

Effects of Manganese on Some Biochemical Indices of Rice (*Oryza sativa* L.) Crop in Acid Soil Condition

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Received: 1.02.2021 | Revised: 4.03.2021 | Accepted: 12.03.2021

ABSTRACT

It's known that Manganese (Mn) is toxic when its concentration goes beyond a critical limit of 2-3ppm in plants. In Assam soil, available Mn concentration is 3-52ppm. There is paucity of information how the excess Mn brings about biochemical changes in upland rice crop grown in acid soil condition of Assam. A dose response study of Mn (0, 10, 20, 30 ppm Mn as $MnSO_4 \cdot H_2O$) on ten upland rice genotypes (Kanaklata, Mulagabharu, Kapilee, Disang, Kolong, Joymoti, Jyoti Prasad, Luit, Lachit and Chilarai) was accomplished applying Mn as foliar spray (each $1000cm^3$) through panicle initiation to heading stage (60-70 days after sowing: DAS). At lower dose of Mn treatment, there were higher increments in total Chlorophyll (27.17%), Chlorophyll-a (26.72%) and Chlorophyll-b (27.77%), NR activity (35.39%), Carbohydrate content in grain (9.04%), and Cell membrane stability (37.06%). The highest dose of Mn treatment (30ppm) reduced all these physiological attributes in the study. However, Mn content in shoots (87%) and grain (87.9%), distribution of Mn contents in intercellular (84.21%), exchangeable locations (74.84%) increased significantly by 30ppm Mn treatment. Based on the overall, Kanaklata followed by Chilarai was superior in terms of the biochemical traits deviated by Mn treatments in the present studies.

Keywords: Acid soil, Carbohydrate, Chlorophyll, Exchangeable, Intercellular, Manganese, Nitrate Reductase, Cell membrane stability.

INTRODUCTION

Manganese (Mn) being an essential micronutrient imparts metabolic roles within different plant cell compartments. However, Mn beyond a critical limit interfere biochemical processes, and Mn becomes toxic to all susceptible plants on acid soils in

contrast to calcareous soils, and in organic soils (Alejandro et al., 2020). The manifestation of both interveinal and marginal leaf chlorosis, necrotic leaf spots are plausible due to the biochemical changes caused by excess Mn in plants (Mora et al., 2009).

Cite this article: Yomso, A., & Bharali, B. (2021). Effects of Manganese on Some Biochemical Indices of Rice (*Oryza sativa* L.) Crop in Acid Soil Condition, *Ind. J. Pure App. Biosci.* 9(2), 103-114. doi: <http://dx.doi.org/10.18782/2582-2845.8613>

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A supra optimal concentration of Mn reduces photo assimilation rate as a result of pigment disruption in plants (Hauck et al., 2003). In acidic soil of Assam (4.7 Mha), Mn is abundant (3-52ppm) with a critical limit of 2-3 ppm, whereas in plants, the critical concentration of Mn is 15-20 ppm g⁻¹ dry weight (Basumatary et al., 2014). Numerous biochemical processes are influenced by Mn in plants, however a little is known about how distribution of Mn in the cellular locations does modulate some of the metabolic processes viz., Chlorophylls synthesis, NR activity in leaf, carbohydrate contents in grain of upland rice crop is scanty. How does level of cell membrane stability supported by cellular Mn fractions become the feature of tolerance or susceptibility of upland rice crop to toxic concentration of Mn, was also poorly understood, and aimed to unveil these in the investigation.

MATERIALS AND METHODS

A pot experiment replicated twice following two factorial 'CRD: completely randomized design' was conducted during the Rabi season of Assam (January-June, 2019) at the Department of Crop Physiology, Assam Agricultural University, Jorhat. The experimental site is situated at 26°45' N latitude, 94°12' E longitude having an elevation of 87 m above mean sea level. The crop growing season was marked by the moderate total rainfall (29.2 mm), cumulative bright sunshine (34.5 hours), and average RH (84-98%). In nature, Assam soil was characterised by low pH (4.92&5.62), higher Mn contents (30.2&27.426ppm) preliminarily and at harvest of the crop respectively. The nursery seedlings of ten rice genotypes viz., Kanaklata, Mulagabharu, Kapilee, Disang, Kolong, Joymoti, Luit, Jyoti prasad, Lachit, and Chilarai were raised in pots using acid mineral soil mixed with FYM @50:50. The seedlings (21 days old) were transplanted in the experimental pots filled with a mixture of acid mineral soil and FYM @50:50. As a part of the crop husbandry, the crop was fertilised

with NPK @60:40:20 Kgha⁻¹ as Urea (23.25g=half dose of N as), SSP(89.25g) & MoP (11.857g) as basal; and rest half of N (11.625g urea) while the crop was at the maximum tillering stage. A regular irrigation (2-3cm water) was supplied from transplanting to harvesting time of the crop. The experimental site was kept weed free and prophylactic measures were followed as and when required. The abaxial leaves were misted with Mn (@0,10,20 and 30ppm) as MnSO₄.H₂O (MW:159.08g) solution (1000cm³) in three splits during tillering to heading stage (60- 70 DAS) using a hand sprayer. All the treatments were given in an isolation manner to avoid any contamination. Chlorophyll contents in leaf were estimated by non-maceration, Dimethyl Sulphoxide (DMSO) method (Arnon, 1949). 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 using the new extraction spectrophotometric method with Methylene Blue (MB) referred by 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). Mn within the leaf cells were separated into intercellular and exchangeable fractions using a sequential elution procedure (Bates, 1992; Badacsonyi et al., 2000; & Bharali & Bates, 2002). Samples of hydrated apices were placed in 100 X 25 mm glass specimen tubes ('master tubes') paired with identical ('slave') tubes to which extracts could be added, and linked to them via fine plastic tubes. Solutions were added and withdrawn from the master tubes using a manifold system attached to a reversible pump, and during incubation, the samples were agitated by bubble streams. Freely soluble ions on the samples were removed with three serial incubations (each 10 min, 20 cm³) with double distilled water (DDW). Exchangeable Mn held on the fixed negative charges of the cell walls were eluted by two treatments (each 1h, 20 cm³) with 25

Mm SrCl₂ (Wells and Brown, 1987). After extraction of Mn, the leaf samples were oven dried (at 80°C) to a constant weight. Cell Membrane stability (CMS) was measured for cellular injury caused by lethal Mn, if any, as suggested by Sullivan and Rose (1972). The CMS was determined using the same sample extracts for inter and exchangeable cellular ions. The electrical conductivity readings of these extracts for the samples collected from the experimental treatments were used to compute CMS.

RESULTS AND DISCUSSION

The soil used in the pots was acidic in nature as the pH (4.92-5.62) was consistent throughout the experiment. Of course, there was 20.1% increase in soil pH at harvest stage of the crop over the initial soil pH. The exchangeable Mn content in soil varied (27.426 ppm to 32.2-ppm) during the crop growth stages. So, the Mn status of the soil was medium (Basumatary et al., 2014). Despite the congenial climatic conditions during the growing season, biochemical deviations were brought about by the supra

optimal concentration of Mn in the upland rice genotypes.

There were significant effects of Mn on total chlorophyll, Chlorophyll-a and Chlorophyll-b contents in the varieties (Table 1, 2, and 3). The highest total chlorophyll content in leaf tissues was found at 10ppm Mn (1.810 mg g⁻¹f.w.) followed by (>) control (1.473 mg g⁻¹f.w.)>20 ppm Mn (1.467 mg g⁻¹f.w.), and the least was at 30ppm Mn (1.410 mg g⁻¹f.w.). On an average, the highest total chlorophyll content in leaf tissues was recorded in Kanaklata (1.927 mg g⁻¹f.w.)> Luit (1.705 mg g⁻¹f.w.)>Chilarai (1.694 mg g⁻¹f.w.), while the lowest was recorded in Kolong (1.109 mg g⁻¹f.w.). The total chlorophyll content increased significantly by 10ppm Mn in Kanaklata (27.17%)>Disang (23.68%) as compared with control. There is disorganisation of the normal arrangement of the grana in chloroplast (Singh et al 2017; Ohki, 1988) in presence of critical Mn concentration. The oxidation of Mn in chloroplasts by light activated chlorophyll generates reactive oxygen species, and thereby it degrades chlorophyll (Balidisserotto et al., 2007).

Table 1: Total chlorophyll content in leaf tissues at maximum tillering to heading stage (~70 days after sowing) of rice crop under different manganese treatments

Total chlorophyll content in leaf tissues (mg g ⁻¹ f.w.)					
Treatments (T)→ Varieties (V)↓	0 ppmMn (Control)	10 ppm Mn	20 ppm Mn	30 ppm Mn	Mean
Kanaklata	1.675	2.300	2.145	1.590	1.927
Mulagabharu	1.535	1.910	1.675	1.352	1.618
Kapilee	1.580	1.930	1.515	1.291	1.528
Disang	1.305	1.710	1.270	1.285	1.342
Kolong	1.210	1.510	1.260	1.150	1.109
Joymoti	1.350	1.505	1.450	1.213	1.379
Luit	1.600	1.958	1.850	1.415	1.705
Jyoti prasad	1.380	1.750	1.505	1.307	1.535
Lachit	1.450	1.680	1.580	1.445	1.588
Chilarai	1.650	1.855	1.781	1.490	1.694
Mean	1.473	1.810	1.467	1.410	
	T	V	T X V		
SEd (±)	0.015	0.010	0.031		
CD	0.031	0.020	0.063		

The highest chlorophyll-a content in leaf tissues was produced, too, by 10ppm Mn (0.900 mg g⁻¹f.w.)> control (0.732 mg g⁻¹f.w.)>20 ppm Mn (0.730 mg g⁻¹f.w.), and the least was at

30ppm Mn (0.710 mg g⁻¹f.w.). On an average, among the varieties, the highest chlorophyll-a content in leaf tissues was recorded in Kanaklata (0.955 mg g⁻¹f.w.)> Luit (0.848 mg g⁻¹f.w.)

¹f.w.)>Chilarai (0.842 mg g⁻¹f.w.), while the lowest was recorded in Kolong (0.541 mg g⁻¹f.w.). So, Chlorophyll-a content increased significantly from 3.17% (Disang) at 10ppm Mn to 26.72% (Kanaklata) up to 20ppm Mn, but at 30 ppm Mn, all the rice varieties showed significant reductions (1.56 to 10.79%) in chlorophyll-a content as compared to the control. In case of Chlorophyll-b, the highest chlorophyll-b content in leaf tissues was recorded in Kanaklata (0.963 mg g⁻¹f.w.) > Luit (0.856 mg g⁻¹f.w.) > Chilarai (0.850 mg g⁻¹f.w.), while the lowest was recorded in Kolong (0.559 mg g⁻¹f.w.). There was significant increase in Chlorophyll-b from 0.44% (Kapili) to 27.77% (Kanaklata) up to 20ppm Mn. However, a drastic reduction of Chlorophyll-b (0.27 to 13.40%) was found in the genotypes at 30 ppm Mn as compared to the control. At an excess of Mn, older leaves of rice developed brown spots and mild interveinal chlorosis in addition to depression in growth. Both deficiency and excess of manganese resulted in low concentration of Chlorophyll-a and Chlorophyll-b as well as reduced Hill reaction activity in leaves (Lidon et al., 2004; & Rezai & Farboodnia 2008).

A significant variation of Mn content of shoot was found among the rice genotypes due to the Mn treatments (Table 4). The highest Mn content was estimated at 30ppm Mn (135.24 mgkg⁻¹ d.w.) > 20ppm Mn (113.36 mgkg⁻¹ d.w.)>10ppm Mn (73.17 mgkg⁻¹ d.w.)> control (37.91 mgkg⁻¹ d.w.). On an average, among the genotypes, the highest Mn content in shoot was recorded in Joymoti (130.0 mgkg⁻¹

¹ d.w.)>Chilarai (125.26 mgkg⁻¹ d.w.)>Kanaklata (103.92 mgkg⁻¹ d.w.), while the lowest was recorded in Jyoti prasad (71.47 mgkg⁻¹ d.w.). Mn content in shoot was increased significantly by 30 ppm Mn in Kanaklata (87.00%) followed by Kanaklata (85.20%) at the treatment 20 ppm Mn as compared to the control. The lowest increase in Mn content in shoot was in variety Lachit (26.02%) at 10 ppm Mn treatment as compared to the control. Fageria et al. (2008) stated that Mn concentrate in shoots and grains commensuration with the increases in Mn concentration in the treatments of rice crop. Similarly, significant variations of Mn contents in grain were found among the genotypes due to the Mn treatments (Table 5). The highest Mn content in grain was estimated in Joymoti (110.55 mgkg⁻¹ d.w.)>Chilarai (111.7 mgkg⁻¹ d.w.)>Kanaklata (88.06 mgkg⁻¹ d.w.), while the lowest Mn content in grain was recorded in Kolong (50.92 mgkg⁻¹ d.w.). However, Mn content in grain increased significantly in 30 ppm Mn treatment in variety Kanaklata (87.9%)>Kanaklata (86.51%) in 20 ppm Mn. The lowest increase in Mn content in grain was recorded in 10 ppm Mn treated Kapilee (13.53%). Overall, there was higher Mn content in grain in the genotypes at 30ppm Mn treated plants as compared to other doses of Mn treatment. Mn content in grains might vary due to differences in concentrations of Mn in treatments or the crop grown at different sites having graded level of Mn in soil (Marcar & Graham, 1987).

Table 2: Variation of chlorophyll-a content in leaf tissues at maximum tillering to heading stage (~70 days after sowing) of rice crop under different manganese treatments

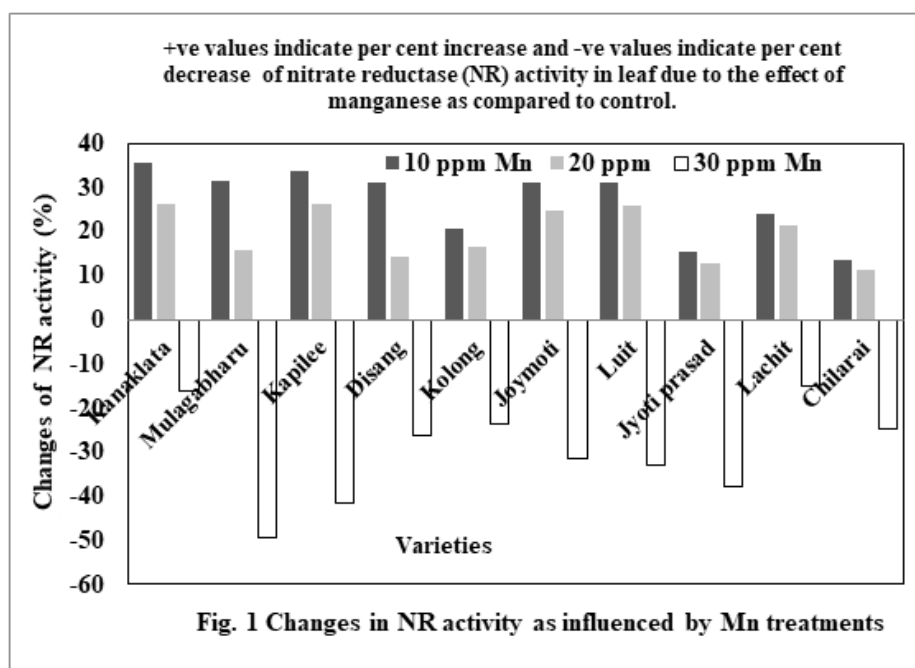
Leaf chlorophyll a contents (mg g ⁻¹ f.w.)					
Treatments (T)→ Varieties (V)↓	0 ppmMn (Control)	10 ppm Mn	20 ppm Mn	30 ppm Mn	Mean
Kanaklata	0.828	1.130	1.070	0.793	0.955
Mulagabharu	0.764	0.952	0.834	0.673	0.796
Kapilee	0.787	0.963	0.743	0.754	0.761
Disang	0.650	0.851	0.630	0.640	0.667
Kolong	0.600	0.750	0.577	0.570	0.541
Joymoti	0.672	0.749	0.722	0.644	0.696
Luit	0.796	0.975	0.733	0.721	0.848
Jyoti prasad	0.688	0.873	0.750	0.651	0.765
Lachit	0.723	0.837	0.787	0.719	0.791
Chilarai	0.821	0.923	0.886	0.741	0.842
Mean	0.732	0.900	0.730	0.710	
	T	V	T X V		
SEd (±)	0.008	0.013	0.026		
CD	0.017	0.026	0.052		

Table 3. Variation of chlorophyll-b content in leaf tissues at maximum tillering to heading stage (~70 days after sowing) of rice crop under different manganese treatments

Chlorophyll b content in leaf tissue (mg g ⁻¹ f.w.)					
Treatments (T)→	0 ppmMn (Control)	10 ppm Mn	20 ppm Mn	30 ppm Mn	Mean
Varieties (V)↓					
Kanaklata	0.832	1.152	1.074	0.797	0.963
Mulagabharu	0.770	0.957	0.840	0.679	0.811
Kapilee	0.793	0.968	0.548	0.760	0.767
Disang	0.634	0.855	0.640	0.644	0.668
Kolong	0.610	0.760	0.687	0.580	0.559
Jyomoti	0.678	0.755	0.728	0.644	0.701
Luit	0.804	0.983	0.929	0.711	0.856
Jyoti Prasad	0.692	0.877	0.754	0.656	0.769
Lachit	0.728	0.843	0.793	0.726	0.797
Chilarai	0.829	0.931	0.894	0.749	0.850
Mean	0.737	0.908	0.736	0.716	
	T	V	T X V		
SEd (±)	0.005	0.008	0.016		
CD	0.011	0.017	0.033		

There were significant reductions of NR activity commensuration with the Mn concentrations in the treatment (**Fig.1**). The highest NR activity in leaf was found at 10ppm (1.59 $\mu\text{mole NO}_3^- \text{g}^{-1} \text{f.w. hr}^{-1}$) > 20ppm (1.43 $\mu\text{mole NO}_3^- \text{g}^{-1} \text{f.w. hr}^{-1}$) > control (1.15 $\mu\text{mole NO}_3^- \text{g}^{-1} \text{f.w. hr}^{-1}$), and the lowest was at 30ppm Mn (0.85 $\mu\text{mole NO}_3^- \text{g}^{-1} \text{f.w. hr}^{-1}$). Among the genotypes, the highest NR activity was recorded in Luit (1.37 $\mu\text{mole NO}_3^- \text{g}^{-1} \text{f.w. hr}^{-1}$) > Jyomoti (1.36 $\mu\text{mole NO}_3^- \text{g}^{-1} \text{f.w. hr}^{-1}$) > Disang (1.34 $\mu\text{mole NO}_3^- \text{g}^{-1} \text{f.w. hr}^{-1}$), while the lowest was recorded in Kolong (1.12

$\mu\text{mole NO}_3^- \text{g}^{-1} \text{f.w. hr}^{-1}$). NR activity increased in Kanaklata at 10ppm Mn (35.39%) and 20ppm Mn (26.41%) treatments, but it declined at 30ppm Mn (15.07 to 49.53%) treated genotypes. Leidi and Gomez (1985) studied the role of Mn in the regulation of soybean NR activity under light and dark conditions. An indirect role of Mn on activation of NR was reported where Mn deficient plants had lower activation of NR than the plants supplied with Mn irrespective of light regimes.



Carbohydrate contents in grains at harvest reduced significantly due to Mn treatments (Fig.2). The highest carbohydrate content in grain was found at 10ppm Mn ($7.69 \text{ mgg}^{-1} \text{ d.w}$) > 20ppm ($7.26 \text{ mgg}^{-1} \text{ d.w}$) > control ($7.24 \text{ mgg}^{-1} \text{ d.w}$), and the lowest was at 30ppm Mn ($6.55 \text{ mgg}^{-1} \text{ d.w}$). On an average, among the genotypes, the highest carbohydrate content was in Kanaklata ($9.06 \text{ mgg}^{-1} \text{ d.w}$) > Chilarai ($8.68 \text{ mgg}^{-1} \text{ d.w}$) > Jyoti prasad ($8.45 \text{ mgg}^{-1} \text{ d.w}$), while the lowest carbohydrate content in grain was recorded in Kolong ($4.91 \text{ mgg}^{-1} \text{ d.w}$). The carbohydrate content in grain was increased significantly by 10ppm Mn in

Kanaklata (9.04%) > Disang (6.89%). In case of treatment 20 ppm Mn, Lachit (5.27%) showed significant increase in the carbohydrate content in grain > Joymoti (4.22%) and Disang (6.04%), However, at 30 ppm Mn treatment, all the rice varieties showed significant reductions (1.49 to 15.72%) in the carbohydrate content in grain as compared to the control. As per the report of Kumar et al. (2017), grain quality including carbohydrate viz., amylase contents in grains can be enhanced with application of Mn @ 5 kg ha^{-1} .

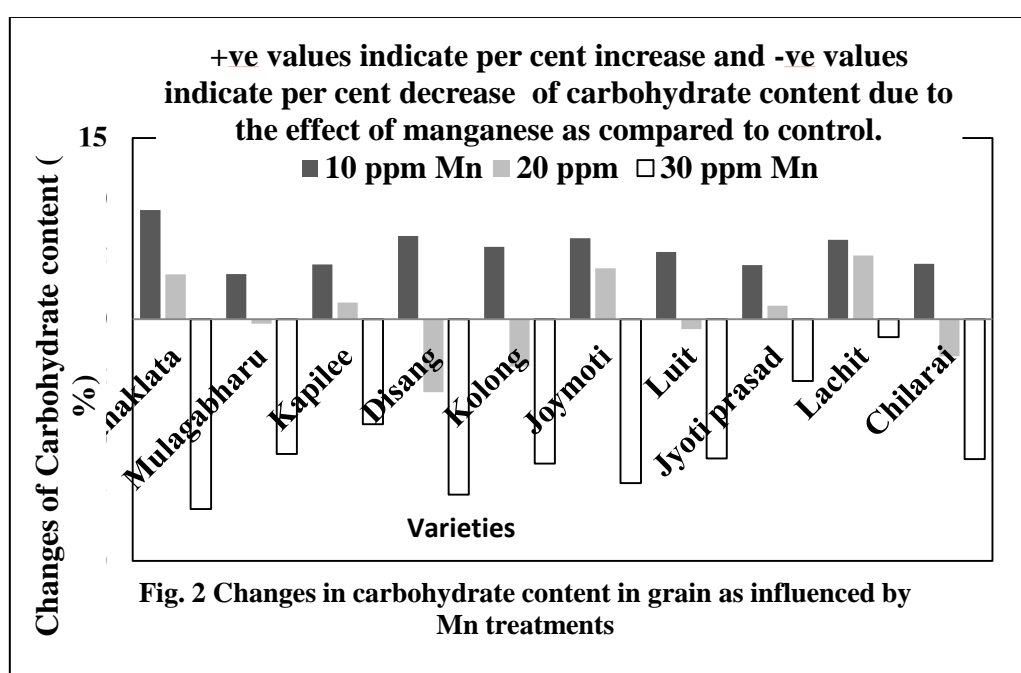


Table 4. Variation of Mn content in shoot at maximum tillering to heading stages (~70 days after sowing) of rice crop under different manganese treatments

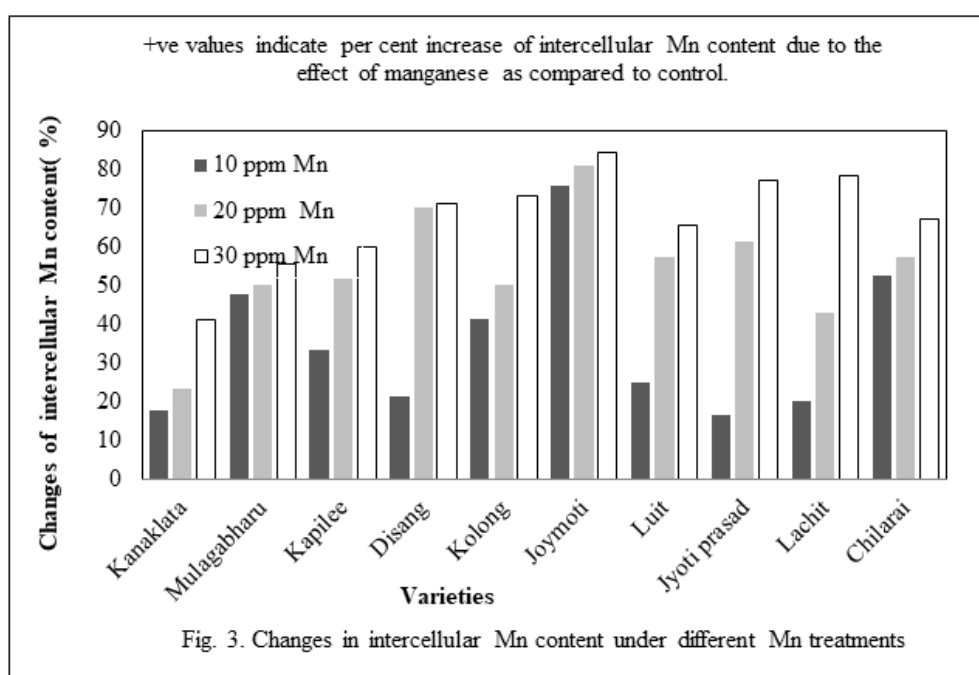
Manganese content in shoot (mg/kg dry weight)					
Treatments (T)→	0 ppm Mn (Control)	10 ppm Mn	20 ppm Mn	30 ppm Mn	Mean
Varieties (V)↓					
Kanaklata	21.230	87.540	143.510	163.400	103.920
Mulagabharu	29.770	48.710	98.950	121.710	74.785
Kapilee	35.450	58.200	72.410	122.460	72.130
Disang	32.610	66.730	117.910	125.490	85.685
Kolong	26.920	47.770	78.110	115.060	66.965
Joymoti	64.830	111.280	162.460	181.420	129.998
Luit	26.920	54.400	100.850	130.240	78.103
Jyoti prasad	24.080	51.550	98.950	111.280	71.465
Lachit	61.980	83.790	104.640	113.170	90.895
Chilarai	55.350	121.700	155.830	168.140	125.255
Mean	37.914	73.167	113.362	135.237	
	T	V	T X V		
S.Ed (±)	2.586	1.635	5.172		
CD	5.246	3.318	10.491		

There were significant changes of intercellular manganese contents due to Mn treatments (Fig.3). The highest intercellular manganese content was detected at 30ppm Mn (0.042 mgg⁻¹d.w.)>20ppm Mn (0.030 mgg⁻¹d.w.)>10ppm Mn (0.021 mgg⁻¹d.w.), and the lowest of intercellular Mn was at controlled plants (0.014 mgg⁻¹d.w.). Among the genotypes, the highest intercellular manganese content was recorded in Chilarai (0.038 mgg⁻¹d.w.)>Joymoti (0.037 mgg⁻¹d.w.)>Kanaklata

(0.030 mgg⁻¹d.w.), while the lowest was recorded in Mulagabharu>Kolong (0.021 mgg⁻¹d.w.). The intercellular Mn content increased significantly in variety Joymoti (84.21%) at 30ppm Mn treatment>Lachit (78.18%). In case of 20 ppm Mn, Joymoti (80.85%) showed significant increase the intercellular Mn>Disang (70.27%). Of course, 10 ppm Mn also enhanced intercellular Mn significantly (16.66 to 75.67%) in the varieties as compared with control.

Table 5. Variation of Mn content in grain at harvest stage of rice crop under different manganese treatments

Mn content in grain (mg/kg dry weight)					
Treatments (T)→ Varieties (V)↓	0 ppmMn (Control)	10 ppm Mn	20 ppm Mn	30 ppm Mn	Mean
Kanaklata	17.440	61.000	129.300	144.500	88.060
Mulagabharu	20.290	28.800	80.000	97.300	56.598
Kapilee	36.400	42.100	55.300	104.700	59.625
Disang	36.400	49.700	102.700	107.500	74.075
Kolong	14.590	27.860	58.540	102.700	50.923
Joymoti	45.800	87.500	145.400	163.500	110.550
Luit	17.440	32.700	83.700	103.700	59.385
Jyoti prasad	18.390	35.400	84.700	93.300	57.948
Lachit	43.000	67.700	88.500	82.800	70.500
Chilarai	46.500	101.800	142.600	155.900	111.700
Mean	29.625	53.456	97.074	115.590	
	T	V	T X V		
S.Ed (±)	1.972	1.247	3.943		
CD	3.999	2.529	7.999		



Similarly, the highest exchangeable manganese content was estimated at 30ppm Mn (0.140 mgg⁻¹d.w.) > 20ppm Mn (0.109 mgg⁻¹d.w.) > 10ppm Mn (0.081 mgg⁻¹d.w.), and the lowest at the control 0ppm Mn (0.057). On an average, the highest exchangeable manganese content was recorded in Joymoti (0.149 mgg⁻¹d.w.) > Chilarai (0.147 mgg⁻¹d.w.) > Kanaklata (0.122 mgg⁻¹d.w.), while the lowest of it was recorded in the Jyoti prasad (0.064 mgg⁻¹d.w.). The exchangeable Mn content increased significantly commensuration to the concentration of Mn in the treatments (**Fig. 4**). So, at 30ppm Mn, variety Lachit (74.84%) followed by (>) Chilarai (73.33%) showed the highest increment of Mn. In case of 20 ppm Mn also, the variety Lachit (72.48%) showed significant increase in the exchangeable Mn > Chilarai (64.86%). Even, at 10 ppm Mn treatment, all the rice varieties showed significant increases in the exchangeable Mn (13.91% to 56.98%) as compared to the control. The concentrations of Mn in cellular locations are highly

dependent on plant species and genotypes (Husted et al., 2009; Broadley et al., 2012; & Fernando & Lynch, 2015). It had been established that excess Mn may be stored in vacuoles (Dučić & Polle, 2007; & Dou et al., 2009), cell walls (Führs et al., 2010), and distributed to different leaf tissues (Fernando et al., 2006a,b). In the present study, higher concentrations of Mn were detected in the intercellular and exchangeable fractions of tissues in case of 30ppm Mn treatment irrespective of the genotypes. Plausibility is that cell membrane permeability increased gradually (with lowering CMS) in commensuration with the increases in Mn concentration in the treatments compared to the control as described below. Plant cells treated with higher Mn concentration had higher alteration of membrane permeability. So, higher leakage of Mn from the protoplast was evidenced by the recovery of more amounts of intercellular and exchangeable Mn contents in the plant tissues as compared to the controlled tissues in the experiment.

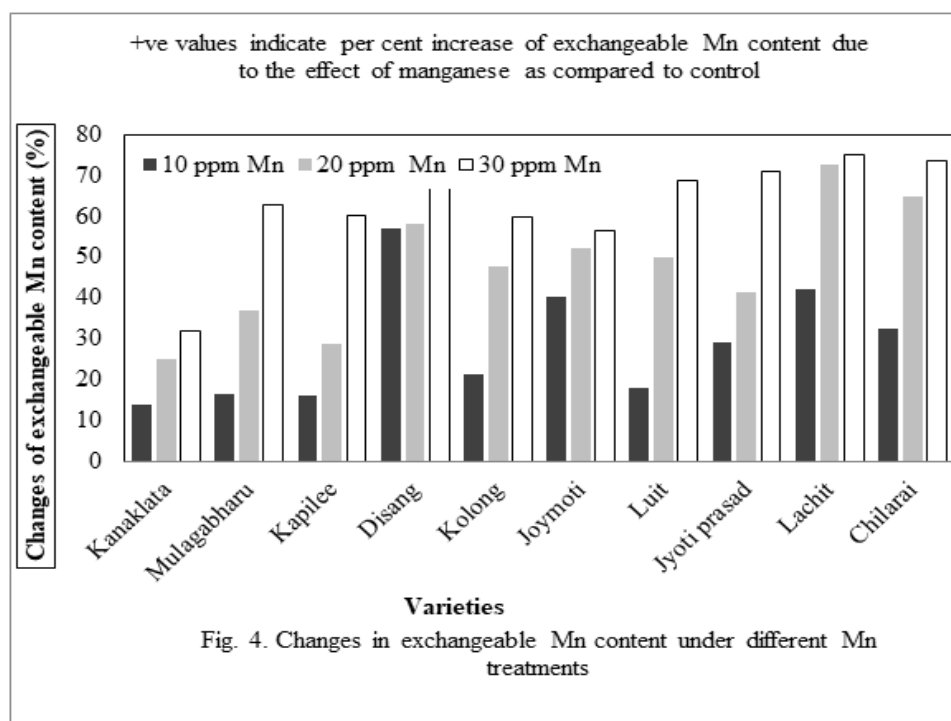


Fig. 4. Changes in exchangeable Mn content under different Mn treatments

There were significant variations of cell membrane stability (CMS) among the genotypes due to Mn treatments (**Fig.5**). The highest CMS index was observed at 10ppm Mn (34.554) > 20ppm (23.109) > control

(28.645), and the lowest CMS was at 30ppm Mn treatment (17.783). Among the genotypes, the highest CMS was recorded in Kanaklata (29.804%) > Chilarai (28.921%) > Joymoti (27.725%), while the lowest CMS was present

in Kolong (22.788%). Overall, there was higher CMS in varieties under treatment of 10ppm Mn as compared to other doses of Mn treatment. The CMS increased significantly in 10ppm Mn treatment in variety Disang (37.06%) followed by Mulagabharu (35.66%) as compared to the control. In case of 20 ppm Mn treatment also, variety Disang (24.27%) showed significant increase in the CMS followed by Mulagabharu (22.47%). However, for the treatment 30 ppm Mn, all the rice varieties showed significant decreases in the CMS (24.14% to 37.61%) as compared to control. Mn in excess of requirements causes oxidative stress in plant cells. Reactive oxygen species (ROS), mainly OH, are produced by lethal doses of Mn in plant cell (Lynch & St. Clair, 2004; & Doncheva et al., 2009). The ROS contributes to the peroxidation of lipids present in plasma membrane leading to fracture of membrane, and causing alteration of permeability properties of the membrane. High concentration of Mn in plant cell causes swelling of thylakoids and changes membrane

stability of the internal organelles like chloroplast (Hauck et al., 2003). In the current study, ROS might have caused lipid breakdown in membrane, induced cellular plasmolysis and lowered CMS (Pryor & Lightsey, 1981, & Bharali et al., 2015ab) in the plants treated with 30ppm Mn. Plants treated with the supra optimal concentration of Mn reduced inter cellular and exchangeable cations viz., calcium, magnesium, and potassium etc in plants (Horst & Marchehner, 1978). Calcium being integral component of membrane helps maintain CMS (Legge et al., 1982, & Bharali & Bates 2004). The plant cell may become vulnerable to solute leakage due to the ROS attack on the membrane. Calcium ions bind with modulator proteins e.g. Calmodulin (Dieter, 1984), and serves as chemical signalling that in some cases equips the plant to resist external stresses (Bharali & Bates, 2004). These possibilities have not been explored meticulously in the present studies.

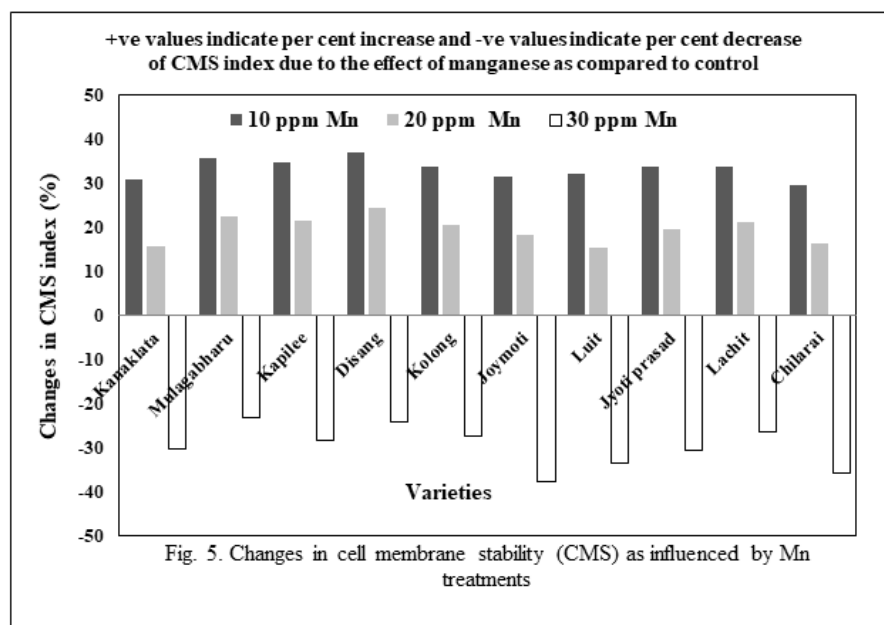


Fig. 5. Changes in cell membrane stability (CMS) as influenced by Mn treatments

From the foregoing studies, it's inferred that Mn was dose responsive which had been judged by the significant changes in biochemical indices. There were increments (↑) in total Chlorophyll (↑27.17%), Chlorophyll-a (↑26.72%) and Chlorophyll-b (↑27.77%), NR activity (↑35.39%), Carbohydrate content in grain (↑9.04%), and

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Cell membrane stability (↑37.06%) when Mn was misted on foliage at vegetative stage (70days after sowing) in lower quantity (10ppm Mn as $MnSO_4 \cdot H_2O$). The highest dose of Mn treatment (30ppm) also influenced positively the Mn content in shoots (↑87%) and grain (↑87.9%), distribution of Mn contents in intercellular (↑84.21%),

exchangeable locations ($\uparrow 74.84\%$). Overall, Kanaklata followed by Chilarai was superior in possessing the biochemical traits even though these were deviated by the Mn treatments in the present study.

Acknowledgements

The authors are grateful to Assam Agricultural University for providing opportunity in accomplishing the Master' research by the first author in the department of Crop physiology at Jorhat. All suggestions received from the members of the student's advisory committee are duly acknowledged.

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