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Salinity Stress Induced Biochemical Changes during Seed Germination of Maize Cultivars

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Abstract: In the present study the two maize cultivars were selected to examine the effect of salinity and the effect of GA_3 on the salinity stressed seeds, the tests were conducted in different conditions with different concentrations. Germination percentage, vigour index and seedling length, chlorophyll content, protein, total free amino acids, protease activity were investigated. GA_3 treatment and combination of GA_3 and NaCl increased the germination percentage, vigour index and seedling length in SNL cultivar and in MYMH cultivar these parameters were reduced. The chlorophyll content was more in both cultivars in control and either salinity or combinations of NaCl and GA_3 have no effect on the synthesis of chlorophyll pigment. Free amino acids, Protein and Protease activity were more in GA_3 200ppm concentration where as in other concentrations these were observed less. The GA_3 played salinity alleviating role and increased the chlorophyll content in the saline treated seeds. In SNL cultivars chlorophyll content was less affected when compared to the MYMH cultivar. Hence, the SNL cultivar in more tolerant compared to the MYMH cultivar.

Keywords: Maize, NaCl, GA_3, Germination percentage, Vigour index, Chlorophyll, Total free amino acids, protein and Protease activity.

I. INTRODUCTION

Maize, also known as corn is a large grain plant. The six major types of corn are dent corn, flint corn, pod corn, popcorn, flour corn, and sweet corn. Maize is the third most important food grain in India after wheat and rice. In India, about 28% of maize produced is used for food purpose, about 11% as livestock feed, 48% as poultry feed, 12% in wet milling industry (for example starch and oil production) and 1% as seed (AICRP on Maize, 2007) . In the last one decade, it has registered the highest growth rate among all food grains including wheat and rice because of newly emerging food habits as well as enhanced industrial requirements.

Maize is an important cereal in many developed and developing countries of the world. It is widely used for animal feed and industrial raw material in the developed countries where as the developing countries use it in general for feed. Among the cereal crops in India, maize with annual production of around 10 million tonnes covering 6 million hectares ranks fifth in area being next to rice, wheat, Jowar and Bajra, fourth production whereas in productivity it ranks third in position. Indian poultry sector has been growing at around 8-10 per cent annually over the last decade with broiler meat production estimated to increase from 0.8 Mt in 2000 to about 3.2 Mt in 2012 and egg production from 37 billion eggs to 66 billion eggs during the same period.

Maize (*Zea mays* L.) is very valuable crop. Globally, it is ranked as third staple food crop after wheat and rice. Maize is a leading commercial crop of high agro- economic importance due to its use in agro-industries. Worldwide, total annual production of maize is 3.341 million tons. Maize is very sensitive to salinity (Maas, 1986) though it has been reported that maize has interspecies inconsistency of salinity resistance (Maas *et al*., 1983).

II. MATERIALS AND METHODS

Gibberllic acid is a plant growth regulator which influences various developmental processes in plant. 100mg and 200mg concentrations of GA_3 were used for the study. Sodium Chloride is also called as salt or halite. It is an ionic compound. This was used for soaking seeds and 200mM and 300mM of sodium chloride were used for the study.

A. Physiological Studies

The cultivars of maize SNL and MYMH seeds were surface sterilized using 0.01% of mercuric chloride ($HgCl_2$) for two minutes. The seeds were washed thoroughly with distilled water for several times and soaked for 6 hours. Two GA_3 concentrations of 100ppm and 200ppm were used to soak the seeds. Two NaCl concentrations of 200mM and 300mM were used to soak the seeds. In the third set a combination of NaCl 200mM+GA_3 100ppm and NaCl 300mM+GA_3 200ppm were made and the seeds were soaked.

The test was conducted by the between paper towel method recommended by ISTA (2009). Hundred seeds of each cultivar were placed on craft paper saturated with known concentrations of salt 200mM and 300mM.

Seeds germinated in distilled water served as control. Each treatment including the control was replicated four times and kept under the temperature of 28±2°C. The number of seeds germinated in each treatment was counted on 7th day of germination and total germination percentage was worked out. On the 14th day root length, shoot length and seedling length were measured and Vigour index was calculated using the germination percentage and mean seedling length.

B. Germination Percentage

Germination refers to the initial appearance of radical by visual observation. It was calculated by using the following formula according to ISTA, 2009.

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

The seedling vigour index was calculated by using the formula proposed by Abdul Baki and Anderson (1973) and expressed in whole number. Seedling Vigour Index (SVI) = % of germination X Mean seedling length. Root length, shoot length and seedling length were calculated from each seedling at different concentrations of NaCl, GA_3 and combined action of NaCl and GA_3 was measured. Each seedling values were added and divided by total number of seeds used for germination.

The chlorophyll content viz., chlorophyll-a and chlorophyll-b and the total chlorophyll were estimated as per the method of Arnon (1949). The total protein content of maize seedlings treated with different doses of NaCl and GA_3 was estimated as per the method of Lowry *et al.*, (1951). Estimation of total free amino acids was carried out as described in Moore and Stein (1948). The activity of proteases was determined following the procedure of Kunitz (1947).

III. RESULTS

A. Germination Percentage

The percentage germination was increased in 200ppm $GA_3$and decreased in 100ppm $GA_3$when compared to control. The germination percentage was also increased in combination of gibberllic acid and NaCl as compared to control. The percentage germination was increased in both the cultivars under salinity stress. But MYMH cultivar showed decreased percentage of germination only under 300mM NaCl. The percentage germination in gibberllic acid as indicated by ANOVA and MYMH cultivar is more sensitive to salinity stress as shown in Table 1.

Table: 1. Effect of different concentrations of NaCl and GA_3 and combined action of NaCl and GA_3on the germination percentage (%) of SNL and MYMH maize cultivars.

Cultivar	Control	NaCl 200mM	NaCl 300mM	GA_3 100ppm	GA_3 200ppm	GA_3 100ppm +NaCl 200mM	GA_3 200ppm +NaCl 300mM
SNL	88^{a}	90.66^{a}	92^{a}	87.66^{a}	92.66^{a}	88.66^{a}	91.33^{a}
MYMH	83.33^{ab}	89.33^{a}	74.66^{b}	82.66^{ab}	83.33^{ab}	79.66^{b}	74.33^{b}

Means followed by the same letter within a row are not significantly different as indicated by Scheffe ($P \leq 0.05$) significant at $P \leq 0.001$.

B. Vigour Index

The vigour index decreased in both the cultivars under salinity stress but SNL cultivar showed increased vigour index only under 300mM NaCl. MYMH cultivar is more sensitive to salinity stress as indicated by ANOVA depicted in the Table 2.

Table: 2**.** Effect of different concentrations of NaCl and $GA_3$and combined action of NaCl and GA_3 on the vigour index of SNL and MYMH maize cultivars.

Cultivar	Control	NaCl 200mM	NaCl 300mM	GA_3 100ppm	GA_3 200ppm	GA_3 100ppm +NaCl 200mM	GA_3 200ppm +NaCl 300mM
SNL	2415.4^{cd}	2357.4^{d}	3061.9^{a}	2805.6^{abc}	2630.8^{bcd}	2592.4^{bcd}	2880.4^{ab}
MYMH	2145.8^{a}	2142.2^{a}	2059.4^{ab}	2114.6^{ab}	1811.9^{b}	2280.9^{a}	1807.4^{b}

Means followed by the same letter within a row are not significantly different as indicated by Scheffe ($P \leq 0.05$) significant at $P \leq 0.001$.

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C. Seedling Length

The root, shoot and seedling length were decreased in both the cultivars under NaCl 200mM concentration where as it increased a little in NaCl 300mM concentration. The root, shoot and seedling length were increased when both the cultivars were treated with GA_3 compared to control. In combination of GA_3 and NaCl there was an increase when compared to control as indicated by the ANOVA and Scheffe ($P \leq 0.05$) as shown in the Table 3.

Table: 3**.** Effect of different concentrations of NaCl and $GA_3$and combined action of NaCl and GA_3 on the Root length, shoot length and seedlings length of SNL and MYMH maize cultivars.

cultivar	Para meter	Control	NaCl 200mM	NaCl 300mM	GA_3 100ppm	GA_3 200ppm	GA_3 100ppm +NaCl 200mM	GA_3 200ppm +NaCl 300mM
SNL	RL	12.46^{ab}	9.28^{bc}	9.0c	11.68^{abc}	13.98^{a}	10.68^{abc}	11.70^{abc}
	SL	14.88^{c}	16.70^{bc}	19.39^{a}	20.32^{a}	19.30^{a}	18.53^{ab}	19.82^{a}
	Sl.L	27.34^{c}	25.98^{c}	28.39^{bc}	32.00^{ab}	33.29^{a}	29.21^{bc}	31.53^{ab}
MYMH	RL	9.41^{ab}	7.30^{c}	6.99^{c}	9.03^{b}	10.64^{a}	10.44^{ab}	9.48^{ab}
	SL	16.33^{ab}	16.70^{ab}	14.75^{b}	16.52^{ab}	18.18^{a}	16.91^{ab}	14.85^{b}
	Sl.L	25.75^{bc}	24.24^{cd}	21.74^{d}	25.86^{bc}	28.62^{a}	27.56^{ab}	24.33^{cd}

Means followed by the same letter within a row are not significantly different as indicated by Scheffe ($P \leq 0.05$) significant at $P \leq 0.001$. (RL-Root length, SL-Shoot length. Sl.L- Seedling length)

D. Chlorophyll Content

Estimation of chlorophyll pigments in SNL and MYMH maize cultivars treated with different concentrations of NaCl and GA_3 has been represented in Table 4. The analysis of variance (ANOVA and Scheffe) performed for these mean values shows a slight difference between the seed which was treated with different concentrations of NaCl and $GA_3$and combination of NaCl and GA_3 compared to control.

Table: 4. Effect of different concentrations of NaCl and $GA_3$and combined action of NaCl and GA_3 on the Chlorophyll (mg/g) content of SNL and MYMH maize cultivars.

cultivar	Para meter	Control	NaCl 200mM	NaCl 300mM	GA_3 100ppm	GA_3 200ppm	GA_3 100ppm +NaCl 200mM	GA_3 200ppm +NaCl 300mM
SNL	Chl-a	0.251^{a}	0.188^{d}	0.144^{e}	0.240^{b}	0.123^{g}	0.135^{f}	0.195^{c}
	Chl-b	0.443^{a}	0.023^{c}	0.166^{d}	0.368^{b}	0.013^{f}	0.015^{e}	0.023^{c}
	Tot.chl	0.611^{a}	0.455^{d}	0.349^{e}	0.586^{b}	0.297^{g}	0.328^{f}	0.471^{c}
MYMH	Chl-a	0.093^{a}	0.739^{c}	0.018^{f}	0.057^{d}	0.423^{e}	0.057^{d}	0.094^{a}
	Chl-b	0.016^{a}	0.014^{d}	0.017^{c}	0.014^{de}	0.013^{f}	0.014^{e}	0.024^{b}
	Tot.chl	0.233^{a}	0.183^{d}	0.227^{c}	0.140^{e}	0.104^{f}	0.140^{e}	0.228^{b}

Means followed by the same letter within a row are not significantly different as indicated by Scheffe ($P \leq 0.05$) significant at $P \leq 0.001$.

E. Protein Content

The total free Amino acids content of SNL cultivar was decreased in response to the salinity, but in MYMH cultivar the total free amino acids content was increased. The total free amino acids content was increased under GA_3treatment and also under combination of GA_3 and NaCl in both the cultivars. In salt sensitive variety more proteins were broken down to amino acids, hence the amino acids content was increased, the MYMH seems to be more tolerant when compared to the SNL cultivar. GA_3 showed the salinity mitigation role as shown in Table 5. The protein content of saline treated seeds was negatively affected and decrease in the protein content was observed in both the varieties. GA_3 treated and combinations of GA3 and NaCl have also shown a decrease in protein in both the maize cultivars. The protease activity was decreased in both the maize cultivars under salinity. The protease content was increased under GA_3 treatment and also in combination of GA_3 and NaCl. Protease activity was more in SNL cultivar compared to MYMH cultivar. So the protease activity was least affected by salinity. The mean values of amino acid, protein and protease activity in both cultivars were more in control when compared to NaCl and GA_3 treatments as indicated by ANOVA and Scheffe test of Table 5.

. Table: 5. Effect of different concentrations of NaCl and $GA_3$and combined action of NaCl and GA_3 on the Total free amino acids (mg g^{-1}), protein (mg g^{-1}) content and protease activity (10-4mM of tyrosine liberated g^{-1} min^{-1}) of SNL and MYMH maize cultivars.

cultivar	Para meter	Control	NaCl 200mM	NaCl 300mM	GA_3 100ppm	GA_3 200ppm	GA_3 100ppm +NaCl 200mM	GA_3 200ppm +NaCl 300mM
SNL	AA	84^{bc}	39^{e}	55^{d}	91^{b}	136^{a}	87^{b}	72^{c}
	Protein	217^{a}	115^{e}	103^{f}	160^{b}	141^{c}	123^{d}	46^{g}
	Protease	99^{d}	91^{e}	88^{e}	128^{b}	137^{a}	92^{e}	106^{c}
MYMH	AA	22^{c}	41^{b}	39^{b}	46^{b}	70^{a}	70^{a}	69^{a}
	Protein	217^{a}	152^{e}	161^{d}	144^{f}	170^{c}	159^{d}	180^{b}
	Protease	52^{e}	78^{d}	49^{e}	101^{ab}	104^{a}	91^{c}	98^{b}

Means followed by the same letter within a row are not significantly different as indicated by Scheffe ($P \leq 0.05$) significant at $P \leq 0.001$.

IV. DISCUSSION

A. *Germination Percentage*

The test was conducted to study the germination percentage of two maize cultivars subjected to salinity stress. As per our investigation there was decrease in germination percentage under saline treatment and there was increase in the germination percentage under GA_3 treatment and when the saline treated seeds were treated with GA_3 the germination percentage was increased, hence GA_3 alleviated the negative effect of NaCl. The higher germination percentage of cultivars in the control was due to lack of salt in the medium. High concentration of NaCl in the salt solution increases its osmotic potential. In addition, high absorption of Na and Cl ions by the seeds during germination can lead to cell toxicity that finally inhibits.

Salinity induces numerous disorders in seeds during germination, it reduces the imbibitions of water because of lower osmotic potential of the medium (Almansouri., *et al*., 2001). Salinity decreased final germination percentage, magnitude of reduction increased with increasing salinity stress (Hakim, *et al*., 2009; Anbumalarmathi, *et al*., 2013 and Mirza Hasamuzzaman *et al*., 2009). The osmotic effect due to salinity on imbibitions of seeds was the main cause for increased or reduced germination was reported by Akbar and Ponnamperuma. (1982).

B. *Vigour Index*

MYMH cultivar showed an increase and SNL cultivar showed a decrease in vigour index under GA_3 treatment. The combination of GA_3 and NaCl also showed an increase in the vigour index. Interaction effect of seed treatment and osmotic potential significantly affected the seedling vigour index. The results of present investigation showed that salt stress reduced the vigor index of maize seedlings. These findings are supported by previous studies as reported by Janmohammadi *et al.* (2008) and Djanaguiraman *et al.* (2003). They reported a significant positive correlation in vigor index and salt stress.

C. *Seedling Length*

As per the result of our investigation, seedling length was decreased under salinity and an increase in the seedling length was found in case of GA_3 treatment. Combination of NaCl and GA_3 also showed increase in Seedling length in both SNL and MYMH cultivars. Seedling length was less in MYMH cultivar under saline treatment, compared to the SNL cultivar.

The root and shoot length are important criteria in salinity studies as these plant parts are in direct contact with soil particles and solution. In this study, when the salinity levels increased, root and shoot length decreased. The gradual decrease in root length with the increase in salinity might be due to more inhibitory effect of NaCl. The root is the first organ exposed to salt stress than shoot. The root was affected negatively by salt stress. Salinity induced growth reduction in maize as noticed by suppressed leaf initiation, expansion and internode growth and by increased leaf abscission. In maize suppression of expansion growth by salinity is principally caused by reduced apoplastic acidification and activity of wall loosening enzymes (Muhammad *et al.*, 2015).

Additional input of the growth hormones like gibberellin increases the cell division and elongation of cells and helps in the increase of seedling length (Parvaneh Rahdari., *et al.*, 2015). GA_3 enhanced the growth by forming new cells in the intercalary meristem as reported by Khadija., *et al.*, (2013).

D. Chlorophyll Pigments

The Chlorophyll a, Chlorophyll b and total chlorophyll content was decreased under salinity in the cultivars. Photosynthesis is the most important process by which green plants covert solar energy into chemical energy in the form of organic compounds synthesized by fixation of atmospheric carbon dioxide (Muhammad *et al.*, 2015). Carbon fixation in maize is very sensitive to salt stress (Omoto *et al.*, 2012). Reduced photosynthetic apparatus, impaired activities of carbon fixation enzymes and reduced stomatal conductance are the key factors limiting carbon fixation capacity of maize plants under salt stress (Omato *et al.,* 2012 and Qu *et al.,* 2012). The total chlorophyll content of maize leaves was reduced by increased level of NaCl treatment. The salinity decreased the total chlorophyll content of two maize varieties. Reduction in photon yield in the salt stressed seedlings of maize was positively correlated to net photosynthetic rate (Pn); in which the significant drop in Pn of salt stressed seedlings resulted in considerable growth reduction (Cha-Um and Kirdmanee 2009).

In GA_3 treatment and combination of GA_3 and NaCl also there was a decreased photosynthetic rate in both the cultivars. Salt stress is known to cause significant degradation of chlorophyll pigments (Jamil *et al.*, 2012b). Salinity stress resulted in a marked degradation of chlorophyll in both cultivars. The degradation of chlorophyll pigments under salt-stress could be linked with increased activity of chlorophyllase or reduced de novo synthesis of chlorophyll (Qu *et al*., 2012).

E. Total Amino Acids Content

Total amino acids content was decreased under salinity and GA_3 treatment which lead to the qualitative and quantitative changes in free amino acids, hence an increase in total amino acids content was observed in seeds treated with GA_3. Total amino acids level in tolerant variety decreased under saline condition (Saikat Paul *et al* 2016). In contrast, Hussein *et al.* (2007) observed that reduced amino acid contents such as arginine, lycine, serine, and glutamic acid, no change for glycine; and enhanced levels of proline in response to salt stress in maize. Salt stress also induced polyamine accumulation, but spermidine was absent, possibly due to its fast turnover (Erdei *et al.*, 1996). In contrast osmoregulation helps maize plants to minimize the effects of salinity-induced osmotic stress. Proline and glycine betaine are the major osmolytes responsible for osmoregulation in maize under salt stress.

F. Protein Content

The test was conducted to study the effect of salinity, on two maize cultivars and action of GA_3 on same cultivars. Both the cultivars showed decrease in the protein content under salinity and combination of NaCl and GA_3. The decrease of protein content under salinity was due to breakdown of surface protein and production of free amino acid (Parvaneh Rahdari., *et al.*, 2015) and also Total protein contents were affected significantly by NaCl driven salt stress (Kumar, *et al.,* 2008). A continuous decrease in protein content with increase in salt stress was observed by Sunita Danai-Tambhale (2011). Protein content decreased in most of the plant species under sodium chloride stress was reported by Misra, *et al.,* (1997). Gibberellin treatment can lead to decreased levels of protein, during the stress condition there will be a decrease in the nitrate reductase enzyme activity in the protein surface tension, and decrease in the protein content is performed (Masroor., *et al.,* 2006).

G. Protease Activity

The protease activity moderately decreased in both the cultivars under salinity and increased by the action of $GA_3$and the combination of NaCl and GA_3. The protease activity was more even under the salinity. The salinity showed positive effect on the protease activity and the lyses of protein was more in the NaCl treated seeds and they could digest long chain of protein under this condition as stated by Cramer *et al.,* (2001).

V. CONCLUSION

The exogenous application of plant hormones and osmoprotectants like gibberellins, cytokinins and others may also improve maize performance under salt stress. Application of these substances helps in osmotic adjustments, nutrient uptake and antioxidant defense system. Further studied are required to arrive at final conclusion.

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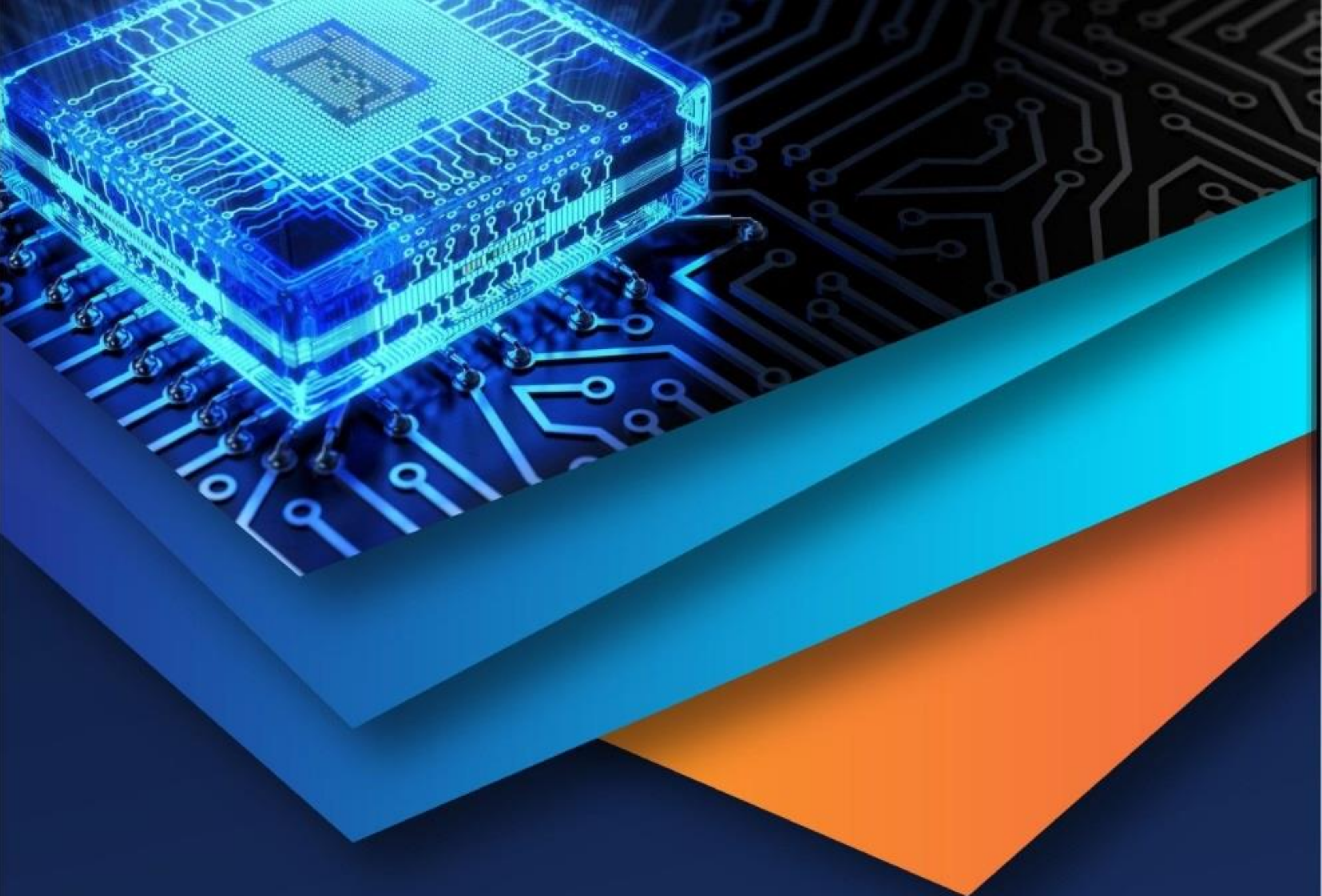

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