Effects of Kinetin, Coconut Milk and Calcium Chloride on Biochemical Indices of Boro Rice (*Oryza Sativa* L.) in Presence of Higher Manganese Condition of Acid Soil

Nibedita Basumatary[1], Bhagawan Bharali[2*]

Department of Crop Physiology, Faculty of Agriculture, Assam Agricultural University, Jorhat
*Corresponding Author

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Abstract— *In North-east India including Assam, available Manganese [Mn^{2+}] in soil is 3- 52 ppm with a critical limit 2-3 ppm. In plants, [Mn^{2+}] is 15-20 ppm g^{-1} dry weight, in excess of which Mn becomes toxic to crop plants (Basumatary et al., 2014). There is a paucity of information on the effects of natural or synthetic plant growth regulating subnstances viz., Cytokinin, Coconut milk and Calcium chloride on amelioration of Mn toxicity of rice crop grown in acid soil condition of Assam. So, a pot experiment was conducted to investigate into the effects of root-dip treatments of rice crop with these substances on biochemical performance of four rice genotypes (Kanaklata, Bishnuprasad, Jyotiprasad and Numoli) cultivated in acid soil enriched with higher Mn (30ppm native Mn plus 20ppm added Mn). At 20 ppm Mn application, there were decreases in total chlorophyll content (14.52%), NR activity (17.77%), Carbohydrate content (3.83%). In contrast, soil treated with 20 ppm Mn as basal at vegetative stage along with the overnight (≈12h) root dip treatments (RDT) of rice varieties with Cytokinin (100ppm), Coconut milk (10 times dilution) and $CaCl_2$ (100ppm), enhenced Cholorophyll a (3-525-19.771%), Chlorophyll b (1.917-19.55%), total chlorophyll contents (4.13-6.35%),) in leaf, NR activity (4.46-10.08%) and carbohydrate content in grain (2.24-10.92%). The variety, Kanaklata was found to be the best based on the biochemical parameters (total Chl:1.861 mg g^{-1} f.w, Chl 'a' (0.924 mg g-1 f.w), Chl 'b' (0.933 mg g-1 f.w), Carbohydrate: 9.202 mg g^{-1}, and [Ca^{2+}] in roots (59.35ppm).*

Keywords— Acid soil, Calcium chloride, Carbohydrate, chlorophyll, Coconut milk, Cytokinin, Manganese, Nitrate reductase.

I. INTRODUCTION

Manganese (Mn) being a micronutrient, at a congenial concentration plays vital roles on growth and development of rice crop cultivated on acidic soil condition. Mn is involved in activating more than 35 enzymes in plants (Mousavi *et al*., 2011), Mn^{2+} along with Ca^{2+} has the power of catalysing the Hill's reaction in the process of photosynthesis (Aref, 2012). Application of Mn especially on older leaves helps in photoassimilation (Agustina, 2011). Because, Mn influences chlorophyll synthesis, and its presence is essential in photosystem II (Diedrick, 2010). However, an excess of Mn^{2+} is toxic for most plants (Millaleo *et al*., 2010). Moreover, Mn^{2+} becomes toxic to all susceptible plants on acid soils in contrast to calcareous soils (high soil pH) and organic soils (Alejandro *et al*., 2020).

Information how the excess Mn^{2+} brings about biochemical changes in upland rice crop grown in acid soil condition of Assam are available (Yomso and Bharali, 2021). Efforts on amelioration of physio-biochemical disorders in Boro rice due to higher Mn^{2+} are lacking. It's known that Cytokinin is involved in cell division, and Calcium is one of the integral components of cell wall and cell membrane in plants (Bharali *et al*., 2015). The hypothesis was that accumulation of root biomass through its

proliferation, and maintenance of membrane integrity either directly by adherence of [Ca^{2+}] or indirectly by the action of growth regulating substances impart tolerance to Mn^{2+} toxicity on rice crop in acid soil condition of Assam. Therefore, the present research work was carried out incorporating Cytokinin, Coconut milk and Calcium chloride as root dip treatments of upland (Boro) rice crop in presence of higher Mn in acid soil condition.

II. MATERIALS AND METHODS

A pot experiment (Jan-July, 2021) replicated thrice, laid out in completely randomized design with two factors i.e. Varieties and Treatments, was executed in the department of Crop Physiology, Assam Agricultural University, Jorhat ($26^0$45' N Latitude, $94^0$12' E Longitude having an altitude of 87 meter above mean sea level). The crop growing season was marked by the moderate rainfall (total 30.5 mm), cumulative bright sunshine (38.5 hours), and average RH (87-98%). The soil was acidic in nature, characterised by low pH (4.89 and 5.60), higher Mn contents (26.5 & 30ppm) initially and at harvest of the crop respectively. Twenty five days old seedlings of four rice varieties (Kanaklata, Bishnuprasad, Jyotiprasad and Numoli) were up-rooted, washed the roots gently so that tender roots were not injured but the excess soil debris were removed. Then, the roots were dipped overnight (≈12 hours) in respective solutions viz. $CaCl_2$ (500ppm), Cytokinin (100ppm), Coconut milk (10 times dilution with distilled water) and control (distilled water). These root dip treated seedlings (n= 3 per pot) were transplanted on to pots. N, P and K fertilizers were applied in the form of Urea, Single super phosphate (SSP) and Muriate of Potash (MoP) at the rate of 60:40:40 Kg ha^{-1}. Accordingly, 17.4g Urea (first dose of nitrogen), 66.9g of SSP and 8.9g of MoP were applied as basal, and the rest of the Urea (17.4g) was applied at the maximum tillering of the crop. A persistent irrigation (2-3cm) was given from the time of transplanting of rice seedlings till one week prior to the harvest. Weeding was done manually as and when required. 20 ppm Mn as $MnSO_4.7H_2O$ was applied as basal at vegetative stage. Chlorophyll contents in leaf were estimated by non-maceration, Dimethyl Sulfoxide (DMSO) method (Hiscox and Israelstam,1979). Mn content in shoot at heading stage (≈70DAS) and in grain at harvest was solubilised by digestion with a mixture of sulphuric and nitric acids, and its contents were estimated spectrophotometrically using Methylene Blue method (Beck *et al*.,2006) In vivo nitrate reductase activity was estimated experimentally at 540nm (Thimmaiah, 1999). Total carbohydrate content in grain was estimated following Anthrone method (Hedge *et al*., 1962). Exchangeable [Ca^{2+}] was estimated using the EDTA (Ethelene Diamine Tetra Acetic Acid) method (Jackson, 1973).

III. RESULTS AND DISCUSSION

The soil used in the pots was acidic in nature throughout the experiment. Of course, there was 12.67% increase in soil pH at harvest stage of the crop over the initial soil pH. The exchangeable Mn content in soil varied (26.5-30ppm) during the crop growth stages. So, the Mn status of the soil was medium (Basumatary *et al*., 2014). There were significant variations in total chlorophyll contents in plants due to the treatments with the growth regulating substances. At maximum tillering stage (Table 1a), the highest (1.795 mgg^{-1} fw) total chlorophyll content was recorded in plants treated with 20 ppm Mn plus 100 ppm Cytokinin, and the lowest (1.471 mgg^{-1} fw) was found in plants grown with 20 ppm Mn. On an average, among the genotypes, Kanaklata recorded the highest total chlorophyll content (1.861 mgg^{-1} fw), and the lowest was in Bishnuprasad (1.335 mgg^{-1} fw). At heading stage (Table 1b), too, the highest (1.762mgg^{-1}fw) total chlorophyll content was recorded in plants under 20 ppm Mn plus 100 ppm Cytokinin treatments, and the lowest (1.418mgg^{-1}fw) was estimated in plants treated with 20 ppm $MnSO_4$. The variety Kanaklata recorded the highest total chlorophyll content (1.791 mgg^{-1}fw)>Nomuli (1.671 mgg^{-1}fw). Overall, Jyotiprasad (1.553 mgg^{-1}fw) had the highest, and Bishnuprasad (1.316 mgg^{-1}fw) possessed the lowest total chlorophyll contents. There was decline in total chlorophyll content by (14.06%) at 20 ppm Mn application. However, the plants treated with 20 ppm Mn as basal plus 500 ppm $CaCl_2$ (RDT) (10.90%) had the highest value followed by (>) 20 ppm Mn as basal plus 100 ppm Cytokinin RDT (6.35%)> 20 ppm Mn as basal plus Coconut milk RDT (3.03%).

TABLE 1

EFFECTS OF CYTOKININ, COCONUT MILK AND CALCIUM CHLORIDE ROOT DIP TREATMENT (RDT) ALONG WITH Mn APPLICATION ON TOTAL CHLOROPHYLL (mg g^{-1}f.w.) AT DIFFERENT GROWTH STAGES

(a) **Maximum tillering stage**						(b) **Heading stage**						
Treatments → Variety ↓	**20 ppm Mnas basal from ($MnSO_4.3H_2O$)**	**20 ppm added Mn as basal + 100 ppm Cytokinin RDT**	**20 ppm added Mn as basal + coconut milk (10X dilution) RDT**	**20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT**	**Control (Without addition of 20ppm Mn as basal and without RDT**	**Mean**	**20 ppm Mnas basal from ($MnSO_4.3H_2O$)**	**20 ppm added Mn as basal + 100 ppm Cytokinin RDT**	**20 ppm added Mn as basal + coconut milk (10X dilution) RDT**	**20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT**	**Control (Without addition of 20ppm Mn as basal and without RDT**	**Mean**
Kanaklata	1.638	2.051	1.897	1.733	1.988	1.861	1.564	1.993	1.874	1.612	1.910	1.791
Bishnuprasad	1.248	1.424	1.348	1.292	1.362	1.335	1.224	1.389	1.344	1.270	1.350	1.316
Jyotiprasad	1.439	1.781	1.572	1.462	1.713	1.593	1.417	1.760	1.550	1.441	1.598	1.553
Nomuli	1.560	1.927	1.674	1.612	1.822	1.7719	1.465	1.905	1.652	1.590	1.743	1.671
Mean	1.471	1.795	1.623	1.525	1.721		1.418	1.762	1.605	1.478	1.650	
	T	**V**	**T X V**				**T**	**V**	**T X V**			
S.Ed (±)	0.024	0.022	0.049				0.023	0.026	0.051			
CD (0.05)	0.049	0.044	0.099				0.046	0.052	0.104			

TABLE 2

EFFECTS OF CYTOKININ, COCONUT MILK AND CALCIUM CHLORIDE ROOT DIP TREATMENT (RDT) ALONG WITH Mn APPLICATION ON CHLOROPHYLL a (mg g^{-1}f.w.) AT DIFFERENT GROWTH STAGES

(a) Maximum tillering stage						(b) Heading stage						
Treatments (T) → Variety (V) ↓	**20 ppm Mnas basal from ($MnSO_4.3H_2O$)**	**20 ppm added Mn as basal + 100 ppm Cytokinin RDT**	**20 ppm added Mn as basal + coconut milk (10X dilution) RDT**	**20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT**	**Control (Without addition of 20ppm Mn as basal and without RDT**	**Mean**	**20 ppm Mnas basal from ($MnSO_4.3H_2O$)**	**20 ppm added Mn as basal + 100 ppm Cytokinin RDT**	**20 ppm added Mn as basal + coconut milk (10X dilution) RDT**	**20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT**	**Control (Without addition of 20ppm Mn as basal and without RDT**	**Mean**
Kanaklata	0.817	1.003	0.946	0.863	0.991	0.924	0.799	0.976	0.934	0.851	0.949	0.902
Bishnuprasad	0.621	0.694	0.68	0.643	0.679	0.663	0.608	0.696	0.669	0.634	0.645	0.65
Jyotiprasad	0.717	0.888	0.779	0.729	0.854	0.794	0.705	0.878	0.769	0.718	0.748	0.74
Nomuli	0.778	0.961	0.834	0.803	0.908	0.857	0.695	0.949	0.822	0.792	0.832	0.818
Mean	0.733	0.886	0.810	0.760	0.858		0.702	0.875	0.801	0.749	0.794	
	T	**V**	**T X V**				**T**	**V**	**T X V**			
S.Ed (±)	0.013	0.012	0.026				0.014	0.012	0.028			
CD (0.05)	0.027	0.024	0.054				0.028	0.025	0.057			

At maximum tillering stage (Table 2a), the highest chlorophyll 'a' content was observed under the treatment 20 ppm Mn plus 100 ppm Cytokinin (0.886 $mgg^{-1}fw$)> Control treatment (0.858 $mgg^{-1}fw$) >20 ppm Mn plus Coconut milk (0.81 $mgg^{-1}fw$)>20 ppm Mn plus 500 ppm $CaCl_2$ (0.76 $mgg^{-1}fw$). The lowest chlorophyll 'a' content was recorded in the treatment with 20 ppm Mn application (0.733 $mgg^{-1}fw$). On an average, among the genotypes, Kanaklata recorded the highest chlorophyll 'a' content (0.924 $mgg^{-1}fw$), followed by genotype Nomuli (0.857 $mgg^{-1}fw$)>Jyotiprasad (0.754 $mgg^{-1}fw$), and the lowest of it was observed under the genotype Bishnuprasad (0.663 $mgg^{-1}fw$). At heading stage (Table 2b), the results revealed significant differences in chlorophyll 'a' content among the treatments. There was decrease in chlorophyll 'a' content (11.5%) at 20 ppm Mn application. Chlorophyll 'a' content increased under the treatments 20 ppm Mn as basal plus 100 ppm Cytokinin (RDT) (14.4%)>20 ppm Mn as basal plus 500 ppm $CaCl_2$RDT (5.66%)> 20 ppm Mn as basal plus Coconut milk RDT (0.87%).

At maximum tillering stage (Table 3a), the highest chlorophyll 'b' content was recorded under the treatment 20 ppm Mn plus 100 ppm Cytokinin (0.9$mgg^{-1}fw$)> Control (0.863 $mgg^{-1}fw$)> 20 ppm Mn plus Coconut milk (0.817 $mgg^{-1}fw$)> 20 ppm Mn plus 500 ppm $CaCl_2$ (0.765 $mgg^{-1}fw$). The lowest was observed under the treatment with 20 ppm Mn application (0.739 $mgg^{-1}fw$). At heading stage (Table 3b), the results revealed significant changes in chlorophyll 'b' content among the treatments. There was decrease in chlorophyll 'b' content by (15.36%) at 20 ppm Mn as basal application. There were increrases in Chlorophyll b in plants treated with 20 ppm Mn as basal plus 500 ppm $CaCl_2$RDT (13.7%)>20 ppm Mn as basal plus 100 pm Cytokinin RDT (4.94%)> 20 ppm Mn as basal plus Coconut milk RDT (4.60%).

One of the classical functions of the Cytokinin is the regulation of plastid development, but the underlying molecular mechanisms remain elusive. Cortleven *et al.,* (2016) employed a genetic approach to evaluate the role of Cytokinin and it's signalling pathway in the light-induced development of chloroplasts from etioplasts in Arabidopsis (*Arabidopsis thaliana*). Cytokinin increases the rate of greening and stimulates ultrastructural changes characteristic for the etioplast-to-chloroplast transition. In younger roots, Ca^{2+} is bound between an exchangeable state in the cell walls and the outside of the plasma lemma before rapidly penetrating the apoplast upon adsorption. Exchange adsorption between the apoplast and xylem tissues of whole stems or mass flow caused by transpiration stream are two ways that calcium might enter chloroplasts (Kirby and Pilbeam,1984). It has been demonstrated that Ca^{2+} in leaves reduces the loss of chlorophyll and the degradation of proteins in corn plants (Poovaiah and leopold,1973). Calcium has an anti-senescence effect on plants (Ferguson, 1983). The concentration of chlorophyll, however, rapidly dropped as the length of the growing season increased. Due to its dual purpose, calcium must have a threshold level at which it no longer benefits plants.

At maximum tillering stage (Table 4a), the treatment with 20ppm Mn plus 100 ppm Cytokinin recorded the highest NR activity (1.52)>20 ppm Mn plus Coconut milk (1.43)>control treatment (1.367)> 20 ppm Mn plus 500 ppm $CaCl_2$ (1.318), and the lowest was observed under the treatment with 20 ppm Mn application (1.124). On an average, comparing all varieties, genotype Kanaklata recorded the highest NR activity (1.442), followed by genotype Nomuli (1.435)> Bishnuprasad (1.302), and the lowest was observed in the variety Jyotiprasad (1.229). At heading stage, the highest NR activity was recorded under the treatment 20 ppm Mn plus 100 ppm Cytokinin (1.596)> 20 ppm Mn plus Coconut milk (1.502)> control plants (1.435)>20 ppm Mn plus 500 ppm $CaCl_2$ (1.3840), and the lowest was observed under the treatment 20 ppm Mn (1.155).

The results revealed significant variation in NR activity among the treatments at maximum tillering stage. There was decrease in NR activity by (17.77%) at 20 ppm Mn application as basal. But there was increase in NR activity under treatments 20 ppm Mn as basal plus 100 ppm Cytokinin as RDT (10.66%), 20 ppm Mn as basal plus Coconut milk as RDT (4.40%). There was decrease in NR activity under treatment 20 ppm Mn as basal plus 500 ppm $CaCl_2$as RDT (3.584%).

At Heading stage (Table 4b), the results revealed significant variation in NR activity among the treatments. There was decrease in NR activity (19.51%) at 20 ppm Mn application as basal. The NR activity at heading stage increased under the treatment 20 ppm Mn as basal plus 100 ppm Cytokinin as RDT (10.08%) and under treatment 20 ppm Mn as basal plus Coconut milk as RDT (4.46%). But NR activity decreased under treatment with 20 ppm Mn as basal plus 500 ppm $CaCl_2$ as RDT (3.55%). According to Santos *et al.,* (2014), cereals treated with Cytokinin exhibited a 40% increase in NR activity as compared to control. Hemalatha (2002) studied regulation of NR activity in rice by growth regulator namely Kinetin. The effect of three growth regulators, namely kinetin, 6 benzyl adenine, 2 chloro ethyl trimethyl ammonium chloride at three concentrations (10-6 M, 5 x 10-5 M 10-4 M) was studied on the catalytic activity of NR enzyme in green and etiolated seedlings. A concentration of 5 x 10^{-5} M was optimal for all the growth regulators treatments. All the growth regulators stimulated NR activity effectively at 5 x 10^{-5}M concentration in both etiolated and green seedlings and had an additive effect when supplemented by NO^{-}_{3} up to 140% to 160%. There were 99.2% and 93.4% inhibition of NR activity in etiolated and green seedlings, respectively when treated with eukaryotic 80S ribosome protein synthesis inhibitor cycloheximide.

TABLE 3

EFFECTS OF CYTOKININ, COCONUT MILK AND CALCIUM CHLORIDE ROOT DIP TREATMENT (RDT) ALONG WITH Mn APPLICATION ON CHLOROPHYLL b (mg g^{-1}f.w.) AT DIFFERENT GROWTH STAGES

(a) Maximum tillering stage						(b) Heading stage						
Treatments (T) → Variety (V) ↓	**20 ppm Mnas basal from ($MnSO_4.3H_2O$)**	**20 ppm added Mn as basal + 100 ppm Cytokinin RDT**	**20 ppm added Mn as basal + coconut milk (10X dilution) RDT**	**20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT**	**Control (Without addition of 20ppm Mn as basal and without RDT**	**Mean**	**20 ppm Mnas basal from ($MnSO_4.3H_2O$)**	**20 ppm added Mn as basal + 100 ppm Cytokinin RDT**	**20 ppm added Mn as basal + coconut milk (10X dilution) RDT**	**20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT**	**Control (Without addition of 20ppm Mn as basal and without RDT**	**Mean**
Kanaklata	0.822	1.028	0.951	0.87	0.996	0.933	0.765	1.017	0.941	0.76	0.96	0.889
Bishnuprasad	0.627	0.714	0.685	0.648	0.683	0.672	0.617	0.704	0.675	0.637	0.671	0.661
Jyotiprasad	0.723	0.893	0.790	0.733	0.860	0.800	0.712	0.882	0.791	0.723	0.849	0.789
Nomuli	0.783	0.966	0.841	0.809	0.914	0.863	0.768	0.956	0.831	0.798	0.904	0.852
Mean	0.739	0.900	0.817	0.765	0.863		0.716	0.890	0.807	0.730	0.846	
	T	**V**	**T X V**				**T**	**V**	**T X V**			
S.Ed (±)	0.012	0.011	0.038				0.019	0.017	0.038			
CD (0.05)	0.024	0.022	0.024				0.039	0.035	0.078			

TABLE 4

EFFECTS OF CYTOKININ, COCONUT MILK AND CALCIUM CHLORIDE ROOT DIP TREATMENT (RDT) ALONG WITH Mn APPLICATION ON NR ACTIVITY (µmole NO_3^- g^{-1} fw hr^{-1}) AT DIFFERENT GROWTH STAGES

(a) Maximum tillering stage						(b) Heading stage						
Treatments (T) → Variety (V) ↓	**20 ppm Mnas basal from ($MnSO_4.3H_2O$)**	**20 ppm added Mn as basal + 100 ppm Cytokinin RDT**	**20 ppm added Mn as basal + coconut milk (10X dilution) RDT**	**20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT**	**Control (Without addition of 20ppm Mn as basal and without RDT**	**Mean**	**20 ppm Mnas basal from ($MnSO_4.3H_2O$)**	**20 ppm added Mn as basal + 100 ppm Cytokinin RDT**	**20 ppm added Mn as basal + coconut milk (10X dilution) RDT**	**20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT**	**Control (Without addition of 20ppm Mn as basal and without RDT**	**Mean**
Kanaklata	1.099	1.649	1.562	1.447	1.452	1.442	1.154	1.731	1.639	1.519	1.525	1.513
Bishnuprasad	1.050	1.452	1.367	1.274	1.369	1.302	1.102	1.525	1.435	1.338	1.437	1.367
Jyotiprasad	1.062	1.364	1.244	1.160	1.316	1.229	1.115	1.432	1.305	1.217	1.382	1.29
Nomuli	1.285	1.616	1.550	1.393	1.331	1.435	1.249	1.696	1.627	1.462	1.397	1.486
Mean	1.124	1.520	1.430	1.318	1.367		1.155	1.596	1.502	1.384	1.435	
	T	**V**	**T X V**				**T**	**V**	**T X V**			
S.Ed (±)	0.015	0.013	0.029				0.025	0.022	0.05			
CD (0.05)	0.030	0.027	0.060				0.051	0.045	0.101			

TABLE 5
CYTOKININ, COCONUT MILK AND CALCIUM CHLORIDE ROOT DIP TREATMENT (RDT) ALONG WITH Mn APPLICATION ON CARBOHYDRATE CONTENT IN GRAINS AT HARVEST

Carbohydrate content ($mgg^{-1}dw$)						
Treatment → / Variety ↓	20 ppm Mnas basal from ($MnSO_4.3H_2O$)	20 ppm added Mn as basal + 100 ppm Cytokinin RDT	20 ppm added Mn as basal + coconut milk (10X dilution) RDT	20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT	Control (Without addition of 20ppm Mn as basal and without RDT	Mean
Kanaklata	8.800	9.620	9.290	9.110	9.120	9.202
Bishnuprasad	4.600	6.780	5.670	5.400	4.890	5.470
Jyotiprasad	8.310	9.240	8.870	8.540	8.720	8.740
Nomuli	8.360	9.510	9.100	8.900	8.500	8.901
Mean	7.530	8.790	8.230	8.010	7.830	
	Treatment	**Variety**	**T X V**			
S.Ed (±)	0.091	0.082	0.182			
CD (0.05)	0.185	0.166	0.370			

The highest carbohydrate content in grain (Table 5) was observed in treatment 20ppm Mn^{2+} plus 100 ppm Cytokinin (8.79)>20ppm Mn + Coconut milk (zeatin):10X dilution (8.23)>20 ppm Mn application (7.83)>20 ppm Mn + 500 ppm $CaCl_2$ (8.01). The lowest carbohydrate content was recorded under control treatment (7.53). On an average, among the rice varieties, the highest amount of carbohydrate content was recorded in the genotype Kanaklata (9.202)>Nomuli (8.901)> Jyotiprasad (8.738), and the least carbohydrate content was recorded in the genotype Bishnuprasad (5.47). Overall, there was higher carbohydrate content in grain in varieties under treatment of 20ppm Mn plus 100 ppm Cytokinin as compared to other treatments. The results revealed significant changes in carbohydrate content among the treatments. As compared to control, there was decrease in carbohydrate content by (3.03%) under 20 ppm Mn as basal treatment. On the otherhand, at RDT with the plant growth regulating substances along with the basal Mn enhenced the carbohydrate content in grains *viz.* 20 ppm Mn plus 100 ppm Cytokinin (10.92%), 20 ppm Mn plus Coconut milk (4.86%) and 20 ppm Mn plus 500 ppm $CaCl_2$ (2.24%). Leaf photosynthesis regulating the production of carbohydrate, accounts for most of the variations in biomass production and yield (Yoshida and Horie 2009; Evans, 1993; Sinclair *et al.,* 2004). Recent studies indicate that growth rate around heading stage is critically related with final yield in rice (Takai *et al.,*2006; Horie *et al.,* 2006). Cytokinins play a key role in preserving the structure and function of the photosynthetic machinery under stress conditions (Cherniad'ev, 2009). Cytokinin increases sink activities by stimulating assimilate accumulation in chloroplasts of older leaves (Criado *et al.,* 2009). Cytokinin has roles in the biosynthesis of Chlorolphyll, stimulation of tetrapyrole biosynthesis, chloroplast transcription (Zubo *et al.,* 2008), and enhancement of photosynthetic efficiency (Yaronskaya *et al.,* 2006).

The highest [Mn] in grain (Table 6a) was found in treatment 20 ppm Mn (103.58)>20 ppm Mn plus 100 ppm Cytokinin (89.47ppm) >20 ppm Mn plus coconut milk (84.81) :10x dilution>20 ppm Mn application plus 500 ppm $CaCl_2$, and the lowest (17.39) was in controlled plants. On an average among genotypes, the highest [Mn] was observed in the genotype Kanaklata (88.442) > Nomuli (80.494), and the lowest was observed in the variety Bishnuprasad (62.116) > Jyotiprasad (68.604). The results revealed significant variations of [Mn] in grains among the treatments (Table 6a). There was increase in [Mn] in grains with all the treatments *viz.* 20 ppm Mn treatment as basal (83.2%), and in treatments with RDT with the plant growth regulating substances along with Mn as basal 20 ppm Mn plus 100 ppm Cytokinin (80.56%), 20 ppm Mn plus Coconut milk (79.49%) and 20 ppm Mn plus 500 ppm $CaCl_2$ (78.13%).

At maximum tillering stage, the highest [Ca^{2+}] in plant roots (Table 6b) was observed in the treatment with 20 ppm Mn plus 500 ppm $CaCl_2$ (89.75ppm)>20 ppm Mn plus 100 ppm Cytokinin (67.22ppm)>treatment with 20 ppm Mn plus Coconut milk (63.395ppm)> 20 ppm Mn application (32.9ppm), and the lowest [Ca^{2+}] in plant roots was observed under the control treatment (22.975ppm). On an average, among the genotypes, the highest [Ca^{2+}] in plant roots was observed in the variety Kanaklata (59.35ppm)>Nomuli (56.093ppm)> Jyotiprasad (54.575), and the least [Ca^{2+}] in plant roots was presented by the variety Bishnuprasad (50.975ppm). The result revealed significant variations in [Ca^{2+}] in roots at maximum tillering stage. There was increase in [Ca^{2+}] in roots with all the treatments *viz.* 20 ppm Mn treatment (28.34%) as basal and at RDT with PGRS along with basal Mn 20 ppm Mn plus 100 ppm Cytokinin (65.82%), 20 ppm Mn plus Coconut milk (63.75%) and 20 ppm Mn plus 500 ppm $CaCl_2$ (74.40%).

TABLE 6

EFFECTS OF CYTOKININ, COCONUT MILK AND CALCIUM CHLORIDE ROOT DIP TREATMENT (RDT) ALONG WITH MN APPLICATION ON Mn CONTENT IN GRAIN AT HARVEST AND Ca^{2+} CONTENT IN ROOTS (ppm) AT MAXIMUM TILLERING STAGE

(a) Mn^{2+} content (ppm) in grain						(b) Ca^{2+} content (ppm) in roots						
Treatments (T) → Variety (V) ↓	**20 ppm Mnas basal from ($MnSO_4.3H_2O$)**	**20 ppm added Mn as basal + 100 ppm Cytokinin RDT**	**20 ppm added Mn as basal + coconut milk (10X dilution) RDT**	**20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT**	**Control (Without addition of 20ppm Mn as basal and without RDT**	**Mean**	**20 ppm Mnas basal from ($MnSO_4.3H_2O$)**	**20 ppm added Mn as basal + 100 ppm Cytokinin RDT**	**20 ppm added Mn as basal + coconut milk (10X dilution) RDT**	**20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT**	**Control (Without addition of 20ppm Mn as basal and without RDT**	**Mean**
Kanaklata	127.500	102.200	100.100	95.200	17.210	88.442	36.633	72.000	68.847	93.930	25.330	59.350
Bishnuprasad	80.030	75.600	71.420	68.500	15.030	62.116	28.567	62.880	57.930	85.360	20.133	50.975
Jyotiprasad	85.900	86.710	80.200	72.800	18.410	68.604	34.100	66.067	62.133	88.540	22.033	54.575
Nomuli	120.900	93.400	87.530	81.710	18.930	80.494	32.300	67.933	64.667	91.167	24.400	56.093
Mean	103.580	89.470	84.810	79.550	17.390		32.900	67.220	63.395	89.750	22.975	
	T	**V**	**T X V**				**Treatment**	**Variety**	**T X V**			
S.Ed (±)	0.973	0.871	1.947				0.324	0.290	0.648			
CD (0.05)	0.420	0.324	1.234				0.658	0.588	1.315			

TABLE 7

EFFECTS OF CYTOKININ, COCONUT MILK AND CALCIUM CHLORIDE ROOT DIP TREATMENT (RDT) ALONG WITH Mn APPLICATION ON Ca^{2+} CONTENTS IN ROOTS (ppm) AT HEADING AND HARVEST STAGES

(a) Ca^{2+} content (ppm) at Heading stage						(b) Ca^{2+} content (ppm) at harvest stage						
Treatments (T) → Variety (V) ↓	**20 ppm Mnas basal from ($MnSO_4.3H_2O$)**	**20 ppm added Mn as basal + 100 ppm Cytokinin RDT**	**20 ppm added Mn as basal + coconut milk (10X dilution) RDT**	**20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT**	**Control (Without addition of 20ppm Mn as basal and without RDT**	**Mean**	**20 ppm Mnas basal from ($MnSO_4.3H_2O$)**	**20 ppm added Mn as basal + 100 ppm Cytokinin RDT**	**20 ppm added Mn as basal + coconut milk (10X dilution) RDT**	**20 ppm added Mn as basal + 500 ppm $CaCl_2$ RDT**	**Control (Without addition of 20ppm Mn as basal and without RDT**	**Mean**
Kanaklata	31.733	65.667	61.633	90.687	22.380	54.420	30.133	61.617	56.367	84.030	18.400	50.11
Bishnuprasad	24.900	58.633	54.600	82.843	18.133	47.822	22.300	54.800	51.76	77.967	14.700	44.307
Jyotiprasad	28.600	61.400	58.367	84.970	19.850	50.637	25.8	56.963	53.067	81.467	17.00	46.859
Nomuli	29.900	63.730	61.133	87.317	22.113	52.839	27.133	59.033	54.707	83.133	18.733	48.56
Mean	28.783	62.358	58.933	86.454	20.618		26.342	58.103	53.992	81.65	17.208	
	Treatment	**Variety**	**T X V**				**Treatment**	**Variety**	**T X V**			
S.Ed (±)	0.354	0.316	0.707				0.389	0.348	0.778			
CD (0.05)	0.717	0.642	1.435				0.789	0.766	1.578			

At heading stage (Table 7a), the maximum [Ca^{2+}] in plant roots was recorded under the treatment 20 ppm Mn plus 500 ppm $CaCl_2$ (86.454ppm)> 20 ppm Mn plus 100 ppm Cytokinin (62.358ppm)>20 ppm Mn plus Coconut milk (zeatin) (58.933ppm)>20 ppm Mn application (28.783ppm), and the lowest [Ca^{2+}] in roots were observed in the control treatment (20.618ppm). On an average, among the genotypes, the genotype Kanaklata recorded the highest [Ca^{2+}] in plant roots (54.42ppm)>Nomuli (52.839ppm)> Jyotiprasad (50.637ppm), and the least [Ca^{2+}] in plant roots was observed in the variety Bishnuprasad (47.822ppm). At heading stage, the results revealed significant variations of [Ca^{2+}]. There was increase in [Ca^{2+}] in roots with all the treatments *viz.* 20 ppm Mn as basal (28.36%) and at RDT with PGRS along with basal Mn 20 ppm Mn plus 100 ppm Cytokinin (66.93%), 20 ppm Mn plus Coconut milk (65.01%) and 20 ppm Mn plus 500 ppm $CaCl_2$ (76.15%).

At the time of harvest (Table 7b), the highest [Ca^{2+}] in plant roots was observed under the treatment 20 ppm Mn plus 500 ppm $CaCl_2$ (81.65ppm)>20 ppm Mn plus 100 ppm Cytokinin (58.103ppm)>treatment 20 ppm Mn plus Coconut milk (53.992ppm)>20ppm Mn application (26.342ppm), and the lowest [Ca^{2+}] was observed under the Control treatment (17.208ppm). At harvest stage, [Ca^{2+}] in roots varied significantly. There was increase in [Ca^{2+}] in roots with all the treatments *viz.* 20 ppm Mn treatment as basal (34.67%), and at RDT along with basal Mn and the growth regulating substances *viz.*20 ppm Mn plus 100 ppm Cytokinin (70.38%), 20 ppm Mn plus Coconut milk (68.128%) and 20 ppm Mn plus 500 pm $CaCl_2$ (78.92%).

Sharma (2002) reported that there were substantial increases in [Ca^{2+}] by 55-97% in the roots after root dip treatments in a pot experiment. The increment was proportional to the increase in the $CaCl_2$ concentration (100-1000 ppm) in the treatment. Sharma (2002) also reported that [Ca^{2+}] in all parts e.g., pods, leaves, stems and roots increased in commensuration with $CaCl_2$ supply.

Ca^{2+} is an important nutrient for root development (White and Broadly, 2003; Mengel and Kirby, 1982; Sharma, 2002). Other way, Ca^{2+} protects membrane from free radical or peroxidative break down when only a large amount of Ca^{2+} binding is present in membranes. Ca^{2+} aids packing of lipids and brings about their aggregation (Ohishi and Ito, 1974). Ca^{2+} bridges membranes by phosphate and COOH- groups to maintain its permeability (Legge *et al.*,1984; Epstein, 1992; Bharali and Bates, 2014; Bharali et al., 2015). Thus, the study enlightened the actions of Cytokinin (i.e. enhancement of root biomass) and Calcium (probably, the membrane stability) which are regarded as the features of the tolerance of rice crops under higher [Mn] in acid soil situation.

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