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The Effect of Exogenous Application of Gibberellic Acid on Two Salt Stressed Paddy Cultivars during Seed Germination

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Abstract: ***Agriculture is a major essential activity in many parts of the world. It provides adequate nutrition and also concerned with economic development especially in developing countries. In India nearly 70% of population directly or indirectly depends on agriculture and contributes almost 16.1% of G.D.P. Cereals are rich in vitamins, minerals, carbohydrates and protein. The starch mobilization of seed reserves and supply substrates essential for growth of the embryonic axis. Rice is a salt sensitive crop; NaCl inhibits mobilization of seed reserves. Gibberellic acid (GA_3) induces the synthesis of α-amylase in embryoless rice seeds. Salinity is the most brutal environmental factor limiting the productivity of crop plants. High salt concentration in soil or in irrigation water causes drastic effect on plant metabolism, and in turn disrupting cellular homeostasis and major physiological and biochemical processes. Mechanisms combining the molecular, physiological, biochemical and metabolic aspects of salt tolerance are essential to develop salt-tolerant crop varieties. Recently, some protectants viz; proline, glycinebetaine, trehalose, gibberellic acids, jasmonic acids, salicylic acid, ascorbic acid, glutathione, tocopherol, nitric oxide, hydrogen peroxide, spermidine, selenium, silicon, etc. have been found to be effective in mitigating the salt induced damage in plant and in turn enhance the plants growth, yield and stress tolerance under salinity. Gibberellic acids (Gibberellins A_3, GA, and GA_3) are concerned with growth. In the present investigation, an attempt has been made to study paddy cultivars primed with GA_3 and challenge with different saline conditions. Seed germination and vigor index has been improved by the application of GA_3. The mean shoot, root and seedling length and also Chlorophyll content and mean protein content of Jaya and Tanu rice cultivars in control and different concentrations of GA_3 treated with NaCl showed significant difference. Biotic stresses form the greatest constraint for crop production worldwide; more than 50% of yield reduction was also due to abiotic stresses (Acquaah 2007). Our results are correlated with the studies of Kumar and Singh (1996). Root, shoot and seedling lengths were also increased upon GA_3 priming under salt stress conditions (Kim and Son 2006). Similarly, Amal et al., (2014) who also reported that, gibberellins treatment on wheat seedlings under salinity stress will increases the Chlorophyll content.***

Keywords: Paddy, GA_3, NaCl, germination percentage, vigour index, chlorophyll, protein and amino acid.

I. INTRODUCTION

Agriculture is a major and vital essential activity in many parts of the world especially in developing countries for providing adequate nutrition and economic development. In India nearly 70% of the population directly or indirectly depends on agriculture and contributes almost 16.1% of G.D.P (CIA World Fact Book, 2015).

Cereals make the largest contribution to human nutrition. They are rich in source of vitamins, minerals, carbohydrates and protein. Major cereals are rice, wheat and maize. The minor cereals are sorghum, barley and millets. These are mostly used as food and animal feed. Hence there is a need for increased production of these cereals.

Crops are often exposed to environmental factors such as high and low soil salinity (Kaur *et al.,* 1998). Starch mobilization of seed reserves, which occurs during early seed germination, is crucial because it supplies substrates for the proper functioning of different metabolic processes that are essential for the growth of the embryonic axis (Mayer and Polijakoff-mayer, 1975). It is well known that rice is a salt sensitive crop. The mechanism of NaCl inhibition of rice seedling growth is unclear, but NaCl inhibits mobilization of seed reserves. Plant hormones are considered as key regulators for seed germination and its development (Davies, 1987). Gibberellic acid is known to induce the synthesis of α-amylase in embryoless rice seeds (Palmiano and Juliano, 1972). Gibberellic acid reduces NaCl inhibition of α-amyalse activity under salt stress (Lin and Kao, 1995). Gibberellins are plant hormones that

regulate growth and influence various developmental processes, including stem elongation, germination, dormancy, flowering, sex-expression, enzyme induction and leaf and fruit senescence.

II. MATERIALS AND METHODS

A. Collection of Seed Samples

The seed samples of two cultivars of paddy viz., Jaya and Tanu were collected from V.C. Farm, Regional Agricultural Research Station, Mandya, Karnataka, India. The cultivars of paddy seeds were surface sterilized using 0.01% mercuric chloride ($HgCl_2$) for two minutes. The seeds were washed thoroughly with distilled water by 3-4 times and soaked in 24 hours in gibberillic acid concentrations of 10ppm, 20ppm and 30ppm. The germination study was conducted by the between paper method recommended by ISTA, 2009. Hundred seeds of each cultivar were placed on Kraft paper saturated with known concentrations of salt (viz 0.01%, 0.02%) Seeds germinated in distilled water served as control. Each treatment including the control was replicated four times. The rolled papers were incubated in seed germinator at 28 $\pm 2^{o}$C. The number of seeds germinated in each treatment was counted on 5^{th} day of germination and total germination percentage was worked out. Germination percentage was calculated by using the formula according to ISTA, 2009. The seedling vigour index was calculated by using the formula proposed by Abdul Baki and Anderson (1973) and expressed in whole number. The chlorophyll content was estimated on 14^{th} day of germination according to ISTA, 2009 viz. chlorophyll – a and chlorophyll – b and total chlorophyll were estimated as per the method of Arnon (1949).

III. RESULTS

A. Impact of GA3 on Germination Percent and vigour Index

The mean germination percentage and vigour index were calculated in Jaya and Tanu paddy cultivars on 5th day old seedlings in control and at different concentrations of GA_3 induced by NaCl significantly different (Table1). The germination percentage and vigour index were gradually increased in both the cultivars on 5^{th} day old seedlings in all the concentrations of GA_3. The Scheffe - post hoc test was also indicated that treated paddy seeds were significantly different.

Table 1. Impact of GA3 on germination percent and vigour index induced by 0.01% and 0.02% of NaCl on Jaya and Tanu paddy cultivars during germination

Concentration of NaCl	paddy cultivars	Parameters	Control	Different concentrations of GA_3		
				10ppm	20ppm	30ppm
0.1%	Jaya	Germination %	88.00^{d}	92.666^{c}	93.333^{b}	95.666^{a}
		Vigour index	218.3^{d}	268.3^{c}	278.7^{b}	304.7^{a}
	Tanu	Germination %	72.00^{d}	84.00^{c}	91.00^{b}	93.00^{a}
		Vigour index	155.9^{d}	194.9^{c}	259.1^{b}	295.5^{a}
0.2%	Jaya	Germination%	88.00^{d}	98.66^{c}	99.33^{b}	100.00^{a}
		Vigour index	218.3^{d}	396.5^{c}	476.4^{b}	714.1^{a}
	Tanu	Germination %	72.00^{d}	91.00^{c}	96.66^{b}	97.66^{a}
		Vigour index	155.9^{d}	345.8^{c}	405.6^{b}	529.4^{a}

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. Impact of GA3 on shoot, root and Seedling Length

The mean shoot, root and seedling length of Jaya and Tanu rice cultivars in control and different concentrations of GA_3 treated with NaCl significantly different (Table1). Screening of paddy cultivars against 0.01% and 0.02% NaCl concentration revealed, that the shoot, root and seedling length increased in both the cultivars at 5th day old seedlings. Therefore compared to varieties the root length, shoot length, seedling length and salt tolerant were more pronounced in Jaya variety.

Table 2. Effect of GA_3 on seedling length of Jaya and Tanu paddy cultivar challenge inoculation with 0.01% and 0.02% NaCl

Concentration of NaCl	Paddy cultivars	Parameters	Control	Different concentrations of GA_3		
				10ppm	20ppm	30ppm
0.1%	Jaya	Root length	1.264^{d}	1.451^{c}	1.495^{b}	1.546^{a}
		Shoot length	1.217^{d}	1.281^{c}	1.321^{b}	1.501^{a}
		Seedling length	4.761^{d}	5.218^{c}	5.296^{b}	5.688^{a}
	Tanu	Root length	1.120^{d}	1.265^{c}	1.430^{b}	1.564^{a}
		Shoot length	1.108^{d}	1.117^{c}	1.242^{b}	1.445^{a}
		Seedling length	2.228^{d}	4.787^{c}	5.152^{b}	5.489^{a}
0.2%	Jaya	Root length	1.292^{d}	2.301^{c}	2.895^{b}	4.249^{a}
		Shoot length	1.219^{d}	2.005^{c}	2.361^{b}	3.254^{a}
		Seedling length	4.920^{d}	6.790^{c}	7.736^{b}	9.983^{a}
	Tanu	Root length	1.120^{d}	1.921^{c}	2.129^{b}	2.886^{a}
		Shoot length	1.108^{d}	1.795^{c}	2.010^{b}	2.442^{a}
		Seedling length	2.228^{d}	6.196^{c}	6.619^{b}	7.808^{a}

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

C. Impact of GA3 on Chlorophyll Content

Chlorophyll estimation at 14th day old seedlings of rice cultivars treated with different concentrations of GA_3 induced with NaCl in 0.01% and 0.02% as shown in Table 3. The mean chlorophyll content of Jaya and Tanu paddy cultivars in control and different concentrations showed significant difference and gradually increased in both the paddy cultivars.

Table 3. Effect of 0.01% and 0.2% NaCl on chlorophyll content of paddy cultivars soaked with GA3 during germination of seeds

Concentration of NaCl	Paddy cultivars	Parameters	Control	Different concentration of GA_3		
				10ppm	20ppm	30ppm
0.1%	Jaya	Chlorophyll- a	0.028^{d}	0.040^{c}	0.043^{b}	0.057^{a}
		Chlorophyll-b	0.052^{d}	0.069^{c}	0.077^{b}	0.113^{a}
		Tot. chlorophyll	0.082^{d}	0.104^{c}	0.114^{b}	0.145^{a}
	Tanu	Chlorophyll- a	0.018^{d}	0.023^{c}	0.032^{b}	0.052^{a}
		Chlorophyll-b	0.026^{d}	0.063^{c}	0.071^{b}	0.100^{a}
		Tot. chlorophyll	0.044^{d}	0.069^{c}	0.104^{b}	0.117^{a}
0.2%	Jaya	Chlorophyll- a	0.038^{c}	0.049^{c}	0.052^{b}	0.061^{a}
		Chlorophyll-b	0.046^{c}	0.075^{c}	0.099^{b}	0.111^{a}
		Tot. chlorophyll	0.072^{c}	0.126^{c}	0.142^{b}	0.183^{a}
	Tanu	Chlorophyll- a	0.021^{c}	0.034^{c}	0.047^{b}	0.058^{a}
		Chlorophyll-b	0.049^{c}	0.068^{c}	0.085^{b}	0.109^{a}

		Tot. chlorophyll	0.073^{c}	0.102^{c}	0.133^{b}	0.169^{a}

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

D. Effect of 0.01% and 0.02% of NaCl on Protein Content

In Jaya and Tanu cultivar the protein contents were gradually increased in pre-soaked with different concentration of GA3 over control treated with 0.01% NaCl as shown in table 4a and 4b. The mean protein content of Jaya and Tanu rice cultivars in control and pre-soaked different concentrations of GA_3 treated with NaCl were significantly different. The protein content **gradually increased** in both the cultivars from day 1 to 5th day in all the concentration of GA_3.

Table 4a. Effect of 0.01% NaCl on protein content paddy cultivars soaked with GA3 during germination of seeds

Parameter	Paddy cultivars	NaCl 0.01%	Control	Different concentration of GA_3		
				10ppm	20ppm	30ppm
Protein	Jaya	1st day	0.241^{d}	0.772^{c}	1.027^{b}	1.208^{a}
		2nd day	0.252^{d}	1.074^{c}	1.125^{b}	1.260^{a}
		3rd day	0.261^{d}	1.218^{c}	1.473^{b}	2.008^{a}
		4th day	0.269^{d}	1.236^{c}	1.573^{b}	2.009^{a}
		5th day	0.272^{d}	1.535^{d}	1.895^{b}	2.074^{a}
	Tanu	1st day	0.164^{c}	0.183^{c}	1.108^{b}	1.176^{a}
		2nd day	0.165^{d}	0.956^{c}	1.164^{b}	1.244^{a}
		3rd day	0.167^{d}	1.348^{c}	1.477^{b}	1.540^{a}
		4th day	0.169^{d}	1.472^{c}	1.647^{b}	1.854^{b}
		5th day	0.172^{d}	1.615^{c}	1.915^{b}	1.990^{a}

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

Table 4b. Effect of 0.02% NaCl on protein content paddy cultivars soaked with GA3 during germination of seeds

Parameter	Paddy cultivars	NaCl 0.02%	Control	Different concentration of GA_3		
				10ppm	20ppm	30ppm
Protein	Jaya	1st day	0.351^{d}	0.787^{c}	1.054^{b}	2.103^{a}
		2nd day	0.354^{d}	1.106^{c}	1.226^{b}	2.106^{a}
		3rd day	0.357^{d}	1.111^{c}	1.247^{b}	2.107^{a}
		4th day	0.359^{d}	1.274^{c}	1.596^{b}	2.115^{a}
		5th day	0.361^{d}	0.677^{c}	2.002^{b}	2.204^{a}
	Tanu	1st day	0.211^{d}	0.694^{c}	0.895^{b}	1.042^{a}
		2nd day	0.215^{d}	0.915^{c}	0.974^{b}	1.218^{a}
		3rd day	0.217^{d}	1.210^{c}	1.269^{b}	1.397^{a}
		4th day	0.219^{d}	1.292^{b}	1.337^{b}	1.541^{a}
		5th day	0.301^{d}	1.308^{b}	1.430^{b}	1.605^{a}

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

Abiotic stresses remain the greatest constraint to crop production worldwide. It has been projected that more than 50% of yield reduction is the direct result of abiotic stresses (Acquaah 2007). The major abiotic stresses like drought, high salinity, cold, and heat negatively influence the survival, biomass production and yield of staple food crops up to 70% (Ahmad and Prasad, 2012); hence, threaten the food security worldwide.

Salinity is one of the most brutal environmental factors limiting the productivity of crop plants because most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil. A considerable amount of land in the world is affected by salinity which is increasing day by day. On the other hand, increased salinity of agricultural land is expected to have destructive global effects, resulting in up to 50% loss of cultivable lands by the middle of the twenty first century (Mahajan and Tuteja 2005).

High salt concentration in the soil or in the irrigation water can also have a devastating effect on plant metabolism, disrupting cellular homeostasis and uncoupling major physiological and biochemical processes. Biochemical and molecular studies of salt stress responses in plants have revealed significant increase of reactive oxygen species (ROS), including singlet oxygen ($^1O_2$), superoxide (O_2^-), hydroxyl radical ($OH^\bullet$) and hydrogen peroxide (H_2O_2) (Tanou *et al*., 2009; Ahmad and Umar 2011) . However, the effect of salt stress on plants depends on the concentration and time of exposure of salt, plant genotypes and environmental factors.

Mechanisms of salt tolerance, not yet completely clear, can be explained to some extent by stress adaptation effectors that mediate ion homeostasis, osmolyte biosynthesis, toxic radical scavenging, water transport and long distance response co-ordination (Hasegawa *et al*., 2000). However, attempts to improve yield under stress conditions by plant improvement have been largely unsuccessful, primarily due to the multigenic origin of the adaptive responses. Therefore, a well-focused approach combining the molecular, physiological, biochemical and metabolic aspects of salt tolerance is essential to develop salt-tolerant crop varieties. Exploring suitable ameliorants or stress alleviant is one of the tasks of plant biologists.

In recent decades exogenous protectant such as osmoprotectants (proline, glycine, betaine, trehalose, etc.), plant hormone (gibberellic acids, jasmonic acids, brassinosterioids, salicylic acid, etc.), antioxidants (ascorbic acid, glutathione, tocopherol, etc.), signalling molecules (nitric oxide, hydrogen peroxide, etc.), polyamines (spermidine, spermine, putrescine), trace elements (selenium, silicon, etc.) have been found effective in mitigating the salt induced damage in plant (Ahmad and Prasad, 2012 ; Yusuf *et al*. 2012). These protectants showed the capacity to enhance the plants growth, yield as well as stress tolerance under salinity.

Gibberellic acids (also called Gibberellin A3, GA, and GA_3) are generally involved in growth and development; they control seed germination, leaf expansion, stem elongation and flowering (Magome *et al*., 2004; Kim and Park 2008). Additionally, GAs interacts with other hormones to regulate various metabolic processes in the plants. However, many conflicting theories have been put forward concerning their interactions (Yang *et al*., 1996; Van Huizen *et al*., 1997). In order to alleviate deleterious effects of salinity, different types of phytohormones have been used. Among them, GA_3 have been the main focus of some plant scientists. Innumerable works have confirmed the potential of GA_3 to synergistically improve crop performance under normal conditions.

Hamayun *et al*. (2010) reported that exogenous GA 3 also mitigated the adverse effects of salt stress in Glycine max by regulating the level of phytohormones, thus aids the plant in resuming its normal growth and development. The application of GA_3 reduced the inhibitory effect of NaCl on growth attributes and photosynthetic pigments in *Hibiscus sabdariffa* by inducing the enzyme activity and enhancing RWC and thus GA_3 helped in the tolerance of plants to salt stress (Ali *et al.,* 2012). During the present investigation, an attempt has been made to study paddy cultivars primed with GA_3 and challenge with different saline conditions. Seed germination and vigor index has been improved by application of GA_3 which is correlated with the studies of Kumar and Singh 1996. Root length, shoot length and seedling length was also increased upon GA_3priming under salt stress conditions similar results to the showed by Kim and Son (2006).

Salinity has toxic effects on plants and causes changes in metabolic activity such as reduced activity of chloroplasts, reduced photosynthetic pigments, reducing the rate of photosynthesis and increase of respiration rate, which ultimately leads to increased production of reactive oxygen species in plant. Content reduction of chlorophyll in plants such as Poulownia imperialis (Astorga et al., 2010), Bean (Beinsan et al., 2003) and Carthamus tinctorius (Siddiqi et al., 2009) was reported. The cause of this reduction was the increasing of destructive enzymes called chlorophyllase. Plant hormones such as gibberellin, has special effects on leaf anatomy and chloroplast structure. Salinity through the influence of peroxidase enzymes and chlorophyll degradation can induce catabolism that Gibberellin reduces the activity of these enzymes. This result was reported with El-Tayeb in 2005 and in Wheat, Younis (1991) in Pisum sativum, Aldesuquy (1992) wheat and El-Bastawisy in (1999) wheat.

Paddy cultivars primed with GA_3 and challenge inoculation with different concentrations of NaCl showed increase of photosynthetic contents in paddy cultivars. Our results are in accordance with Amal *et al.,* (2014) who reported that treatment with gibberellins hormone in wheat seedlings under salinity stress increased the chlorophyll content.

V. CONCLUSION

Finally, it can be inferred that GA_3 counteracts with salinity by improving membrane permeability and nutrient levels in leaves which ultimately leads to better seedling growth. GA_3 induced physiochemical changes responsible for induction of salt tolerance in paddy especially Jaya and Tanu. It is, therefore, possible that exogenous applications of GA_3 could be a useful tool in promoting

good seedlings growth and establishment under saline soil conditions. It was concluded from the present investigation that with the increase in NaCl and GA_3 concentration, applied to different paddy cultivars, the quality enhanced with regards to germination percentage, vigour index, photosynthetic pigments and protein content. However, the effect of salt stress on plants depends on the concentration and time of exposure to salt, plant genotypes and environmental factors.

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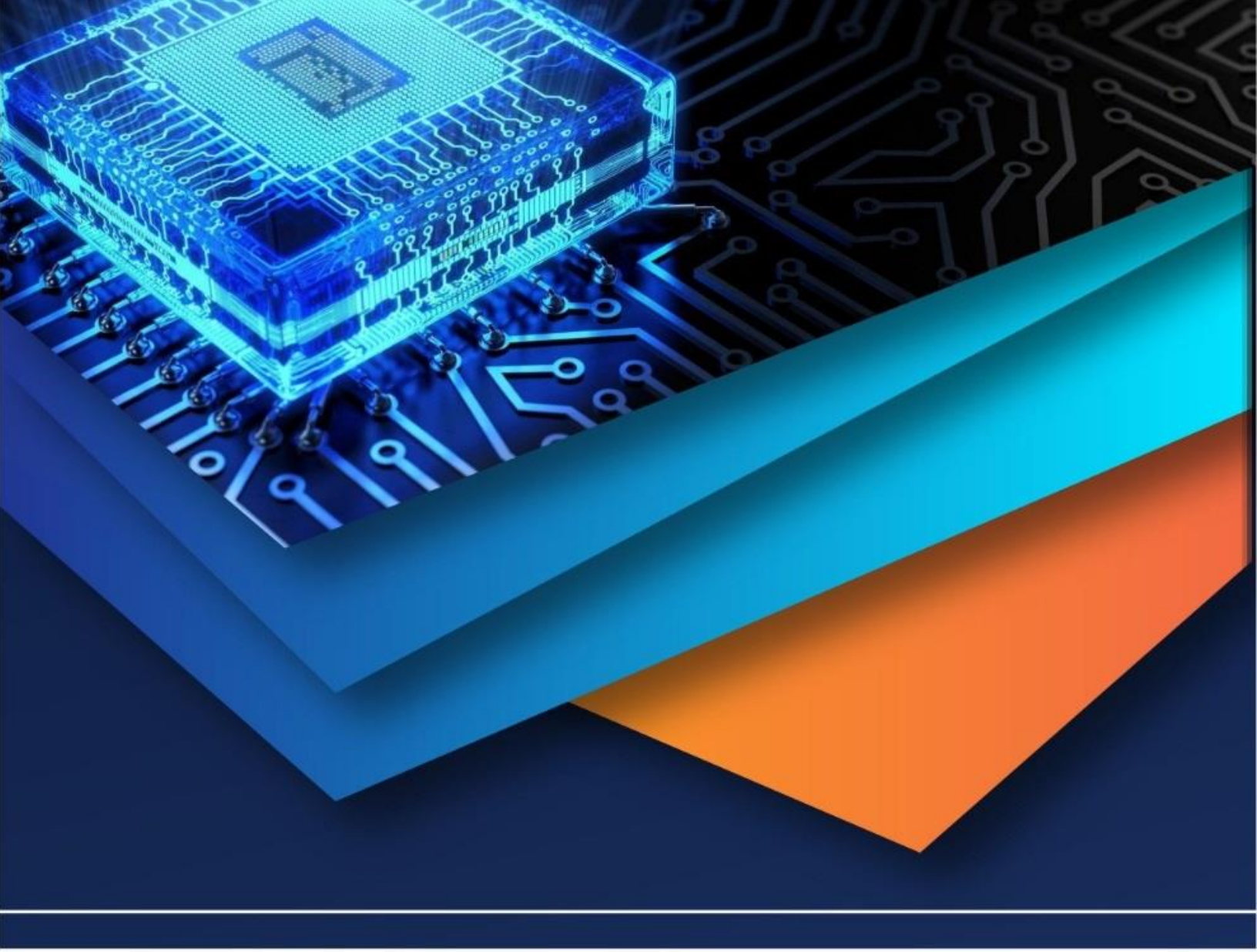

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