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Hydro, Mg $(NO_3)_2$ and kinetin primed seeds mitigate the inhibitory effects of $CdCl_2$ in germinating rice

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Abstract
Seed priming is a technique of regulating the germination process by managing the temperature and moisture content of seeds. It is used to improve germination%, reduce the duration of germination and improve plant establishment and yield. In the present investigation efforts were made to see the effect of heavy metal stress imposed during germination by using solution of $CdCl_2$ in three different concentrations (4, 12 & 30 ppm) in petridishes on primed and non-primed seeds of rice. Priming of seed was done with distilled water (hydro), Mg $(NO_3)_2$ and kinetin. Different germination parameters such as germination percentage, radicle and plumule lengths, and contents of soluble sugar and activity of α-amylase in endosperm were investigated at different study periods. All the germination parameters of primed seeds were increased in respect to non-primed seeds. However, the use of primed seeds have shown to overcome the inhibitory effects of heavy metal stress imposed in form of $CdCl_2$ solution during the period of germination. Hence the work elucidates the mitigating effects of priming under heavy metal stress.

Keywords: Priming, cadmium chloride, magnesium nitrate, kinetin, sugar metabolism and germination

Introduction
Heavy metals are widespread pollutants and they are non-degradable; these metals are used in various industries from which effluents are discharged into the environment. Heavy metals are those having densities higher than 5 g cm^{-3}. Heavy metals act as persistent pollutants in the atmosphere and have toxic effects. It adds either by natural or anthropogenic activities. Those metals are considered more hazardous to plants which have immobilized properties than those which can be dissolved. The former group includes Mn, Fe, Cd, As, Pb and Hg. Cd is a dangerous heavy metal which have density 8.642 gcm^{-3} at 20 degree Celsius. Cadmium is a divalent heavy metal cation (Cd^{2+}) and causes phytotoxicity in plants via its uptake and accumulation, makes it to enter in the food chain which effect food chain and causes dangerous diseases (Sghayar,S.2014) [12]. Due to agricultural practices, transportation, industrial activities and waste disposal have increased the concentrations of toxic elements such as Cd in agricultural soils. According to International Agency for Research on Cancer (IARC, 1993) [7] Cd is considered as a class 1 human carcinogen, its transfer may occur from plants to human. Among the top toxins cadmium is ranked seven due to its negative influence on the cell's enzymatic systems (ATSDR, 1999) [2], and it has been estimated that 70% of the Cd intake by human beings comes from plant foods (Wagner, 1993). Yang *et al*. (1995) [15] suggested that critical Cd level in nutrient solution for conventional crop plants is reported to be 8 µmol L^{-1}. Nevertheless, cereals such as maize, rice and barley tolerate Cd as much as 100 µmolL^{-1} (da Silveira *et al*., 2008) [5]. Presence of high amount of Cd may cause a decrease in uptake of nutrient elements, induction of oxidative stress including alterations in enzyme of the antioxidant defence system (Sandalio *et al*., 2001) [11]. The first phase in plant's life is germination at that time seed may face the utmost benefits /problems of any element present in the soil where it is sown. Germination is found to be affected in presence of any heavy metal. In recent years a technology referred as seed priming is noted to have capacity to mitigate the adverse effects of various kinds of stresses including the phytotoxic one (Kumar *et al*., 2016) [9]. But the literature regarding use of primed seeds to mitigate the adverse effects of heavy metal Cd on germination of various crops are limited. Therefore in present piece of work non-primed, distilled water, Mg $(NO_3)_2$ and kinetin primed seeds were used for mitigation purpose of $CdCl_2$ induced stress on germination of rice variety.

Material and Methods

Healthy and bold seeds of rice were surface sterilized by keeping them in 0.01% $HgCl_2$ (Mercuric chloride) solution for 5 minutes and then thoroughly washed with distilled water for 5-6 times. These sterilized seeds were used for experimental purpose. For priming purpose the sterilized seeds were kept in distilled water and solution of 5mM concentration of Mg $(NO_3)_2$ and 5ppm kinetin separately for 10 hours. After 10 hours these seed were gently washed with distilled water and then seeds were further dried back to its original weight at the room temperature by placing them under forced air. Thereafter these seeds were packed in paper bags and then they were used as per experimental requirements within two months. Hundred seeds of each type of primed and non-primed seeds were placed in each petridishes, and then they were provided with a shock of three different concentrations (4, 12, 30 ppm) of $CdCl_2$ solution. Thereafter rice seed/seedlings were removed from $CdCl_2$ solution and washed with distilled water for 2 times. These seeds were kept in petridishes in distilled water and for comparison non-primed control, hydro, Mg $(NO_3)_2$ and kinetin primed seeds were also kept in the same condition. All these studies were conducted in Seed Priming and Seed Physiology Laboratory of Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.

The treatment detail of present experiment were as follows:

P_0=Non-primed P1=Hydro-primed P_2=Magnesium nitrate primed P3=Kinetin primed T_0=Control T_1=$CdCl_2$ (4ppm) T_2= $CdCl_2$ (12ppm) T_3= $CdCl_2$ (30ppm)

The Germination studies were carried out in Petri dishes to measure germination parameters (germination percentage, plumule and radicle length). Germinated seeds were counted daily and germination percentage was evaluated by counting the number of normal seedlings at the end of the standard germination test.

Germination% = Number of seeds germinated/ total number of seeds sown × 100

The radicle length (cm) was measured with the help of meter scale and distance lying between the portion of the shoot just touching the soil and the tip of the plants were used for this and the plumule length (cm) was determined with the help of meter scale by measuring distance lying between the portion of the shoot just touching the soil and the tip of the plants.

The α-amylase activity& soluble sugar contents were measured in the endosperm, obtained from 3rd, 5th & 7th of germinated wheat seeds using the methods of Bernfeld (1955) and Dubios *et al*. (1956) respectively. Statistical analysis were done as per requirement of experiment.

Results

Table No 1 represents the germination percentage of primed and non- primed seeds of rice variety HUBR-10-9. The germination percentage was dropped with the increased concentration of $CdCl_2$ in primed and non-primed sets. The hydro-primed and $Mg(NO_3)_2$ primed sets showed at par results in some of the study periods with 12 and 30 ppm of $CdCl_2$ soaked sets but it was less than that of absence of cadmium and 4 ppm of Cd. However, the mean values clearly indicated that all primed and non-primed sets showed a decrease in germination% with increasing concentrations of $CdCl_2$ and same trend was found at 24 to 108 hr. At 48 & 84 hours the germination percent of 30ppm $CdCl_2$ is higher than 12ppm in hydro primed sets. In kinetin primed sets the germination percent decreased from control to higher conc. of CdCl2 but at 60hr the germination percent of 30ppm $CdCl_2$ is higher than 12ppm $CdCl_2$. At 108hr the germination percent was highest in $Mg(NO_3)_2$ primed sets & lowest in non-primed sets, while no stress was imposed to seeds. Further it was noted that from 36 to 108 hours non primed seeds showed only 68 to 80 percent germination where as in hydro primed as well as in $Mg(NO_3)_2$ and kinetin primed sets 92to 94 percent germination were visualized at 108 hrs. From a critical look to the mean values it has been cleared that all the primed sets were able to improve the germination in presence/absence of CdCl2 in respect to non-primed seeds in the same condition, in the later one the CdCl2 reduced germination with increase in concentration from 4 to 30ppm.

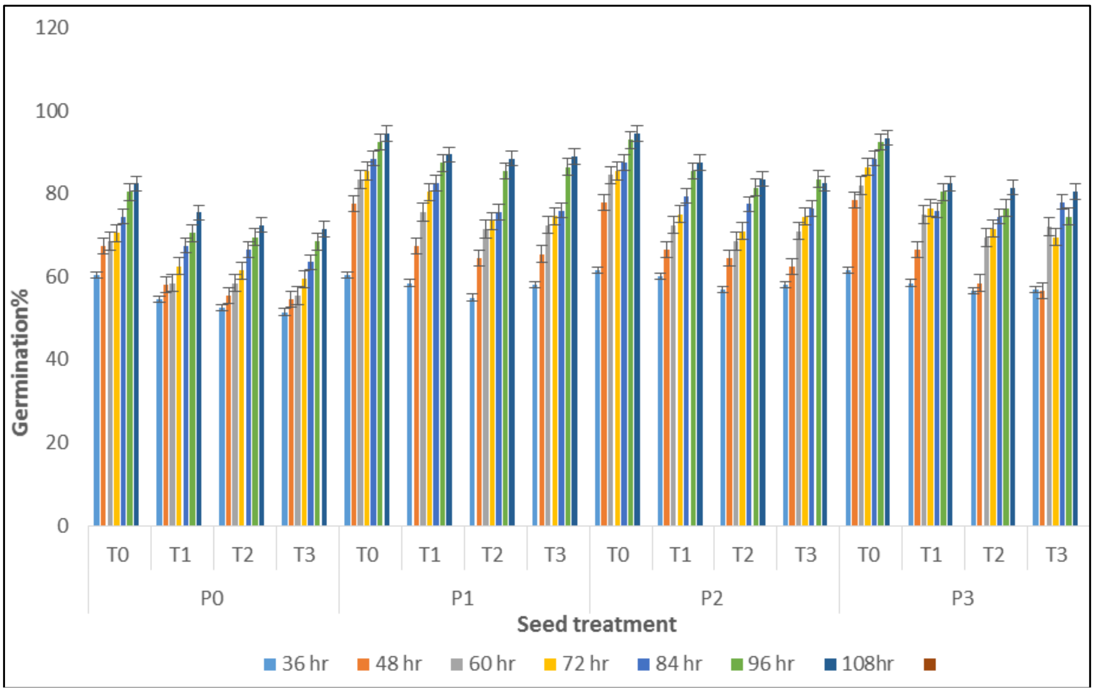
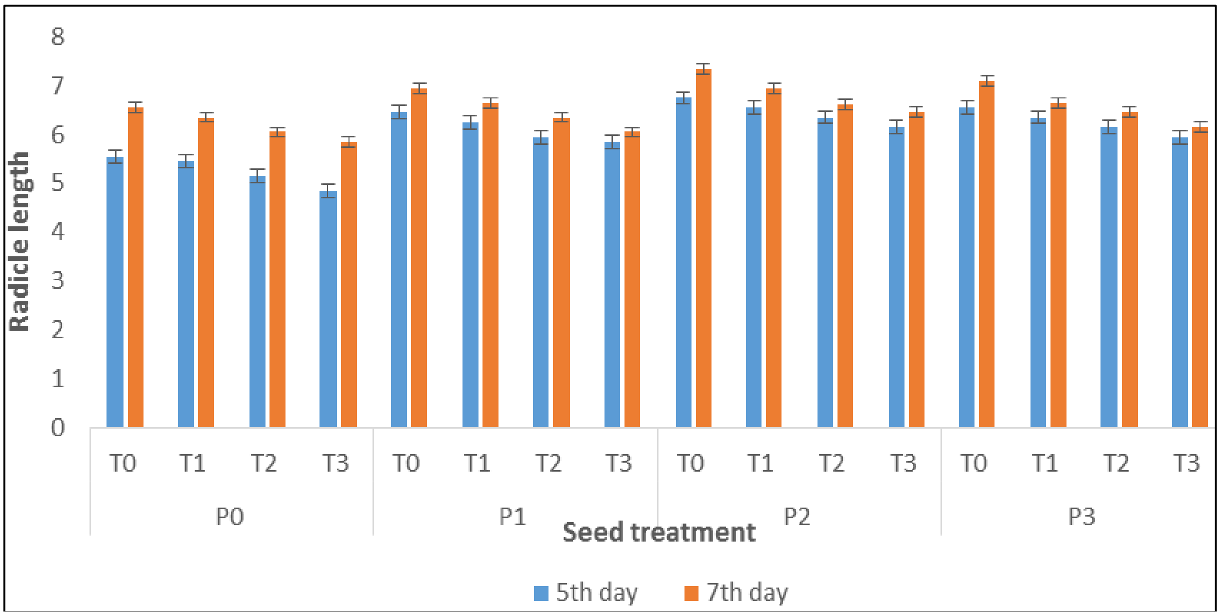


Fig 1: Effect of varying concentrations of $CdCl_2$, using non-primed (control), hydro, magnesium nitrate & kinetin primed seeds on germination% in germinating rice

Table-1: Effect of varying concentration of $CdCl_2$, using non-primed (control) hydro, magnesium nitrate &kinetin primed seeds on germination% in germinating rice

Priming(P)/Treatment(T)		Germination percentage								
		24 h	**36 h**	**48 h**	**60 h**	**72 h**	**84 h**	**96 h**	**108h**	**Mean**
P0	T0	27.50	60.50	67.50	68.50	70.50	74.50	80.50	82.50	66.50
	T1	20.50	54.50	58.00	58.50	62.50	67.50	70.50	75.50	58.44
	T2	17.50	52.50	55.50	58.50	61.50	66.50	69.50	72.50	56.75
	T3	16.50	51.50	54.50	55.50	59.50	63.50	68.50	71.50	55.13
P1	T0	28.50	60.50	77.50	83.50	85.50	88.50	92.50	94.50	76.38
	T1	27.00	58.50	67.50	75.50	80.50	82.50	87.50	89.50	71.06
	T2	26.75	55.00	64.50	71.50	73.50	75.50	85.50	88.50	67.59
	T3	27.50	58.00	65.50	72.50	74.55	76.00	86.50	89.00	68.63
P2	T0	29.25	61.50	78.00	84.50	85.50	87.50	93.00	94.50	76.72
	T1	28.50	60.00	66.50	72.50	75.00	79.50	85.50	87.50	69.38
	T2	28.00	57.00	64.50	68.50	71.00	77.50	81.50	83.50	66.44
	T3	29.00	58.00	62.50	71.00	74.50	76.50	83.50	82.50	67.19
P3	T0	28.25	61.50	78.50	82.00	86.50	88.50	92.50	93.50	76.41
	T1	27.00	58.50	66.50	75.00	76.50	76.00	80.50	82.50	67.81
	T2	26.00	56.50	58.50	69.50	71.50	74.50	76.50	81.50	64.31
	T3	26.50	57.00	56.50	72.00	69.50	78.00	74.50	80.50	64.31
P0		20.50	54.75	58.88	59.50	63.25	68.00	72.25	75.50	59.08
P1		27.44	58.00	68.75	75.75	78.38	80.63	88.00	90.38	70.91
P2		28.69	59.13	67.88	74.13	76.50	80.25	85.88	87.00	69.93
P3		26.94	58.38	65.00	74.63	76.00	79.25	89.63	84.50	69.29
T0		28.38	61.00	75.38	78.88	81.75	84.75	89.63	91.25	73.88
T1		25.75	57.88	64.63	70.38	73.63	76.38	81.00	83.75	66.67
T2		24.56	55.25	60.75	67.00	69.38	73.50	78.25	81.50	63.77
T3		24.88	56.13	59.75	67.75	69.38	73.50	78.25	80.88	63.81
Grand Mean		25.89	57.56	65.13	71.00	73.53	77.03	82.86	84.34	67.17
Seem±	P	0.41	0.21	0.13	0.47	0.22	0.41	0.47	0.16	0.31
	T	0.41	0.21	0.13	0.47	0.22	0.41	0.47	0.16	0.31
	P×T	0.83	0.41	0.26	0.95	0.44	0.81	0.94	0.31	0.62
CD at 1%	P	1.60	0.80	0.50	1.83	0.86	1.58	1.81	0.61	1.20
	T	1.60	0.80	0.50	1.83	0.86	1.58	1.81	0.61	1.20
	P×T	3.20	1.61	1.01	3.67	1.71	3.16	3.63	1.22	2.40

Table No. 2 represents the radicle and plumule lengths of the germinating seeds at 5th& 7th days of germination. Here the seeds were used in form of hydro, nitrate salt & kinetin primed and non-primed form and those were subjected to induced stress of different concentrations of $CdCl_2$. Radicle length was noted to decrease with increasing concentrations of $CdCl_2$, whereas seed priming sets have more root length as compared to non- primed sets. Use of primed seeds have the capacity to enhance the radicle length in normal as well as in $CdCl_2$ treated sets. The same trend was observed in case of plumule length at all the two mentioned days of study.



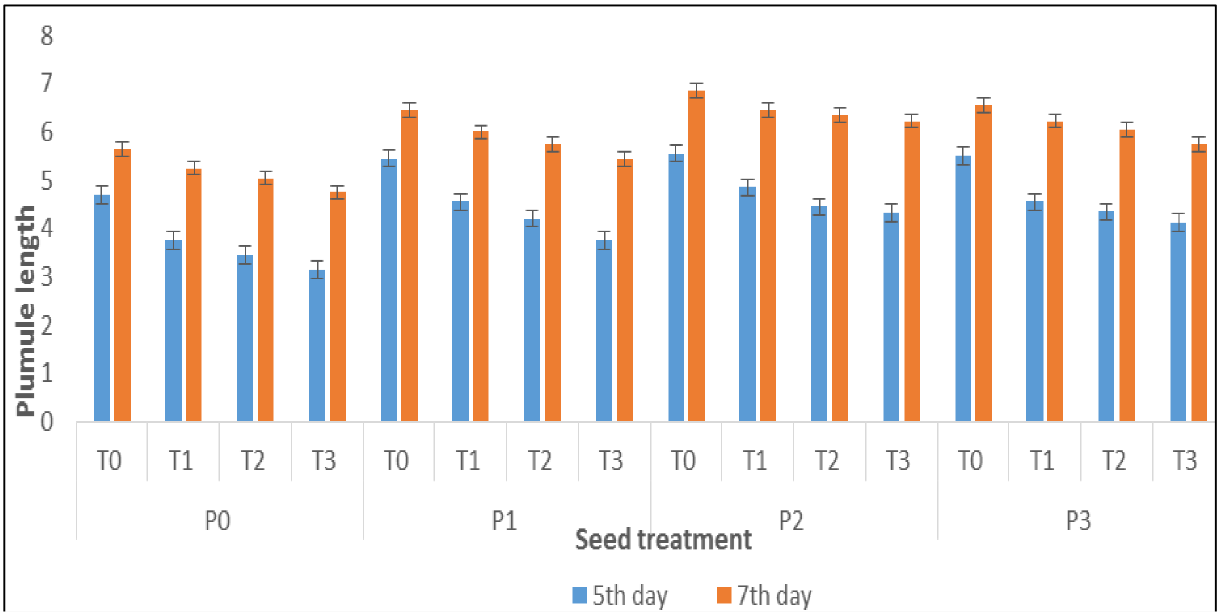


Fig-2: Effect of varying concentration of $CdCl_2$, using non-primed (control), hydro, magnesium nitrate & kinetin primed seeds of rice on radicle and plumule lengths (cm) during germination at different days**.**

Table-2: Effect of varying concentration of $CdCl_2$, using non-primed (control), hydro, magnesium nitrate & kinetin primed seeds of rice on radicle and plumule lengths (cm) during germination at different days

Treatment	Concentration	Radicle length			Plumule length		
		5th day	7th day	Mean	5th day	7th day	Mean
P0	T0	5.55	6.55	6.05	4.70	5.65	5.18
	T1	5.45	6.35	5.90	3.75	5.25	4.50
	T2	5.15	6.05	5.60	3.45	5.05	4.25
	T3	4.85	5.85	5.35	3.15	4.75	3.95
P1	T0	6.45	6.95	6.70	5.45	6.45	5.95
	T1	6.25	6.65	6.45	4.55	6.00	5.28
	T2	5.95	6.35	6.15	4.20	5.75	4.98
	T3	5.85	6.05	5.95	3.75	5.45	4.60
P2	T0	6.75	7.35	7.05	5.55	6.85	6.20
	T1	6.55	6.95	6.75	4.85	6.45	5.65
	T2	6.35	6.60	6.48	4.45	6.35	5.40
	T3	6.15	6.45	6.30	4.33	6.23	5.28
P3	T0	6.55	7.10	6.83	5.50	6.55	6.03
	T1	6.35	6.65	6.50	4.55	6.23	5.39
	T2	6.15	6.45	6.30	4.35	6.05	5.20
	T3	5.95	6.15	6.05	4.13	5.75	4.94
P0		5.25	6.20	5.73	3.76	5.18	4.47
P1		6.13	6.50	6.31	4.49	5.91	5.20
P2		6.45	6.84	6.64	4.79	6.47	5.63
P3		6.25	6.59	6.42	4.63	6.14	5.39
T0		6.33	6.99	6.66	5.30	6.38	5.84
T1		6.15	6.65	6.40	4.43	5.98	5.20
T2		5.90	6.36	6.13	4.11	5.80	4.96
T3		5.25	6.20	5.73	3.76	5.18	4.47
Grand Mean		6.02	6.53	6.28	4.42	5.93	5.17
SEm±	P	0.01	0.02	0.02	0.02	0.01	0.02
	T	0.01	0.02	0.02	0.02	0.01	0.02
	P×T	0.02	0.03	0.03	0.03	0.03	0.03
CD at 1%	P	0.05	0.06	0.06	0.06	0.06	0.06
	T	0.05	0.06	0.06	0.06	0.06	0.06
	P×T	0.10	0.13	0.12	0.13	0.11	0.12

It was noted from fig No 3 that soluble sugar content in endosperm in the germinating seeds of rice was found to decrease with increasing concentration of $CdCl_2$ studied at 3rd, 5th & 7th days. With the use of primed seeds the soluble sugar of endosperm increased which has been clearly visualized in the (table-3) but at 7th days in magnesium nitrate primed sets soluble sugar was found less as compared to hydro primed that might be due to its proper mobility of soluble sugar from endosperm to growing embryo. It was noted that maximum soluble sugar content in P_2T_1, P_2T_0 & P_1T_1 at 3rd, 5th & 7th days of Mg $(NO_3)_2$ primed sets & minimum soluble sugar content was in non-primed control in respected days.

Table 3: Effect of varying concentration of CdC_2, using non-primed (control), hydro, magnesium nitrate & kinetin primed seeds of rice on soluble sugar (mg/g DW) content in germinating endosperm at different days

Priming(P)/Treatment(T)		Soluble sugar			
		3rd Days	5th Days	7th Days	Mean
P0	T0	0.150	0.162	0.170	0.161
	T1	0.149	0.164	0.168	0.160
	T2	0.147	0.158	0.162	0.156
	T3	0.145	0.155	0.160	0.153
P1	T0	0.168	0.180	0.195	0.181
	T1	0.171	0.178	0.196	0.182
	T2	0.160	0.179	0.190	0.176
	T3	0.158	0.174	0.188	0.173
P2	T0	0.174	0.185	0.190	0.183
	T1	0.176	0.182	0.193	0.184
	T2	0.169	0.179	0.187	0.178
	T3	0.167	0.176	0.185	0.176
P3	T0	0.170	0.183	0.180	0.178
	T1	0.167	0.180	0.178	0.175
	T2	0.168	0.178	0.179	0.175
	T3	0.163	0.176	0.174	0.171
P0		0.147	0.160	0.165	0.157
P1		0.164	0.178	0.192	0.178
P2		0.172	0.180	0.189	0.180
P3		0.167	0.179	0.178	0.175
T0		0.165	0.177	0.183	0.175
T1		0.166	0.176	0.184	0.175
T2		0.166	0.173	0.179	0.173
T3		0.158	0.170	0.177	0.168
Grand Mean		0.163	0.174	0.181	0.173
SEm±	P	0.01	0.01	0.01	0.01
	T	0.01	0.01	0.01	0.01
	P×T	0.01	0.02	0.02	0.02
CD at 1%	P	0.03	0.03	0.04	0.03
	T	0.03	0.03	0.04	0.03
	P×T	0.05	0.07	0.07	0.06

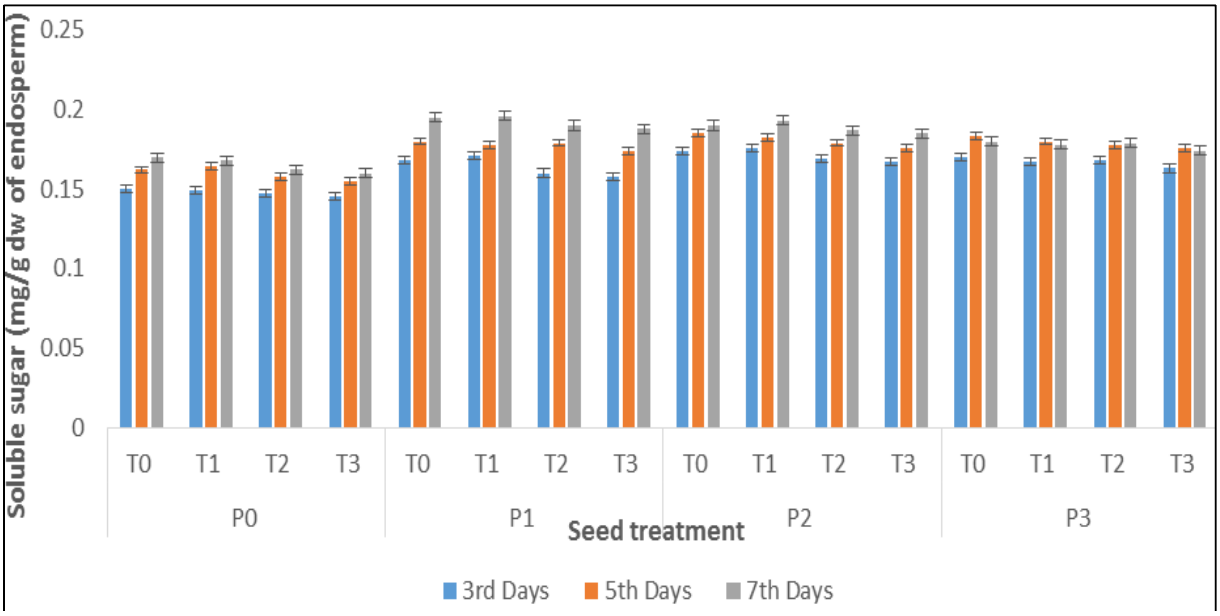


Fig 3: Effect of varying concentration of $CdCl_2$, using non-primed (control), hydro, magnesium nitrate & kinetin primed seeds of rice on soluble sugar (mg/g DW) content in germinating endosperm at different days

The α-amylase activity of endosperm during the germination of rice seeds was found to show a decreasement with increasing concentration of $CdCl_2$, while studied at 3rd, 5th & 7th days but somewhere it slightly increased in primed & non-primed sets. The result regarding mean values clearly showed that the increasing concentrations of $CdCl_2$ inhibited the activity of enzyme in respect to control non primed seeds; however the use of hydro and nitrate primed seeds were found to improve the activity of enzyme α- amylase even in presence of higher concentration of $CdCl_2$. It is also cleared from the result that primed sets are always have more α-amylase activity in the germinating endosperm of rice in respect to the non-primed set at different studied period. The mean data suggested that the Mg $(NO_3)_2$ was best among all the sets in improving the amylase activity.

Table 4: Effect of varying concentration of $CdCl_2$, using non-primed (control), hydro, magnesium nitrate & kinetin primed seeds of rice on the activity of α-amylase (mg maltose g^{-1} h^{-1}FW) in germinating rice endosperm at different days

Priming(P)/Treatment(T)		α-amylase			
		3rd Days	5th Days	7th Days	Mean
P0	T0	0.174	0.161	0.149	0.161
	T1	0.171	0.159	0.147	0.159
	T2	0.165	0.155	0.143	0.154
	T3	0.164	0.153	0.140	0.152
P1	T0	0.189	0.179	0.165	0.178
	T1	0.186	0.176	0.163	0.175
	T2	0.184	0.174	0.160	0.173
	T3	0.181	0.171	0.158	0.170
P2	T0	0.196	0.181	0.167	0.181
	T1	0.192	0.178	0.162	0.177
	T2	0.187	0.175	0.159	0.174
	T3	0.185	0.173	0.157	0.172
P3	T0	0.199	0.177	0.159	0.178
	T1	0.188	0.174	0.156	0.173
	T2	0.186	0.170	0.152	0.169
	T3	0.183	0.165	0.150	0.166
P0		0.185	0.175	0.162	0.174
P1		0.190	0.177	0.161	0.176
P2		0.189	0.172	0.154	0.172
P3		0.169	0.157	0.145	0.157
T0		0.190	0.175	0.160	0.175
T1		0.184	0.172	0.157	0.171
T2		0.181	0.168	0.153	0.167
T3		0.178	0.166	0.151	0.165
Grand Mean		0.183	0.170	0.155	0.170
SEm±	P	0.01	0.00	0.01	0.007
	T	0.01	0.00	0.01	0.007
	P×T	0.01	0.00	0.01	0.007
CD at 1%	P	0.01	0.00	0.01	0.007
	T	0.01	0.01	0.01	0.010
	P×T	0.01	0.01	0.01	0.010

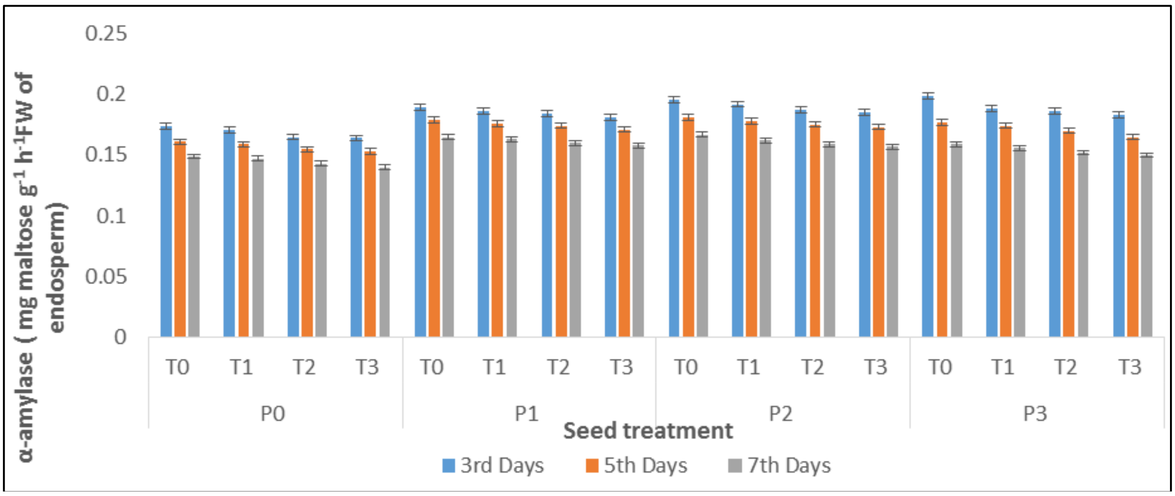


Fig 4: Effect of varying concentration of $CdCl_2$, using non-primed (control), hydro, magnesium nitrate & kinetin primed seeds of rice on the activity of α-amylase (mg maltose g^{-1} h^{-1}FW) in germinating rice endosperm at different days

Discussion

The result suggested that imposing heavy metal stress using $CdCl_2$ showed its toxicity in inhibiting the germination in non-primed sets in rice which was found to remove with the use of hydro, $Mg(NO_3)_2$ and kinetin primed seeds. However overall result regarding germination percentage suggested that $Mg(NO_3)_2$, hydro and kinetin priming have more effect in improving the said parameter even in presence of $CdCl_2$. In 1982, Bose *et al.* while working with maize, primed with $Ca(NO_3)_2$ they observed an improvement in germination percentage and that was well correlated with protease activity and soluble nitrogen content in endosperm during germination; Anayatullah and Bose (2007) [1] observed that use of $Mg(NO_3)_2$ primed seeds improved germination percentage, amylase activity and soluble sugar content during germination of wheat under cold stress. Use of kinetin to seeds is found to alter the adverse effect of Cd and increased germination, this study agrees with finding of Singh and Amritphale (1993) [13] with soyabean. However, Garg *et al.*, (2010) [6] observed that in the rice cultivar germination was inhibited with application of $HgCl_2$ (heavy metals). The same was observed in present case, where $CdCl_2$ inhibited percent germination in non-primed control seeds but it improved with the use of primed seeds. Yaksha *et al.* (2011) further suggested that putrescence hardened (a kind of priming) seeds of Brassica showed a good percentage of germination even in presence of heavy metals. This finding supports the present study where all the primed sets showed an ability to overcome the heavy metal toxicity by improving their percent germination. In the present case the result suggested that the cadmium chloride treatment reduced the length of plumule and radicle both (fig 2) but the use of primed seeds reduced the extent of loss in length in respect to non-primed cadmium chloride treated sets. It was also reported that increase in cadmium levels decrease the plant growth of *Dalbergia sisso* Roxb (Iqbaland Mehmood 1991) [8] this reduction was formed in meristematic cells present in this region and reduced levels of certain enzymes present in the cotyledon and endosperms which in turn result in the reduction in seedlings length under heavy metal treatments. The soluble sugar content and α-amylase activity both were found to be decreased with increased concentrations of cadmium chloride in primed and non-primed sets but the extent of reduction was more in non-primed sets (fig 3 & 4). It was noticed by Bose *et al.*(1982) [4] while working with maize seed germination under the influence of various nitrogenous salts reported that nitrogenous salts are able to improve not only degradation of the stored material but also it induces the rate of mobilization of degraded materials towards embryo. It was found that nitrate primed seeds enhanced the α-amylase activity and soluble sugar content in germinating wheat seeds under cold and imposed heavy metal stress respectively (Anayatullah and Bose 2007; Kumar *et al.* 2016) [1, 9]. The same might be occurring in the present case where the rate of degradation of sugar in nitrate and kinetin primed seeds were noted more, realized from the data of the α-amylase activity of different period and mobilization of soluble sugar might be higher which is also noticed from the study of soluble sugar content at different periods (table no-3, fig no-3). Further it is well established that starch hydrolysis in germinating cereal seeds provides essential soluble sugars for the emerging seedling prior to the beginning of photosynthesis. Priming of wheat seeds either with water only or with $Ca(NO_3)_2$ solution may induce the activity as well as synthesis of α -amylase in germinating wheat seeds compared to non-primed control seeds (Mondal and Bose 2012). In the present case a positive correlation between α-amylase and soluble sugars also confirms the assumption that increased α-amylase activity resulted in increased reducing sugars (Anaytullah and Bose 2007) [1] in presence and absence of $CdCl_2$.

Conclusion

Study concludes that the use of hydro, $Mg(NO_3)_2$ and kinetin primed seeds may overcome the adverse effects/phyto toxicity of the heavy metal Cd (used as a $CdCl_2$) in the germinating seeds of rice thereby improving α-amylase activity and enhanced availability of soluble sugar in endosperm. So seed priming proves to be a fruitful technology which improves germination as well as may also mitigate phytotoxic role of cadmium chloride in germinating rice seeds.

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