutase activity beside improving the germination, vigor index and α –amylase activity of rice as compared to other primed sets. It may be presumed that kinetin while applied as priming purpose may accumulate the nitrate present in seeds at a point to activate/ induce the activity of nitrate reductase enzyme, as kinetin acts as the accumulator of resource at the place of sink development. However no other reports are available in the literature in this respect, it may be the first report of its kind of study.

Table 1: Effect of distilled water (hydro) and Kinetin (hormonal) priming on germination percentage of seeds of rice varieties at different hours (h) of germination

Treatments	Germination %														
	18h			24h			36h			48h			60h		
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1	32.00	30.00	31.00	69.67	61.33	65.50	82.00	79.33	80.67	85.00	85.33	85.17	87.00	87.67	87.33
T_2	31.67	32.00	31.83	68.33	66.00	67.17	83.33	80.67	82.00	85.67	86.67	86.17	87.78	88.67	88.22
$T3C_1$	32.67	33.00	32.83	66.67	64.33	65.50	83.00	80.67	81.83	86.67	85.67	86.17	88.67	87.67	88.17
T_3C_2	36.33	33.00	34.67	72.67	70.33	71.50	87.00	83.67	85.33	89.33	88.33	88.83	91.33	90.33	90.83
T_3C_3	**57.00**	**54.00**	**55.50**	**75.00**	**73.00**	**74.00**	**96.00**	**94.33**	**95.17**	**97.33**	**95.67**	**96.50**	**99.33**	**97.67**	**98.50**
T_3C_4	54.00	51.67	52.83	60.33	58.33	59.33	90.00	88.33	89.17	94.67	93.33	94.00	96.67	95.33	96.00
T_3C_5	42.00	39.67	40.83	66.67	39.00	52.83	84.67	82.67	83.67	90.33	90.33	90.33	92.33	92.33	92.33
T_3C_6	25.33	24.00	24.67	52.00	49.67	50.83	74.67	72.33	73.50	84.33	84.33	84.33	86.33	86.33	86.33
Mean	38.88	37.17		66.42	60.25		85.08	82.75		89.17	88.71		91.18	90.75	

TABLE OF C.D. AND SEM

Particulars	18h		24h		36h		48h		60h	
	C.D.	SEm(±)	C.D.	SEm(±)	C.D.	SEm(±)	C.D.	SEm(±)	C.D.	SEm(±)
Factor(V)	0.78	0.28	0.66	0.24	0.97	0.35	0.69	0.25	0.70	0.25
Factor(T)	2.47	0.88	2.10	0.74	3.07	1.09	2.18	0.78	2.22	0.79
V X T	3.50	1.24	2.97	1.05	4.35	1.54	3.09	1.10	3.14	1.11
Factor(C)	1.47	0.52	1.22	0.43	1.78	0.63	1.28	0.45	1.35	0.37
V X C	2.08	0.73	1.72	0.60	2.53	0.89	2.16	0.64	2.21	0.78
TX C	2.55	0.90	2.10	0.75	3.09	1.09	2.22	0.79	2.28	0.81
V X T X C	3.60	1.28	2.96	1.05	4.37	1.55	3.14	1.11	3.20	1.63

Where (1): V_1= HUBR-10-9, V_2= HUR-105 rice varieties

(2): T_1, T_2 and T_3 are non primed, Hydro, Kinetin primed seeds respectively

(3): C_1,C_2,C_3,C_4,C_5 and C_6 are different concentrations (1.5, 2.5, 5,10,15 and 20 ppm) of Kinetin

(4) CD at 5%

Table 2: Effect of distilled water (hydro) and Kinetin (hormonal) priming on vigour index of rice varieties at different hours (h) of germination/seedling growth

Treatments	Vigor index								
	24h			36h			48h		
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1	1.02	1.27	1.15	1.47	1.58	1.52	1.74	1.76	1.75
T_2	1.10	1.24	1.17	1.27	1.85	1.56	1.78	1.80	1.79
$T3C_1$	1.16	1.27	1.22	1.41	1.74	1.58	1.81	1.94	1.88
T_3C_2	1.30	1.44	1.37	1.64	1.81	1.72	2.05	2.11	2.08
T_3C_3	**1.67**	**1.85**	**1.76**	**1.76**	**2.23**	**2.00**	**2.36**	**2.42**	**2.39**
T_3C_4	1.56	1.74	1.65	1.74	1.85	1.79	2.40	2.33	2.36
T_3C_5	1.37	1.52	1.45	1.97	2.01	1.99	2.32	2.36	2.34
T_3C_6	1.32	1.46	1.39	1.79	1.91	1.85	2.24	2.29	2.27
Mean	1.31	1.47		1.63	1.87		2.09	2.13	

TABLE OF C.D. AND SEM

Particulars	24h		36h		48h	
	C.D.	SEm(±)	C.D.	SEm(±)	C.D.	SEm(±)
Factor(V)	N/A	0.02	0.12	0.04	N/A	0.01
Factor(T)	0.22	0.08	0.37	0.13	0.09	0.03
V X T	N/A	0.11	N/A	0.19	N/A	0.05
Factor(C)	0.11	0.04	0.08	0.03	0.06	0.02
V X C	N/A	0.06	N/A	0.04	N/A	0.03
TX C	N/A	0.07	N/A	0.05	0.09	0.03
V X T X C	0.27	0.16	N/A	0.07	N/A	0.05

Note: Detail of the conditions has given in table 1.

Table 3: Effect of distilled water (hydro) and Kinetin (hormonal) priming on α- amylase activity of endosperm of rice varieties at 72 and 96 hours (h) of germination

Treatments	α- amylase (µg maltose h^{-1} g^{-1} FW)					
	72h			96h		
	V_1	V_2	Mean	V_1	V_2	Mean
T_1	0.214	0.200	0.207	0.096	0.090	0.093
T_2	0.225	0.204	0.215	0.105	0.093	0.099
$T3C_1$	0.238	0.216	0.227	0.111	0.097	0.104
T_3C_2	0.245	0.227	0.236	0.106	0.108	0.107
T_3C_3	**0.276**	**0.264**	**0.270**	**0.125**	**0.114**	**0.119**
T_3C_4	0.260	0.245	0.253	0.117	0.107	0.112
T_3C_5	0.219	0.204	0.212	0.103	0.096	0.100
T_3C_6	0.210	0.195	0.203	0.096	0.087	0.091
Mean	0.214	0.200		0.096	0.090	

TABLE OF C.D. AND SEM

Particulars	72h		96h	
	C.D.	SEm(±)	C.D.	SEm(±)
Factor(V)	N/A	0.005	0.005	0.002
Factor(T)	0.046	0.016	0.015	0.005
V X T	N/A	0.023	N/A	0.008
Factor(C)	0.035	0.010	0.009	0.003
V X C	N/A	0.014	N/A	0.004
TX C	N/A	0.017	N/A	0.005
V X T X C	N/A	0.024	N/A	0.008

Note: Detail of the conditions has given in table 1.

Table 4: Effect of distilled water (hydro) and Kinetin (hormonal) priming on soluble and insoluble sugar contents of endosperm of rice varieties at 72 and 96 hours (h) of germination

Treatments	Sugar(mg g^{-1}dw)											
	Soluble						Insoluble					
	72h			96h			72 h			96h		
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1	0.197	0.144	0.170	0.230	0.202	0.216	0.415	0.453	0.434	0.313	0.315	0.314
T_2	**0.225**	**0.154**	**0.190**	**0.240**	**0.208**	**0.224**	**0.332**	**0.400**	**0.366**	**0.303**	**0.310**	**0.307**
$T3C_1$	0.245	0.230	0.238	0.254	0.212	0.233	0.412	0.436	0.424	0.272	0.298	0.285
T_3C_2	0.238	0.245	0.241	0.261	0.275	0.268	0.335	0.400	0.368	0.204	0.283	0.244
T_3C_3	**0.276**	**0.246**	**0.261**	**0.282**	**0.288**	**0.285**	**0.341**	**0.360**	**0.350**	**0.192**	**0.259**	**0.225**
T_3C_4	0.260	0.232	0.246	0.285	0.271	0.278	0.358	0.393	0.375	0.245	0.263	0.254
T_3C_5	0.219	0.195	0.207	0.238	0.221	0.229	0.379	0.419	0.399	0.255	0.265	0.260
T_3C_6	0.210	0.144	0.177	0.201	0.216	0.208	0.385	0.439	0.412	0.295	0.274	0.284
Mean	0.234	0.199	0.216	0.249	0.237	0.243	0.370	0.413	0.391	0.260	0.283	0.272

TABLE OF C.D. AND SEM

Particulars	Soluble				Insoluble			
	72h		96h		72h		96h	
	C.D.	SEm(±)	C.D.	SEm(±)	C.D.	SEm(±)	C.D.	SEm(±)
Factor(V)	0.015	0.005	0.006	0.002	0.002	0.001	0.001	0.001
Factor(T)	0.047	0.017	0.019	0.007	0.007	0.002	0.003	0.005
V X T	0.031	0.023	0.039	0.010	0.01	0.004	0.004	0.008
Factor(C)	0.027	0.010	0.016	0.006	0.028	0.010	0.025	0.009
V X C	N/A	0.013	N/A	0.008	N/A	0.014	N/A	0.013
TX C	N/A	0.017	N/A	0.010	N/A	0.017	N/A	0.016
V X T X C	N/A	0.023	N/A	0.014	N/A	0.024	N/A	0.022

Note: Detail of the conditions has given in table 1.

CONCLUSION

On the basis of study we conclude that seed priming with 5 ppm concentration of Kinetin was found best for both the varieties and improved all germination parameters except insoluble sugar content over non primed control.

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