



Effects of gibberellic acid (GA) under cadmium chloride stress on maize (*Zea mays* L.)

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Abstract

The maize is major cash crop of Pakistan after wheat and rice. It is exposed to many biotic and abiotic stresses throughout the life. Worldwide the contamination of soils with heavy metals is a serious environmental issue. Among the heavy metals the Cadmium (Cd) is a highly toxic metal because of its negative effects on plant growth and development. To determine the effect of GA and Cd, a pot experiment was conducted in the Old Botanical Garden University of Agriculture Faisalabad. Two different maize varieties were used (Neelam and Desi makai). The two concentrations of cadmium chloride (0 μ M and 200 μ M) and Gibberellic acid (0mg and 0.25 mg) were applied. The experiment was performed under CRD with three replications. Stress was applied after every 10 days and gibberellic acid was applied once in three leaves stage. After 60 days the plants were harvested. Different physiological parameters like chlorophyll (a, b, a/b), carotenoids contents, photosynthetic rate, transpiration rate, stomatal conductance, CO₂ conductance, POD and total soluble proteins were recorded. All studied parameters were negatively affected by cadmium chloride while the foliar application of gibberellic acid improved these traits under cadmium chloride stress. Overall, Desi Makai performed better than Neelam in both stress and spray conditions.

Key word: cadmium chloride, gibberellic acid, Maize

Introduction

The maize is major cash crop of Pakistan after wheat and rice. It is exposed to many biotic and abiotic stresses throughout the life. Worldwide the contamination of soils with heavy metals is a serious environmental issue (Yang et al., 2004). Among the heavy metals the Cadmium (Cd) is a highly toxic metal because of its negative effects on plant growth and development. Because of heavy use of fertilizers, chemical sprays, urban compost, irrigation with industrial water the concentration of cadmium is elevated in agricultural lands. Cadmium is oxidative metal, due to this cadmium activated all of the non-redox stress in different plants at the cell levels. It caused termination of plant cell due to the process of lipid peroxidation and protein oxidations (Gill et al., 2011). Cadmium is not harmful to plants at low level but the higher amount of cadmium chloride may cause decreased in the root growth and all of the growth processes taking place in plants (Jiang et al., 2001). Cadmium toxicity manifestations are like iron deficiency cause the yellowing of leaves in plants and moreover cadmium initiate complete cell death and cell shrinkage. Plant growth regulators are natural compounds that can advance, repress or adjust physiological procedures in plants (Tanu et al., 2008). Gibberellins are a vast group of regular items that direct numerous formative procedures in plants, including seed germination, stem elongation, and flower blooming (Shani et al., 2013). It plays a vital role in plant growth and development at all life stages. Plant growth regulators are natural compounds that can advance, repress or adjust physiological procedures in plants (Tanu et al., 2008). Gibberellins are a vast group of regular items that direct numerous formative procedures in plants, including seed germination, stem elongation, and flower

blooming (Shani *et al.*, 2013). The chlorophyll contents, proline accumulation are used to evaluate the effect of environmental stresses on plants.

The objectives of this study were (i) to examine the physiological changes in maize seedling because of Cd accumulation ii) to determine the effect of Gibberellic acid at the physiological traits of maize seedlings

Materials and methods

The current study was performed in Old Botanical Garden of UAF Pakistan. The Seeds of two maize cultivars (Neelum and Desi Makai) were taken from Sargodha city. The research trial was performed under complete randomized design (CRD) with three replications. The seeds were grown in artificial tubs containing 7 kilo gram sand in per pot. Seedlings were thinned after the germination, ten seedlings per pot of almost uniform size were kept. There were used two levels i.e. (0 μ m and 200 μ m) CaCl₂ and GA i.e. (0mg and 0.25 mg). The following traits like chlorophyll (a, b, a/b), carotenoids contents, photosynthetic rate, transpiration rate, stomatal conductance, CO₂ conductance, POD and total soluble proteins were recorded.

Results and discussion

Photosynthetic pigments

Chlorophyll “a” (mg/g fresh weight):

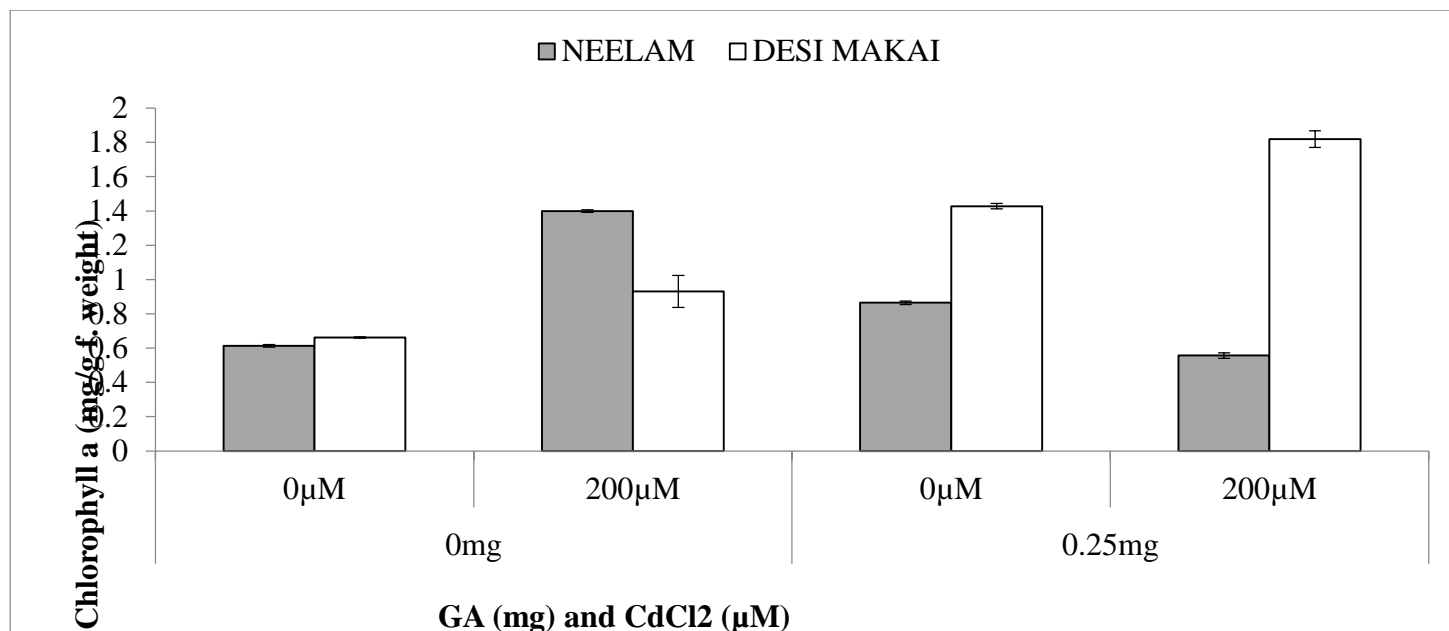
The Analysis of variance for Chlorophyll a of 2 maize Cultivars (Neelum and Desi makai) grown in control condition and Cadmium chloride tension is given in (Tab. 1). The foliar application of Gibberellic acid significantly ($p \leq 0.05$) improved in Chlorophyll a of Desi makai and highest decline was detected in chlorophyll a of Desi makai when 200 μ m Cadmium chloride was applied (Fig. 1). Both Neelum and Desi makai performed better under control conditions. Moreover, highest improved in chlorophyll a was observed in Desi makai at 0.25mg GA spray (Fig. 1). The varietal significant ($p \leq 0.05$ difference was detected between both cultivars. (Table. 1).

Table 1: ANOVA table for chlorophyll a of two maize cultivars (Neelum and Desi makai) under CdCl₂ stress with foliar application of Gibberellic acid.

Source of variation	df	MS	F-Value	p-value
Stress	1	0.423207	71.847724	.0000 ***
Spray	1	0.485926	82.495508	.0000 ***
Varieties	1	0.740962	125.79289	.0000 ***
Stress X Spray	1	0.354051	60.107132	.0000 ***
Stress X varieties	1	1.892255	321.24753	.0000 ***
Spray X varieties	1	0.012467	2.1165257	.1651 ns
Stress x spray x varieties	1	0.5548	94.188225	.0000 ***
Error	16	0.0058903		

Non significant=^{ns}. ***, **, * significant at 0.001, 0.01, 0.05 separately

Fig 1: of Chlorophyll a (mg/g f. weight) two maize cultivars (Neelum and Desi makai) under CdCl₂ stress with foliar application of GA.



Chlorophyll "b" (mg/g fresh weight):

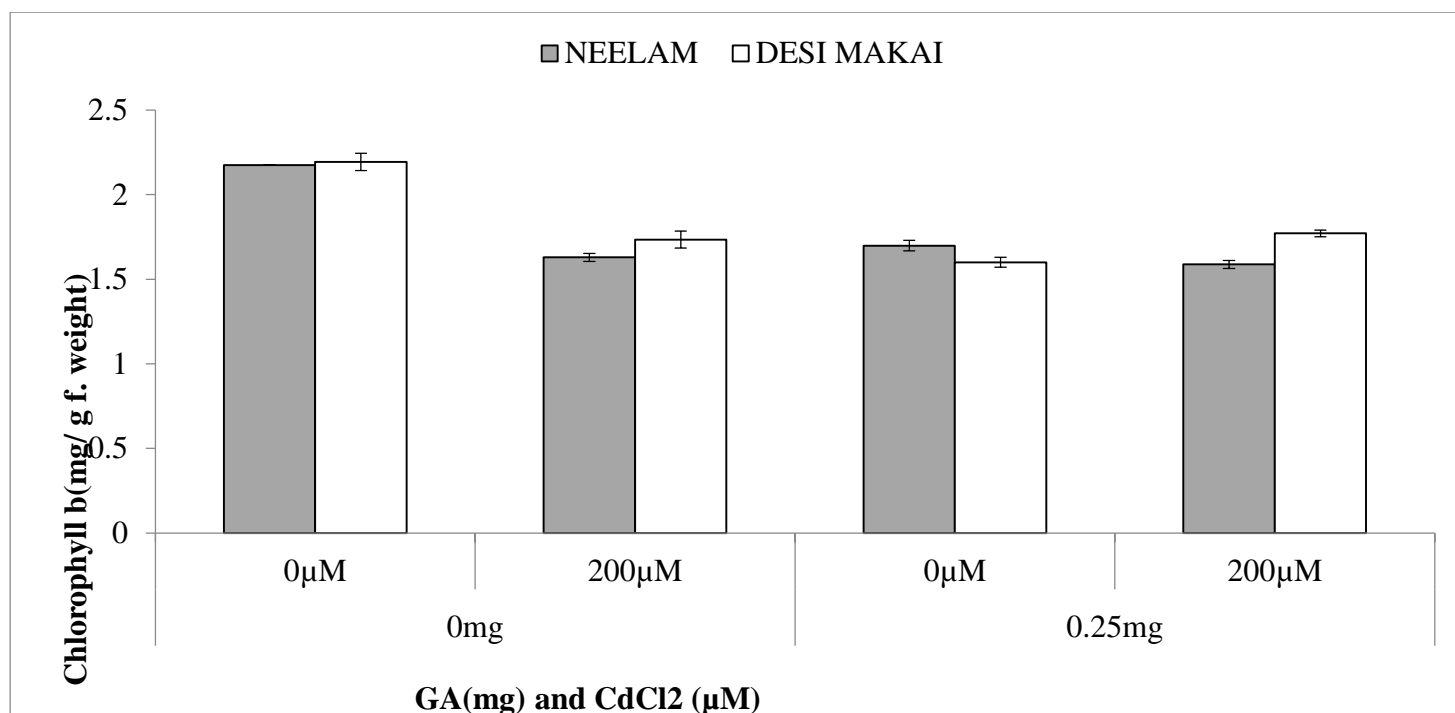
The Analysis of variance Chlorophyll b of 2 maize Cultivars (Neelam and Desi makai) grown in control condition and Cadmium chloride tension is given in (Tab. 2). The foliar application of Gibberellic acid significantly ($p \leq 0.05$) enhanced in chlorophyll b of both Neelam or Desi makai but maximum growth in Chlorophyll b was observed in Desi makai when 200µm Cadmium chloride was applied (Fig. 2). On the other hand maximum increased in chlorophyll b was observed in Desi makai at 0.25mg GA spray and 200µm Cadmium chloride (Fig. 2). Varietal significant ($p \leq 0.05$) difference was detected between both cultivars. (Table. 2).

Table 2: ANOVA table for chlorophyll b of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of Gibberellic acid.

Source of variation	df	MS	F-Value	p-value
Stress	1	0.083426	28.602806	.0001 ***
Spray	1	0.383801	131.58706	.0000 ***
Varieties	1	1.145377	392.69509	.0000 ***
Stress X Spray	1	0.146797	50.329695	.0000 ***
Stress X varieties	1	1.023827	351.0214	.0000 ***
Spray X varieties	1	4.3682134	1497.6518	.0000 ***
Stress x spray x varieties	1	0.062526	21.437194	.0003 ***
Error	16	0.0029167		

Non significant=^{ns}. ***, **, * significant at 0.001, 0.01, 0.05 separately

Fig 2: of Chlorophyll b (mg/g f. weight) two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of GA.



Carotenoids (mg/g fresh weight):

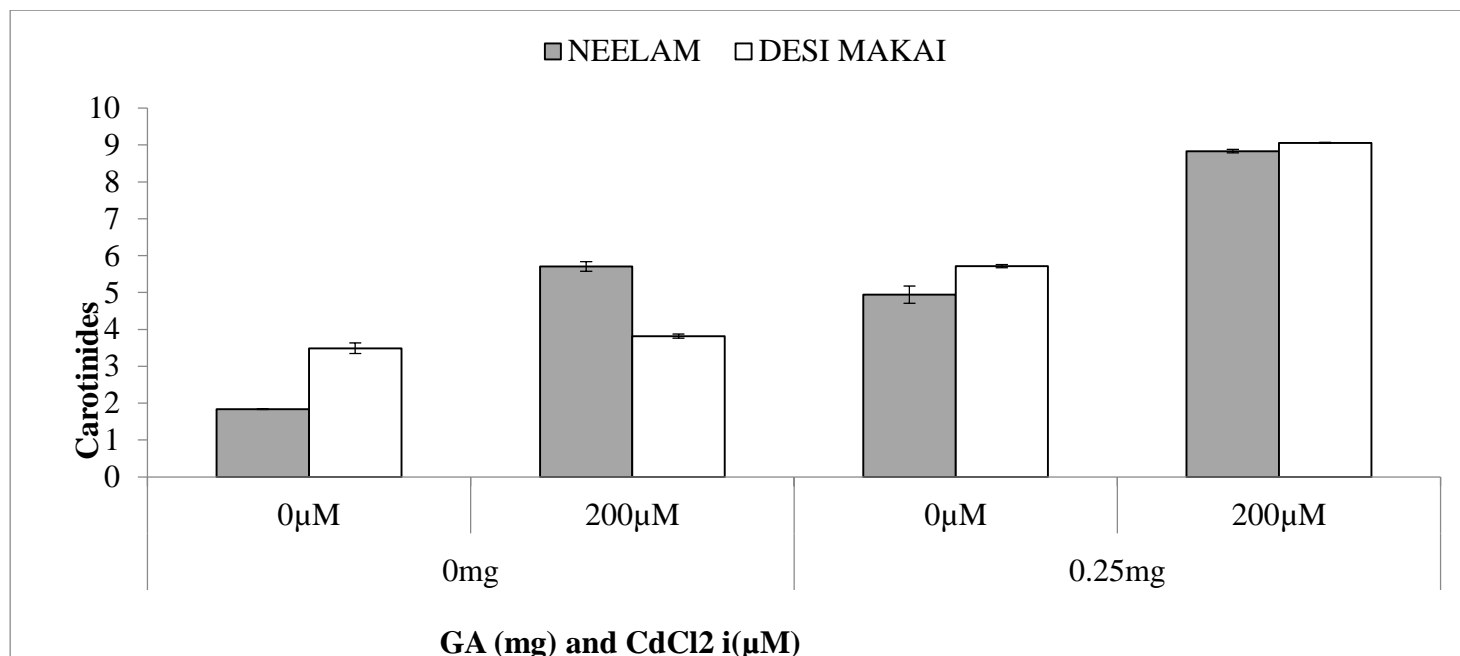
The Analysis of variance for carotenoids of 2 maize Cultivars (Neelam or Desi makai) grown in control condition and CdCl₂ tension is given in (Tab. 3). The foliar application of Gibberellic acid significantly ($p \leq 0.05$) declined in carotenoids of both Neelam and Desi makai, maximum growth was observed in both varieties when 200µm Cadmium chloride was applied was given in (Fig 3). On the other hand maximum increased in both varieties was observed at 0.25mg GA spray and 200µm Cadmium chloride (Fig. 3). Varietal significant ($p \leq 0.05$) difference was detected between both cultivars. (Table. 3).

Table 3: ANOVA table for carotinoides of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of Gibberellic acid.

Source of variation	df	MS	F-Value	p-value
Stress	1	70.331243	1405.888	.0000 ***
Spray	1	49.064254	980.7712	.0000 ***
Varieties	1	0.219778	4.393263	.0523 ns
Stress X Spray	1	3.433753	68.63911	.0000 ***
Stress X varieties	1	0.581363	11.621174	.0036 **
Spray X varieties	1	6.282584	125.5859	.0000 ***
Stress x spray x varieties	1	3.365506	67.27488	.0000 ***
Error	16	0.0500262<-		

Non significant=^{ns}. ***, **, * significant at 0.001, 0.01, 0.05 separately

Fig 3: Carotenoid (mg/g f. weight) two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of GA.



Total Chlorophyll (mg/g fresh weight):

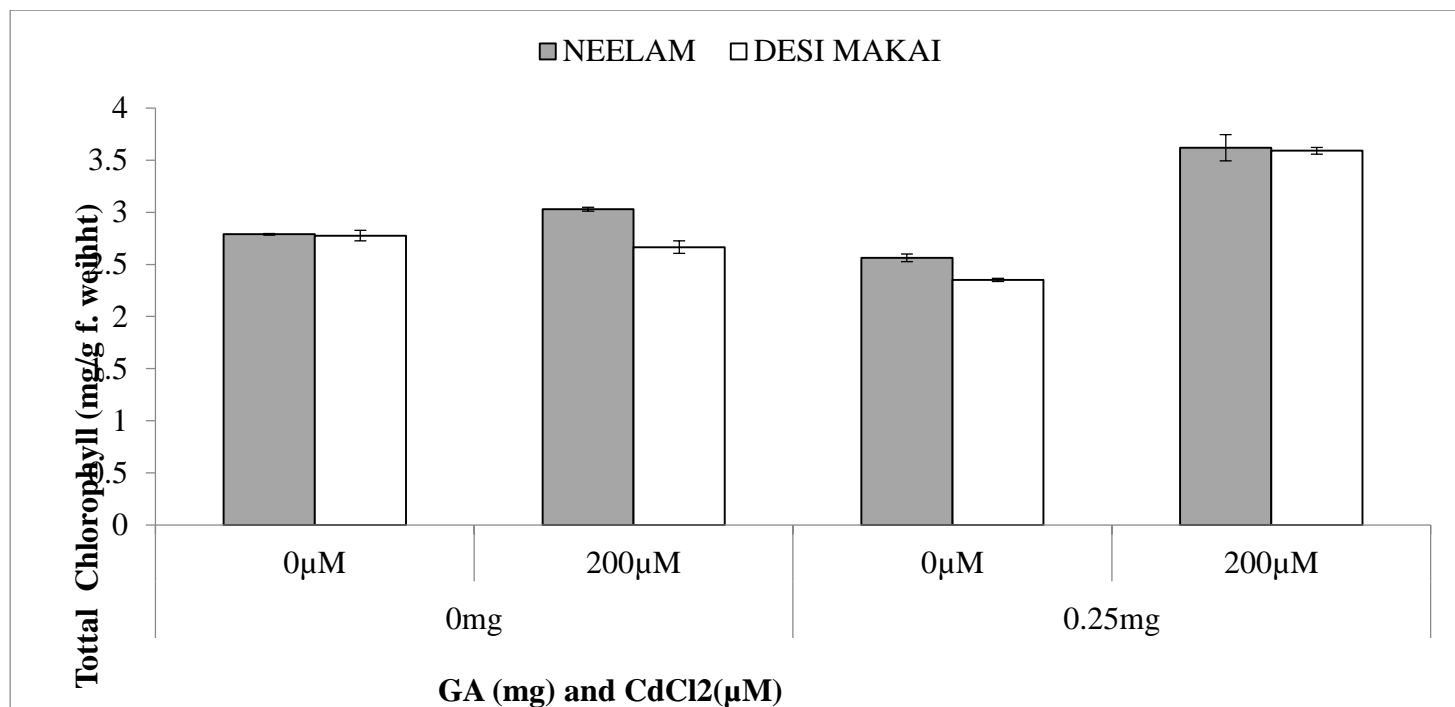
The Analysis of variance for total chlorophyll of 2 maize Cultivars (Neelam and Desi makai) grown in control condition and CdCl₂ tension is given in (Tab. 4). The foliar application of Gibberellic acid significantly ($p \leq 0.05$) decline in total chlorophyll of both Neelam or Desi makai, highest decline was detected in total chlorophyll of both varieties when 0.25mg GA was applied (Fig. 4). On the other hand maximum increased in total chlorophyll of both varieties was observed at 0.25mg GA spray and 200µm Cadmium chloride (Fig. 4). Varietal significant ($p \leq 0.05$) difference was detected between both cultivars. (Table. 4).

Table 4: ANOVA table for Total chlorophyll of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of Gibberellic acid.

Source of variation	df	MS	F-Value	p-value
Stress	1	0.28145	22.41930	.0002 ***
Spray	1	2.20644	175.7576	.0000 ***
Varieties	1	0.14306	11.39620	.0039 **
Stress X Spray	1	1.75770	140.01283	.0000 ***
Stress X varieties	1	0.00697	0.55520	.4670 ns
Spray X varieties	1	0.01045	0.83307	3749 ns
Stress x spray x varieties	1	0.10626	8.46485	0102 *
Error	16	0.0125539<-		

Non significant=^{ns}. ***, **, * significant at 0.001, 0.01, 0.05 separately

Fig 4: Tottal Chlorophyll (mg/g f. weight) of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of GA.



Chlorophyll a and b ratio (mg/g fresh weight):

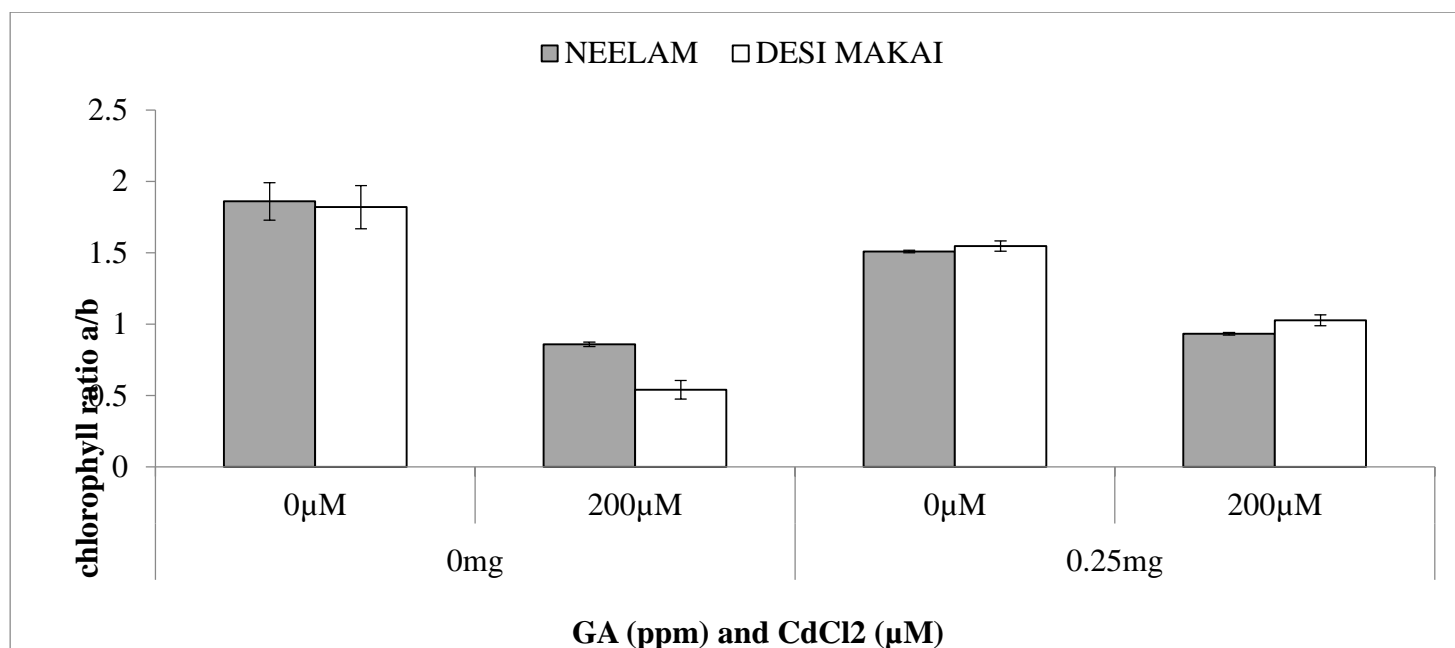
The Analysis of variance for Chlorophyll a and b ratio of 2 maize Cultivars (Neelam and Desi makai) grown in control condition and CdCl₂ tension is given in (Tab. 5). The foliar application of Gibberellic acid significantly ($p \leq 0.05$) effected the chlorophyll a and b ratio of both Neelam and Desi makai, maximum reduction was observed in ratio of both varieties when 200µm Cadmium chloride was applied (Fig. 5). On the other hand maximum increased in both varieties was observed at 0.25mg GA spray and (Fig. 5). Varietal significant ($p \leq 0.05$) difference was detected between both cultivars. (Table. 5).

Table 5: ANOVA table for chlorophyll ratio a/b of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of Gibberellic acid.

Source of variation	df	MS	F-Value	p-value
Stress	1	0.42511	17.954214	.0006 ***
Spray	1	2.11657	89.390994	.0000 ***
Varieties	1	0.22445	9.4797021	.0072 **
Stress X Spray	1	1.79037	75.614401	.0000 ***
Stress X varieties	1	0.83469	35.252211	.0000 ***
Spray X varieties	1	0.55827	23.578211	.0002 ***
Stress x spray x varieties	1	0.16561	6.9943305	.0177 *
Error	16	0.0236778<-		

Non significant=^{ns}. ***, **, * significant at 0.001, 0.01, 0.05 separately

Fig 5: chlorophyll ratio a/b of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of GA.



Net photosynthetic rate (A) ($\mu\text{mol m}^{-2}\text{s}^{-1}$)

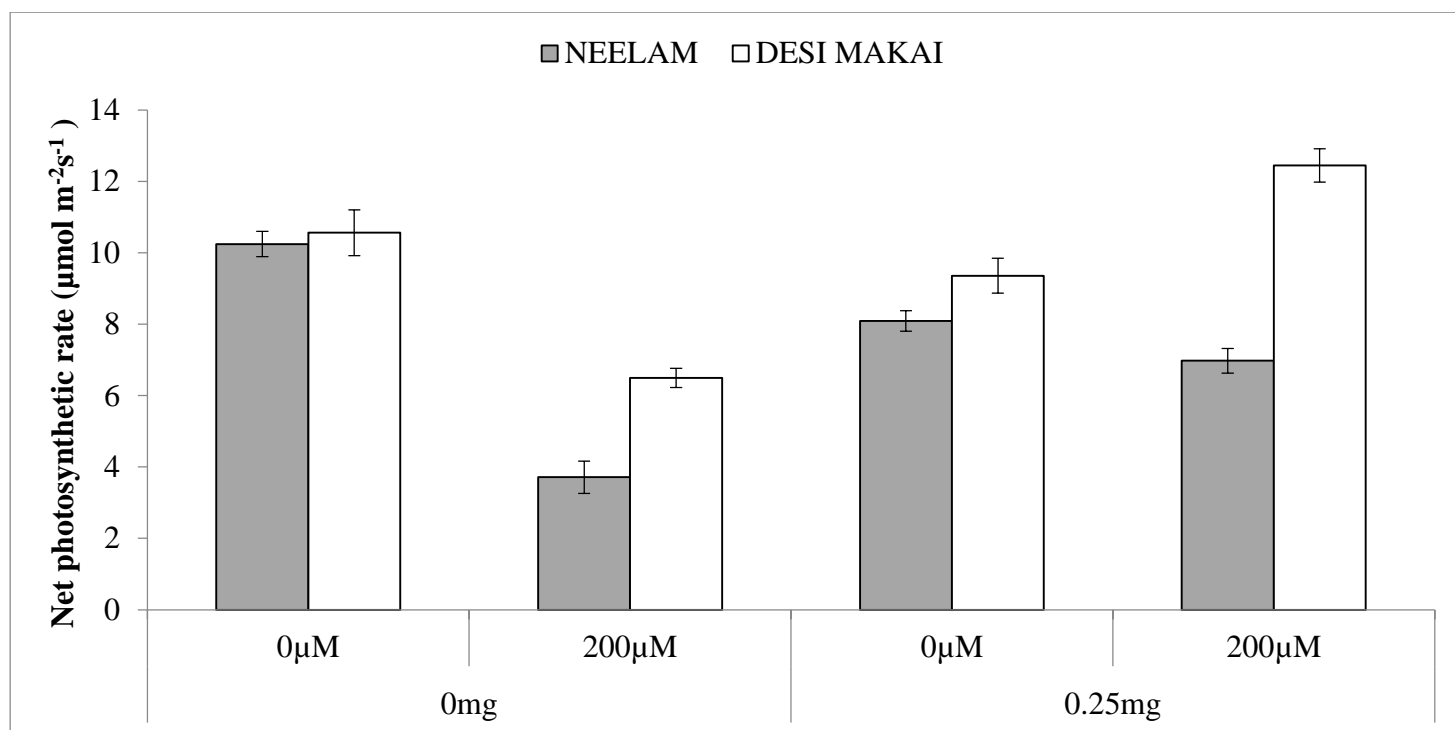
The Analysis of variance for Net photosynthetic rate of 2 maize Cultivars (Neelam and Desi makai) grown in control condition and CdCl₂ tension is given in (Tab. 6). The foliar application of Gibberellic acid significantly ($p \leq 0.05$) increased in Net photosynthetic rate of Desi makai at 0.25 GA, maximum decreased was observed in the Net photosynthetic rate of both varieties at 200µm Cadmium chloride (Fig. 6). On the other hand maximum growth was observed in Neelam and desi makai at 200µm Cadmium chloride and 0.25mg GA (Fig. 6). varietal significant ($p \leq 0.05$) difference was detected between both cultivars. (Table. 6).

Table 6: ANOVA table for Net photosynthetic rate of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of Gibberellic acid.

Source of variation	df	MS	F-Value	p-value
Stress	1	19.296267	18.428514	.0006 ***
Spray	1	47.88375	45.730419	.0000 ***
Varieties	1	9.00375	8.5988516	.0098 **
Stress X Spray	1	39.578017	37.798194	.0000 ***
Stress X varieties	1	43.148017	41.207651	.0000 ***
Spray X varieties	1	2.6666667	2.5467467	.1301 ns
Stress x spray x varieties	1	0.2242667	0.2141814	.6497 ns
Error	16	1.0470875		

Non significant=^{ns}. ***, **, * significant at 0.001, 0.01, 0.05 separately

Fig 6: Net photosynthetic rate of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of GA.



Transpiration rate (E) (m mol m⁻²s⁻¹):

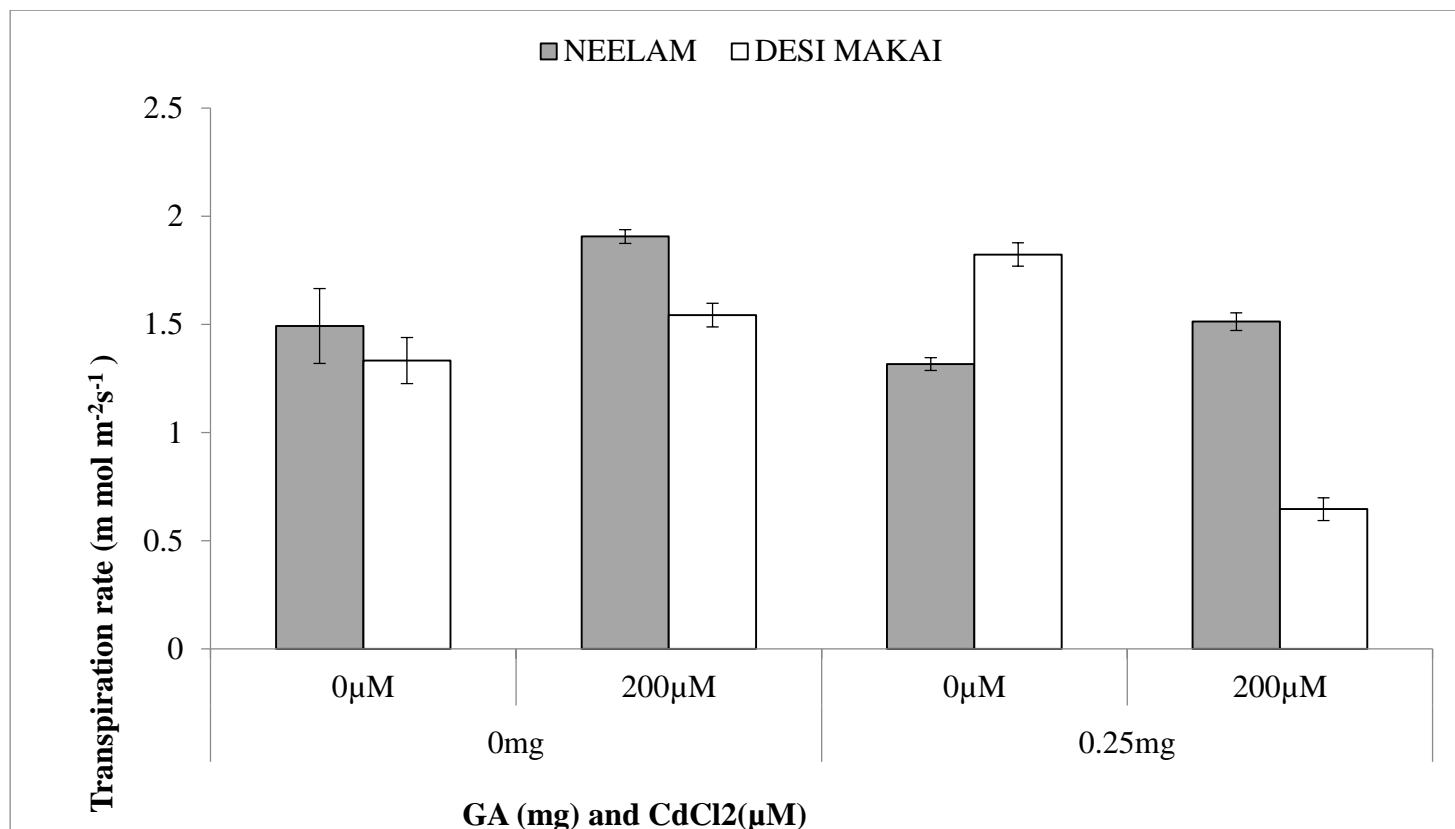
The Analysis of variance for transpiration rate of 2 maize Cultivars (Neelam or Desi makai) matured in control environment or CdCl₂ tension is given in (Tab. 7). The foliar application of Gibberellic acid significantly ($p \leq 0.05$) declined in transpiration rate of both Neelam and Desi makai at 0.25 GA, maximum decreased was observed in the growth of both varieties when 200µm Cadmium chloride and 0.25mf GA was applied (Fig. 7). On the other hand maximum growth was observed in Neelam and desi makai at 200µm Cadmium chloride was given in (Fig. 7). Varietal significant ($p \leq 0.05$) difference was detected between both cultivars. (Table. 7).

Table 7: ANOVA table for Transpiration rate two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of Gibberellic acid.

Source of variation	df	MS	F-Value	p-value
Stress	1	0.3577042	13.418099	.0021
Spray	1	0.0477042	1.7894655	.1997 ns
Varieties	1	0.2926042	10.976068	.0044 **
Stress X Spray	1	0.9640042	36.161457	.0000 ***
Stress X varieties	1	0.0100042	0.3752735	.5488 ns
Spray X varieties	1	0.9322042	34.968584	.0000 ***
Stress x spray x varieties	1	0.5133375	19.256174	.0005 ***
Error	16	0.0266583		

Non significant=ns. ***, **, * significant at 0.001, 0.01, 0.05 separately

Fig 7: Transpiration rate of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of GA.



Stomatal conductance [gs] (m mol m⁻² s⁻¹):

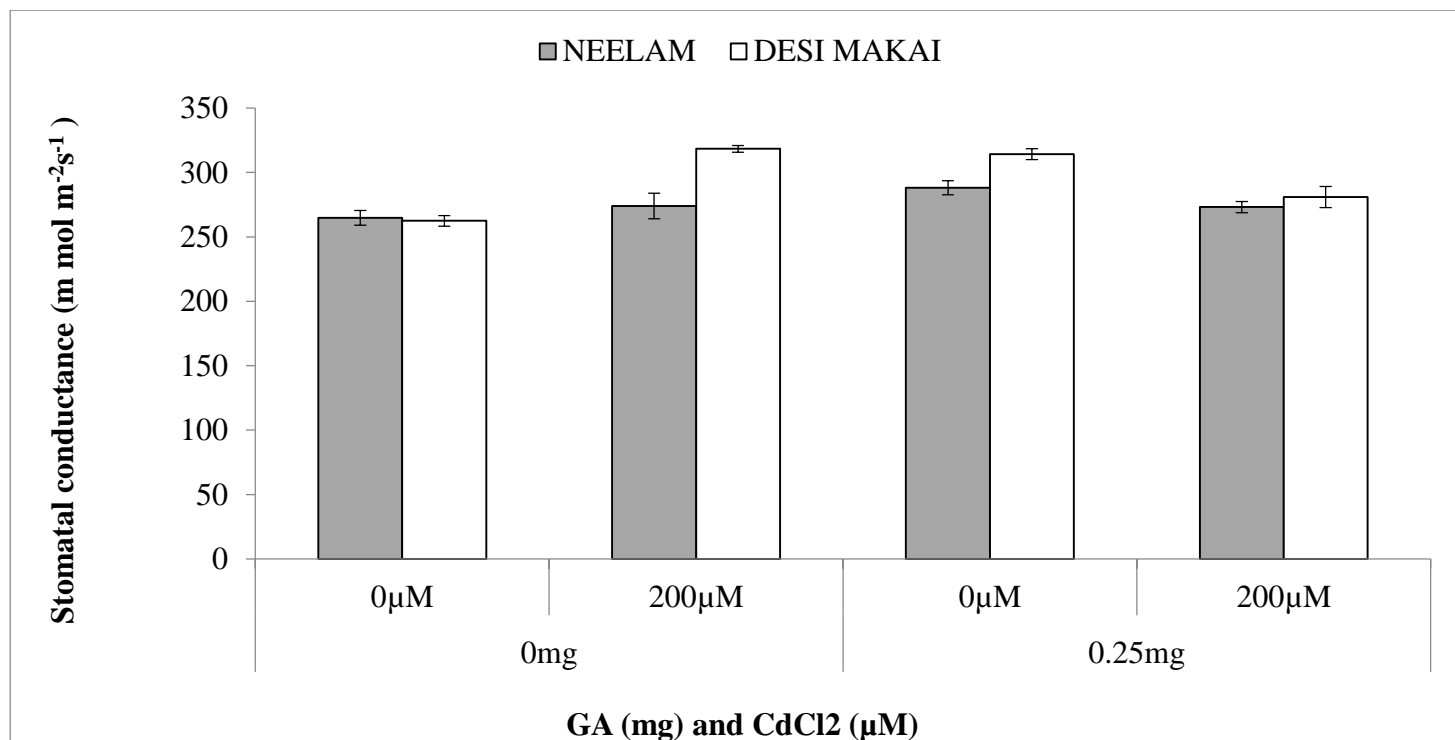
The Analysis of variance for stomatal conductance of 2 maize Cultivars (Neelam and Desi makai) grown in control condition or CdCl₂ tension is given in (Tab. 8). Application of Gibberellic acid cause significantly ($p \leq 0.05$) enhanced in [GS] of both Neelam and Desi makai at 0.25 GA but maximum increased in stomatal conductance was observed in the growth of both varieties when 200μm Cadmium chloride was applied (Fig. 8). On the other hand maximum growth was observed in Neelam and desi makai at 200μm Cadmium chloride was given in (Fig. 8).

Table 8: ANOVA table for Stomatal conductance of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of Gibberellic acid.

Source of variation	df	MS	F-Value	p-value
Stress	1	0.0352667	3.5849216	.0765 ns
Spray	1	0.0266667	2.7107158	.1192 ns
Varieties	1	0.0240667	2.446421	.1374 ns
Stress X Spray	1	0.02535	2.5768742	.1280 ns
Stress X varieties	1	0.05415	5.5044473	.0322 *
Spray X varieties	1	0.0368167	3.742482	.0709 ns
Stress x spray x varieties	1	0.0192667	1.9584922	.1808 ns
Error	16	0.0098375		

Non significant=^{ns}. ***, **, * significant at 0.001, 0.01, 0.05 separately

Fig 8: Stomatal conductance of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of GA.



Gas Conductance [Ci] ($\mu \text{ mol mol}^{-1}$)

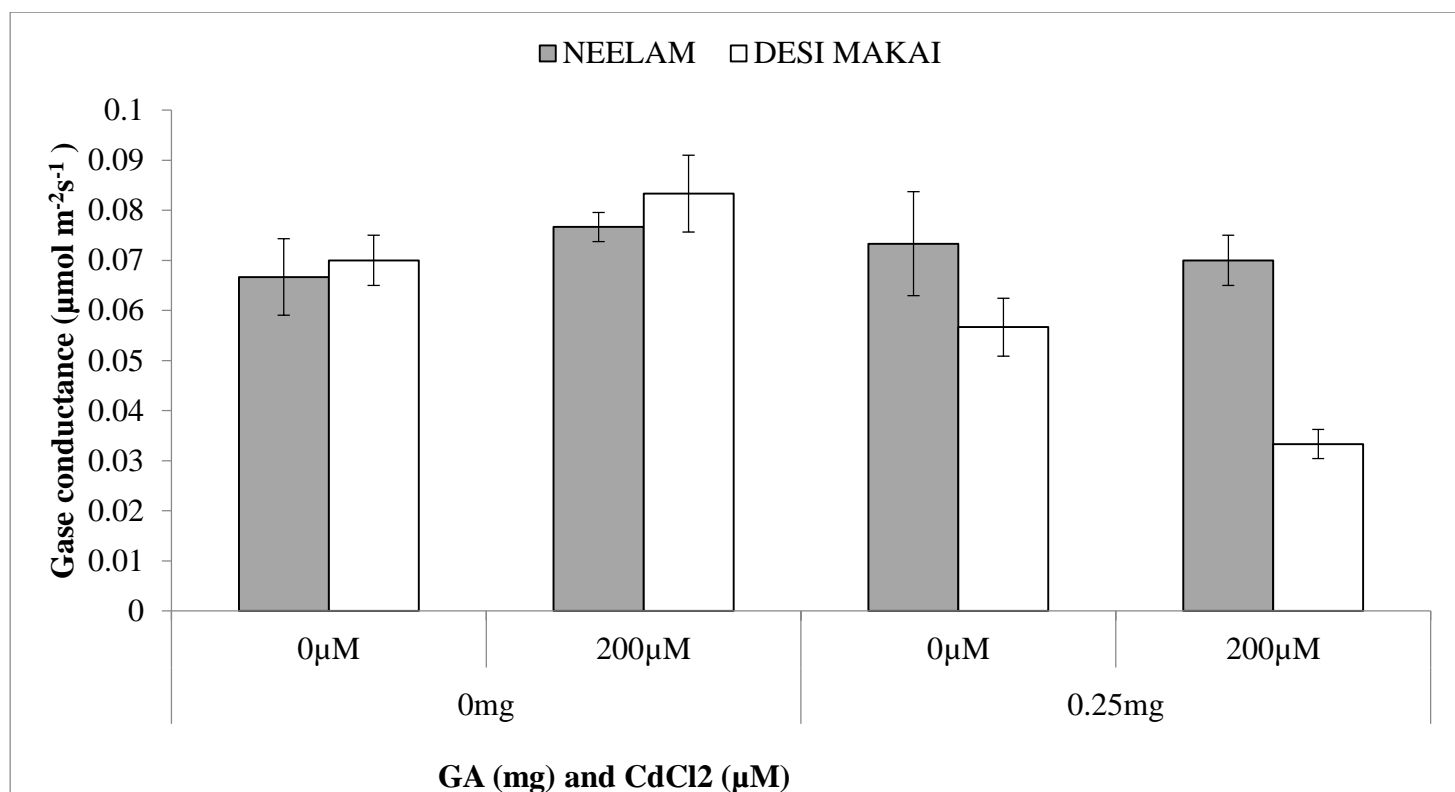
The Analysis of variance for gas conductance of 2 maize Cultivars (Neelam and Desi makai) grown in control condition or CdCl₂ tension is given in (Tab. 9). Treatment of Gibberellic acid cause significantly ($p \leq 0.05$) increased in gas conductance of both Neelam and Desi makai at 0.25 GA, but maximum increased was observed in the growth of both varieties when 200 μM Cadmium chloride was applied (Fig. 9). On the other hand maximum growth was observed in Neelam and desi makai at 200 μM Cadmium chloride (Fig. 9). Varietal significant ($p \leq 0.05$) difference was detected between both cultivars. (Table. 9).

Table 4.9: ANOVA table for Gas conductance of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of Gibberellic acid.

Source of variation	df	MS	F-Value	p-value
Stress	1	0.0352667	3.5849216	.0765 ns
Spray	1	0.0266667	2.7107158	.1192 ns
Varieties	1	0.0240667	2.446421	.1374 ns
Stress X Spray	1	0.02535	2.5768742	.1280 ns
Stress X varieties	1	0.05415	5.5044473	.0322 *
Spray X varieties	1	0.0368167	3.742482	.0709 ns
Stress x spray x varieties	1	0.0192667	1.9584922	.1808 ns
Error	16	0.0098375		

Non significant=^{ns}. ***, **, * significant at 0.001, 0.01, 0.05 separately

Fig 9: Gas conductance of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of GA



Total soluble Proteins (mg/g fresh weight):

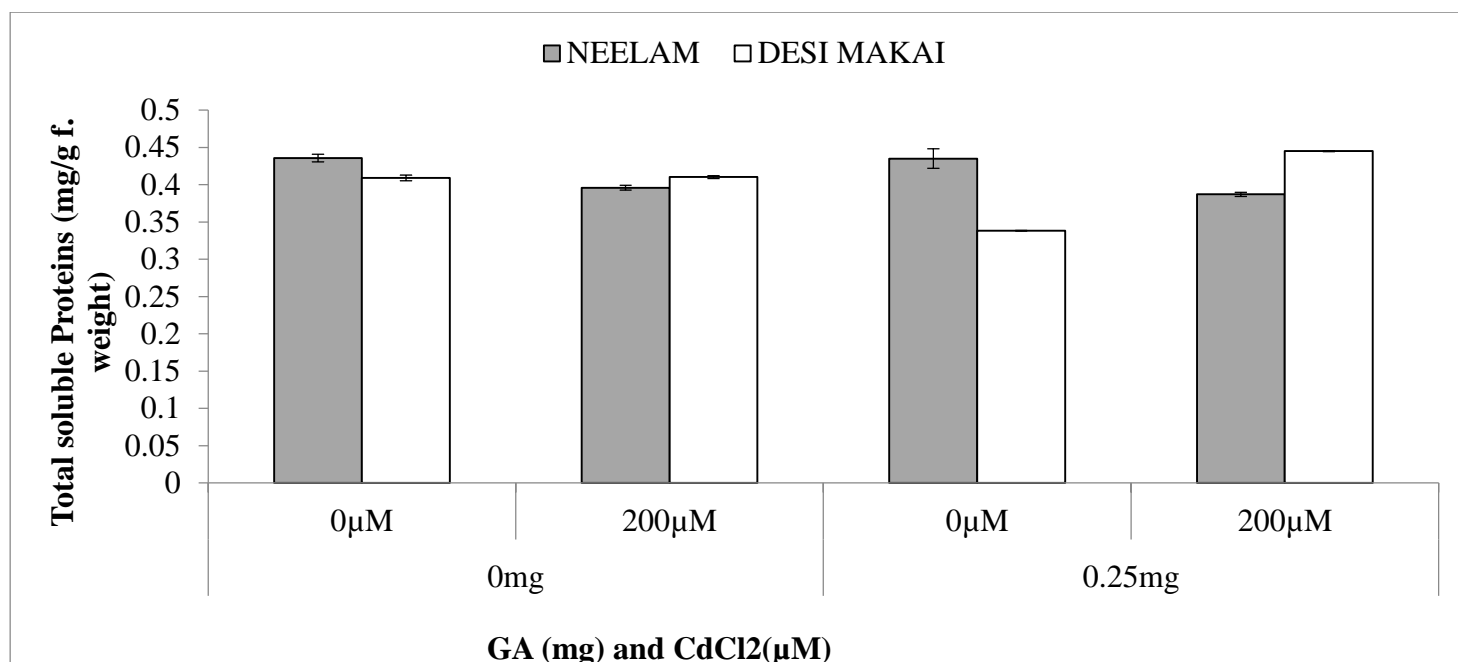
The Analysis of variance for proteins of 2 maize Cultivars (Neelam or Desi makai) matured in control environment or CdCl₂ tension is given in (Tab. 10). Application of Gibberellic acid cause significantly ($p \leq 0.05$) enhanced in proteins of both Neelam and Desi makai at 0.25 GA, there were no decreased in growth in both of the varieties (Fig. 10). On the other hand maximum growth was observed in Neelam and desi makai at 200µm Cadmium chloride and 0.25mg GA (Fig. 10. Varietal significant ($p \leq 0.05$) difference was detected between both cultivars. (Table. 10).

Table 10: ANOVA table for Total soluble Proteins two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of Gibberellic acid.

Source of variation	df	MS	F-Value	p-value
Stress	1	7.8204	6.6486008	.0202 *
Spray	1	1.5504	1.3181013	.2678 ns
Varieties	1	9.7538	8.2922423	.0109 *
Stress X Spray	1	0.00352	829.996812	.0001 ***
Stress X varieties	1	2.6004	2.2107687	.1565 ns
Spray X varieties	1	0.014357	122.05774	.0000 ***
Stress x spray x varieties	1	0.004845	41.190577	.0000 ***
Error	16	1.1762		

Non significant=^{ns}. ***, **, * significant at 0.001, 0.01, 0.05 separately

Fig 10: Total soluble proteins of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of GA.



Peroxidase (POD) (mg g⁻¹ fresh weight)

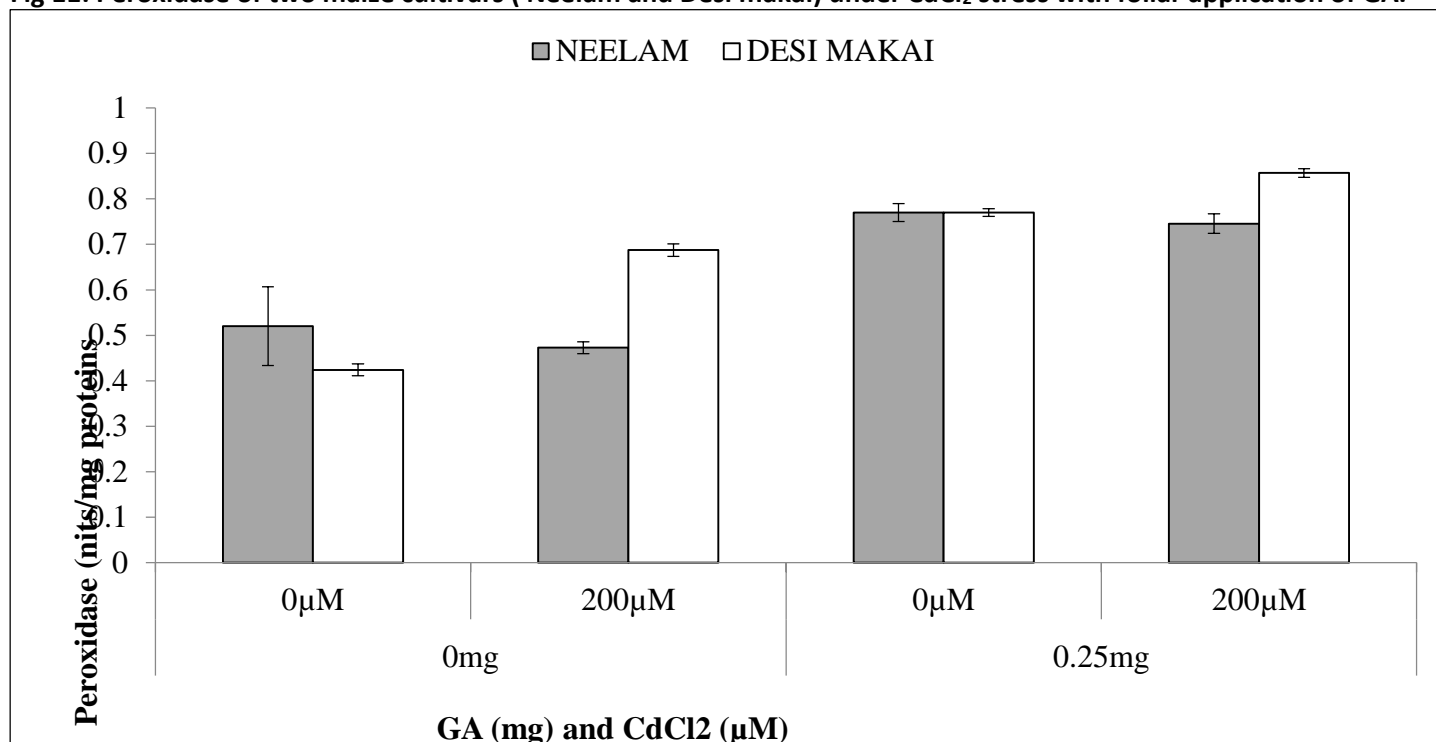
The Analysis of variance for POD of 2 maize Cultivars (Neelam or Desi makai) matured in control environment or CdCl₂ tension is given in (Fig. 11; Tab. 4.20). Application of Gibberelic acid cause significantly ($p \leq 0.05$) enhanced in the POD of both Neelam or Desi makai at 0.25 GA, maximum decreased was observed in the growth of both varieties when 200µm Cadmium chloride (Fig. 11). On the other hand maximum growth was observed in Neelam and desi makai at 200µm Cadmium chloride and 0.25mg GA (Fig. 11). Varietal significant ($p \leq 0.05$) difference was detected between both cultivars. (Table. 11).

Table 11: ANOVA table for Peroxidase two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of Gibberellic acid.

Source of variation	df	MS	F-Value	p-value
Stress	1	0.404301	89.61152	.0000 ***
Spray	1	0.0291904	6.469916	.0217 *
Varieties	1	0.01978	4.384157	.0526 ns
Stress X Spray	1	0.008855	1.962680	.1803 ns
Stress X varieties	1	1.8375e-5	0.004072	.9499 ns
Spray X varieties	1	0.066676	14.77844	.0014 **
Stress x spray x varieties	1	0.0148504	3.291519	.0884 ns
Error	16	0.0045117<-		

Non significant=^{ns}. ***, **, * significant at 0.001, 0.01, 0.05 separately

Fig 11: Peroxidase of two maize cultivars (Neelam and Desi makai) under CdCl₂ stress with foliar application of GA.



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