iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 2 **Issue:** V **Month of publication:** May 2014

DOI:

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

ISSN: 2321-9653

INTERNATIONAL JOURNAL FOR RESEARCH IN APPLIED SCIENCE AND ENGINEERING TECHNOLOGY (IJRASET)

Plant Growth Promoting Traits of Diazotrophic Bacteria Effects on Growth and Yield of Rice Crops

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Abstract- The search for diverse plant growth promoting (PGP) nitrogen fixing bacteria is increasing day by day as efforts are increasing to exploit them as bio fertilizers for various economically important crops. In Chhattisgarh, about 83% population depends on agriculture for their livelihood. The area sown under rice crop is 3.8 million hectare. The average productivity of rice in Chhattisgarh is only 2.1 tons per hectare. The prime cause of low productivity of rice in the state is that, only 21.7% area is under irrigated and rest is rain fed [1].High temperature (heat) stress is considered to be one of the major environmental factors limiting crop growth and yield. This stress induces many biochemical, molecular, and physiological changes and responses that influence various cellular and whole plant processes that affect crop yield and quality. The impacts of environmental stress, particularly those of drought and heat, have been studied, independently. Rice is arguably the most important cereal crop in the world, feeding more than 50% of the world's population However, the population is growing at a rapid rate; therefore, rice yields will need to be enhanced to match the increased consumption. Achieving these higher yields by 2020 will require at least double the amount of N fertilizers currently being used because, after water, N is the most limiting nutrient for rice growth.

Keywords: plant growth, diazotrophic PGPR, bio fertilizer, DNA hybridization, Rice Seedlings

I. INTRODUCTION

The N requirement for wheat is higher than that for rice, because of its higher grain protein content. Wheat yields vary widely from 1 to 7 t ha21 depending on inherent soil fertility, the amount of applied fertiliser, wheat variety, diseases such as take-all, other management practices and environmental conditions [2]. Thus, the estimated amount of N removed by wheat crops varies between 26 and 200 kg N ha21, depending on yield. To maximise wheat yields in soils that are not capable of supplying enough N, chemical N fertilisers such as urea are used to enhance N supply. The N rate applied to wheat crops ranges between 30 and 225 kg N ha21 depending on soil fertility, wheat variety and targeted yield. Bacterial inoculants biofertilisers can, in principle, be used to supplement the use of urea-N. There are comparatively fewer reliable reports of the successful field applications of biofertiliser for wheat than for rice. It seems possible that in dryland production of wheat, water stress may increase the difficulty of obtaining such benefits, although this possible limitation has not yet been experimentally tested. A range of diazotrophs including strains of Azospirillum, Azotobacter, Azorhizobium, Bacillus, Herbaspirillum and Klebsiella can supplement the use of urea-N in wheat production either by BNF or growth promotion [3]. The estimated amount of BNF by such wheat–bacterial associations was between 10 and 30 kg N ha21 for each crop. about 10% of their total-N requirement. However, successful cases of inoculation of wheat on a continuing basis are known to the reviewers.

- The special case of sugarcane

Brazil is the world leader in replacing chemical N fertilizer with BNF for sugarcane production. Sugarcane (Saccharum officinarum) is an important crop grown for sugar and ethanol production. It requires approximately 1.45 kg N ha21 to produce 1 t of moist biomass or about 7 kg N ha21 for 1 t of dry cane (i.e. 116–274 kg N ha21). The yield of millable sugarcane biomass varies from 80 to 190 t ha21 depending on soil fertility, amount of fertilizer applied, and the cultivar.The large amount of N removed by sugarcane from soil and the extended period of cultivation without replenishing N-fertiliser applications can deplete the soil-N concentration alarmingly[4]. Generally 150–250 kg urea-N ha21 is applied for sugarcane cultivation depending on soil fertility, genotype and the targeted yield Evidence from Brazil indicates fertiliser-N can be reduced to half by exploiting BNF systems, claimed to be based on diazotrophic PGPR such as Acetobacter (Gluconace to bacter) and Herbaspirillum The 15N natural abundance technique established that the BNF contributes up to 60% of the total assimilated N by sugarcane varieties not receiving fertiliser-N Both obligate and facultative diazotrophs live in the roots, stems and leaves of sugarcane, and can fix up to 150 kg N ha21 of atmospheric N Because of the limits of accuracy for the 15N natural abundance technique, it is possible that some of the extra N obtained is from a growth-promotion effect, which contributes to the efficient N uptake from soil. The diazotrophs commonly present in sugarcane plants are Acetobactor diazotrophicus, Azospirillum brasilense, A. lipoferum, A. amazonense, B. brasilensis, B. tropicalis, H. seropedicae and H. rubrisubalbicans Where fertiliser-N is applied, the numbers of these diazotrophs markedly decline in sugarcane rendering the plant more dependent on fertiliser-N. The endophytes colonise sugarcane spontaneously, promoted by the vegetative mode of propagation of sugarcane [5]. It seems likely that continued study of the sugar cane system will yield information of use in establishing the use of diazotrophs with other crops [6].

- PGPR effects and crop yields

Additional data can be quoted that shows significant beneficial PGPR effects improving the yields of a broad range of field crops although many failures to obtain such responses may have been unreported. Both green house and field experiments support the ability of organisms such as Azospirillum to increase yield in the range 5–30% in about 70% of inoculation trials But not all such trials are successful and there are even cases where declines in yield were associated with inoculation [7]. This may reflect incompatibilities between bacterial strains and plant cultivars, as well as adequate soil-N for nutrition, as noted earlier with maize. It is also noteable that a large number of different diazotrophic as well as non diazotrophic species may contribute to the beneficial effects on the growth and yield of cereals, including those listed in as well as Pseudomonas, Klebsiella, Citrobacter, Clostridium, Azoarcus, Azorhizobium and others mentioned earlier. Some, such as Acetobacter, may be more restricted in the range of plants they can associate with because of special nutritional needs such as high sugar concentration [8]. There is little evidence of clearly preferred combinations of plant and microbial species to obtain beneficial effects, although some studies have suggested variation in response based on genotype.However, there are many questions that remain to be addressed before there can be sufficient confidence in the possible agronomic role of such in oculant bio fertilizers to recommend their widespread adoption. Unfortunately, no studies have yet been reported to test if re-inoculation with PGPR is needed for each successive crop [9].

II. OBJECTIVE

The bio fertilizer product must be shown to contain beneficial strains of microorganisms. Strain selection is the first step of inoculation technology[10]. According to criteria for successful selection of bio fertilizer strains for rice are.

- the strain should be abundant in the soil,
- it should have high activity (e.g. BNF, phytohormone production, P solubilising activity, etc.),
- the strain should be as fast-growing as possible, improving the success rate if non-sterile carrier media must be used,
- the strain must be shown not to cause root disease and finally
- In laboratories where freeze-drying is not available, the strains should be reselected, if necessary, to show their effectiveness has been maintained. Confirmation of the correct identity of biofertiliser strains used to prepare bio fertiliser inoculants can be based on cultural characteristics, PCR methods, immunodiagnostic tests or immunoblots and DNA hybridisation techniques.

III. LITERATURE SURVEY

At Wageningen in the Netherlands, it has been found that plants rely on a complex community of soil microbes to defend

themselves against pathogens much the way mammals harbor a raft of microbes to avoid infections (Mendes et al 2011)[10]. In six soils under sugar beets with varying degrees of suppressiveness to Rhizoctonia solani, genomic tools detected more than 33,000 bacterial and archaeal species with all six soils having more or less the same types of bacteria. However, in terms of abundance of bacteria, each had a unique fingerprint. All the samples in which disease was suppressed had a greater abundance of 17 unique types of bacteria. These included well-known fungal fighters such as Pseudomonas, Burkholderia,Xanthomonas and Actinobacteria. In addition, other types of bacteria that have no demonstrated ability to fight pathogens on their own were found to act synergistically to suppress plant disease. Based on this, they concluded that an uptick in several bacterial types is a more important indicator of disease suppression than the presence of one or two bacteria that are especially good at killing pathogens [3]. The complex phenomenon of disease suppression in soils cannot simply be attributed to a single bacterial group, but is most likely controlled by a community of organisms acting together as a tight knit army. This means that attempts to improve soil health by microbial inoculation should consist of inoculation with a number of organisms as a consortia rather than simply single organisms like Pseudomonas as widely practiced. Heterotrophic organisms require organic materials as both carbon and energy sources, and higher microbial populations have been measured by a number of workers in organically managed soils. As a generalization, organically managed soils maintain higher biodiversity and have been shown to have lesser incidence of soil borne diseases compared to conventional farming [9]. Higher incidence of my corrhiza in organically managed soils has also been known since long. In a long term experiment at Frick, Switzerland, soil microbial biomass and enzymes activities were higher in organic than in conventional soils. My corrhiza were 40% more, biomass and abundance of earthworms were higher by a factor of 1.3 to 3.2. Average density of carabids, staphylinids and spiders in the organic plots was almost twice that in conventional plots and their beneficial role in biological control is known (Mader et al). In a comparison of various soil management practices in UK, differences in microbial communities in soils under conventional, organic, and integrated systems were subtle, rather than dramatic (Shannon et al). Greater amount of readily extractable ATP, increased numbers of viable but nonculturable bacteria, total and vital fungal bio-volumes in soil in organically managed soils pointed to greater physiological diversity of microorganisms in such soils. As compared to organic or conventional farming, microbial biomass was higher in integrated farming systems whereas fungal biomass was higher in organic soils [2] . Very large number of microbes are only required if added organic material has to be broken down to provide minerals for plant growth. One of most consistent observations in temperate soils has been the inability of organically farmed soils to provide sufficient N required for rapid vegetative growth in spring wheat during peak demand, which prove that despite the build up, there are probably insufficient microbial numbers to mineralize SOM in soils of organic farms. A more important benefit of organic farming is the development of 'suppressiveness' of diseases, as discussed later. In our recent studies at a sandy soil site in Rajasthan, we found consistently higher populations of copiotrophic bacteria (that consume high amount of substrate and have higher growth rates) and oligotrophic bacteria ((bacteria that thrive on very low amount of substrate and have slow growth rates) actinomycetes, glomalin, biological activity (dehydrogenase, FDA hydrolysis) and activity of soil enzymes (acid phosphatase, ß-glucosidase) in organic cropping and orchards compared to conventional cropping. In an experiment on low-input farming at the ICRISAT Center, near Hyderabad, no- tillage along with addition of rice straw mulch or farm waste mulch (microbial inoculants and bio-pesticides were applied in both) was compared with conventional tillage system with (i) NP fertilizers plus chemical pesticides and (ii) an integrated system involving chemicals and biomass mulch (Rupela et al 2005). After 5 years, SOM content, microbial biomass and soil respiration were higher in the two organic plots (with no-till and biomassmulch) as compared to conventional, chemical farming with normal tillage, but highest in the integrated (chemical + plant biomass residue) treatment with normal tillage .Although the effects of organics and not ill were compounded in this treatment, yet it is clear that majority of the benefits were attributable to greater inputs of plant biomass nutrients (27-52% more N and 50-58% more P)[9,10]. It is important to note that microbial activity per se did not differ in the organic and conventional plots. For example, the respiratory quotient (qCO2) in organic–1, (rice-straw based), organic-2 (farm waste based), conventional chemical and integrated (chemical and farm waste based) were similar as was dehydrogenate activity. The C:N ratio of microbial biomass was also similar as was the proportion of microbial biomass as a fraction of organic C. This supports the findings of Gunapala et al (1998) and many others that the ability of soil microorganisms to decompose added organic matter was the same in organic or conventional systems

and that microbial diversity was not compromised by chemical farming with the conclusion that integrated systems are the best.

IV. METHODOLOGY

INOCULATION OF DIAZOTROPH BACTERIA TO RICE SEEDLINGS

The eight biofertiliser treatments were: T1 (Sphingomonas azotifi gens); T2 (Pseudomonas putida); T3 (Herbispirillum sp.); T4 (Pantoea agglomerans); T5 (Stenotrophomonas maltophila); T6 (Acinetobacter radioresistance): T7 (mixed inoculum of T1+T2+T3+T4+T5+T6) and T8 (control without any diazotroph inoculation). The standard inoculam of the strain was prepared by growing the bacteria in 250 ml glucose-peptone broth for 72 h at 28 0C on a rotatory shaker. The cells in active growth stage were harvested by centrifugation at 8000 rpm for 10 mins and resuspended the pellets in sterilized distilled water to attain a concentration of 108cfu ml-1. Seed bacterization was done by taking twenty five ml of bacterial inoculum containing 1x108 cfu ml-1 and added to a 100 ml Erlenmeyer conical flask. One hundred mg of CMC was added as adhesive material. Ten grams of seeds were soaked in bacterial culture for 12 h on a rotary shaker at 150 rpm. The bacterial suspension was drained off and the seeds were dried overnight aseptically in laminar air flow. Seeds soaked in distilled water amended with CMC served as control. The seeds were then placed in plastic pots filled with air-dried paddy field soil. The pots were then kept in green house with a light/dark cycle beginning with 12h darkness followed by 12 h light. One ml overnight culture was applied to the soil near the root zone on day 15 and 30. The crop was uprooted 45 days post sowing and growth parameters and nutrient uptake was measured. The experiment was conducetd on six replicates for each treatment and was completely randomized. The AR activity of the rhizospheric soil sample was determined by the method of Yim et al.[7].1 g rhizosphere soil from each treatment was placed in a 120 ml vial containing 40 ml of semisolid Media. Following incubation, the gas phase in the headspace was replaced with acetylene [10% (v/v)] and incubated again at 30 0C for 24 h. Ethylene production was measured using gas chromatograph (Konic model). Values were expressed as nM C2H4 h-1 g-1 dry soil. After 45 days post sowing the plants was uprooted with intact roots, washed thoroughly to remove the adhered soil and taken for the measurement of growth and yield parameters. The height of five randomly chosen plants from each replicate plot in a treatment block was measured from the root to the panicle tip and the average height was expressed as cm/plant. The root and the shoot length of plants were measured separately from the replicate samples and the average length was expressed as cm/plant. Number of tillers/plant (average) was counted in five randomly chosen plants in each replicate plot of a treatment and the values were expressed as average of replicate samples. Plant dry weight (g/plant) was recorded after drying the samples in an oven for 24 h at 70 0C. The grain yield was determined by measuring the weight of all the grains of individual plants and the yield (g/plant) was expressed as average of replicate samples of a treatment. The N-content of the shoot was evaluated by micro-kjeldahl method [7] and the chlorophyll content of the leaves was determined by Arnon (1949) method. Protein content of grains was determined by Lowry's method[2]. These parameters are expressed as average of five randomly collected plants from each replicate plots in a treatment block.

V.RESULTS

The plant growth promoting ability of all the eleven diazotrophic strains was studied. The maximum activity of nitrogenase enzyme was showed by Sphingomonas azotifigens (K23) (290.22 nm ethylene/h/mg protein) followed by Pseudomonas putida (K4) (280 nm ethylene/h/mg protein), Herbispirillum sp. (K16) (250 nm ethylene/h/mg protein), Acinetobacter radioresistence (K34) (210 nm ethylene/h/mg protein) and Stenotrophomonas maltophila (K6) (150 nm ethylene/h/mg protein) (Table 1). The strains produced IAA, ranging from 1.08 µg/ml in Stenotrophomonas maltophilato 2.23 µg/ml in Pseudomonas putida. Among all the isolates Sphin Sphingomonas azotifigens, Pseudomonas putida, Acinetobacter radioresistance and Pantoea agglomerans were capable of solubilising phosphorous.

Table1.Effect of diazotrophs on the growth and yield of rice

Treatment	Plant Height (cm)	No.of tillers /Plants	Fresh weight/Plant(g)	weight of 100 grans/plants(g)	Dry biomass/plant(g)
T1	104.2	25	155	4.25	38.25
T2	132.21	21	143	3.5	30.54
T3	136.1	22	146.77	4.10	37.24

T4	126.4	18	134	3.89	25.06
T5	119.8	17	131	2.12	29.89
T6	128.41	21	136.21	2.23	39.27
T7	120.8	14	123	2.11	28.56
T8	110.21	13	119	2.03	27.96

Plant inoculation experiment

Based on the PGPR activity of the isolated strains i.e 6 strains (Stenotrophomonas maltophila, Pseudomonas putida, Herbispirillum sp, Pantoea agglomerans, Stenotrophomonas maltophila and Acinetobacter radioresistance) were selected for their effect on the growth and yield of the rice. The effect of inoculation of the isolated strains over control was observed after 45 days post sowing and it was observed that the plant height, shoot length, root length, number of tillers per plant, plant fresh weight, weight of 100 grains, dry biomass/plant, chlorophyll content of leaves, nitrogen content of shoot, protein content of grains and root NR activity were increased over un-inoculated control rice plots. In cultivated varieties Sphingomonas azotifigens was found to increase the plant height, root length, number of tillers per plant, plant fresh weight, weight of 100 grains and dry biomass/plant of autumn rice at a maximum rate. But in case of local variety Herbispirillum sp was found to increase all the parameters at a maximum rate than all the other strains taken under study. In cultivated varieties root length, shoot length, plant height, number of tillers per plant, plant fresh weight and weight of 100 grains per plant were increased by inoculating Sphingomonas azotifigens but dry biomass/plant was increased by inoculating with Pantoea agglomerans. Again in local varieties Herbispirillum sp was found to increase all the growth parameters in maximum amount followed by Sphingomonas azotifigens and Pseudomonas putida. Maximum yield is shown by Sphingomonas azotofigens followed by Herbispirillum sp, Pseudomonas putida, and Pantoea agglomerans. All the biochemical parameters of growth were found to increase compared to the control after inoculating with the diazotrophs. Among the six strains taken Herbispirillum sp was found to increase the chlorophyll content, nitrogen content of shoot, protein content of grains and root NR activity to a maximum amount, followed by Sphingomonas azotifigens and Pseudomonas putida.

VI.CONCLUSION AND DISCUSSION

The main objective behind this research work is fulfilled which Increment of agricultural performance over the past three decades has been achieved by degrading the environment and the emergence of problems such as soil erosion, pollution from chemical fertilizers and pesticides, water resources and have reduces biological diversity in plants and animals in the world. Therefore, low input farming systems as an aim to achieve maximum production in a short period of time is different to conventional system. Its aim is to achieve a stable level of production for long-term environmental compatibility to low energy inputs and small amounts of chemicals. Using these two types of bacteria in this study makes a better availability of nitrogen and phosphorus to plants that stimulate the issue of better growth, increase tolerance of plants against diseases and biotic and abiotic stress in order to stimulate further growth. The use of PGPR's increased the amount of cytokinin, gibberlin, auxin which led to better growth and increase rice yield. In fact, these bacteria by producing metabolites similar to growth regulatory substances directly increase plant growth and development. Based on the results of this study it can be stated that when phosphorus and nitrogen exist in soils the presence of PGPR's increase the absorption of elements in rice. Therefore the use of biological fertilizers results in yield increment decrease the use of chemical fertilizers so that the least adverse impact on the environment is achieved.

VII. FUTURE WORK

The future work compilation of specific plant growth promoting characters of diverse diazotrophic strains suggest that these particular organisms can promote plant growth by more than one mechanism and they can be better exploited if the PGPB are selected for use. However any practical application of these results should be proceeded by further evaluation under field conditions. Because it is important to examine the adaptability of these diazotrophic PGPB to environmental conditions before they are utilized as inoculants strains.

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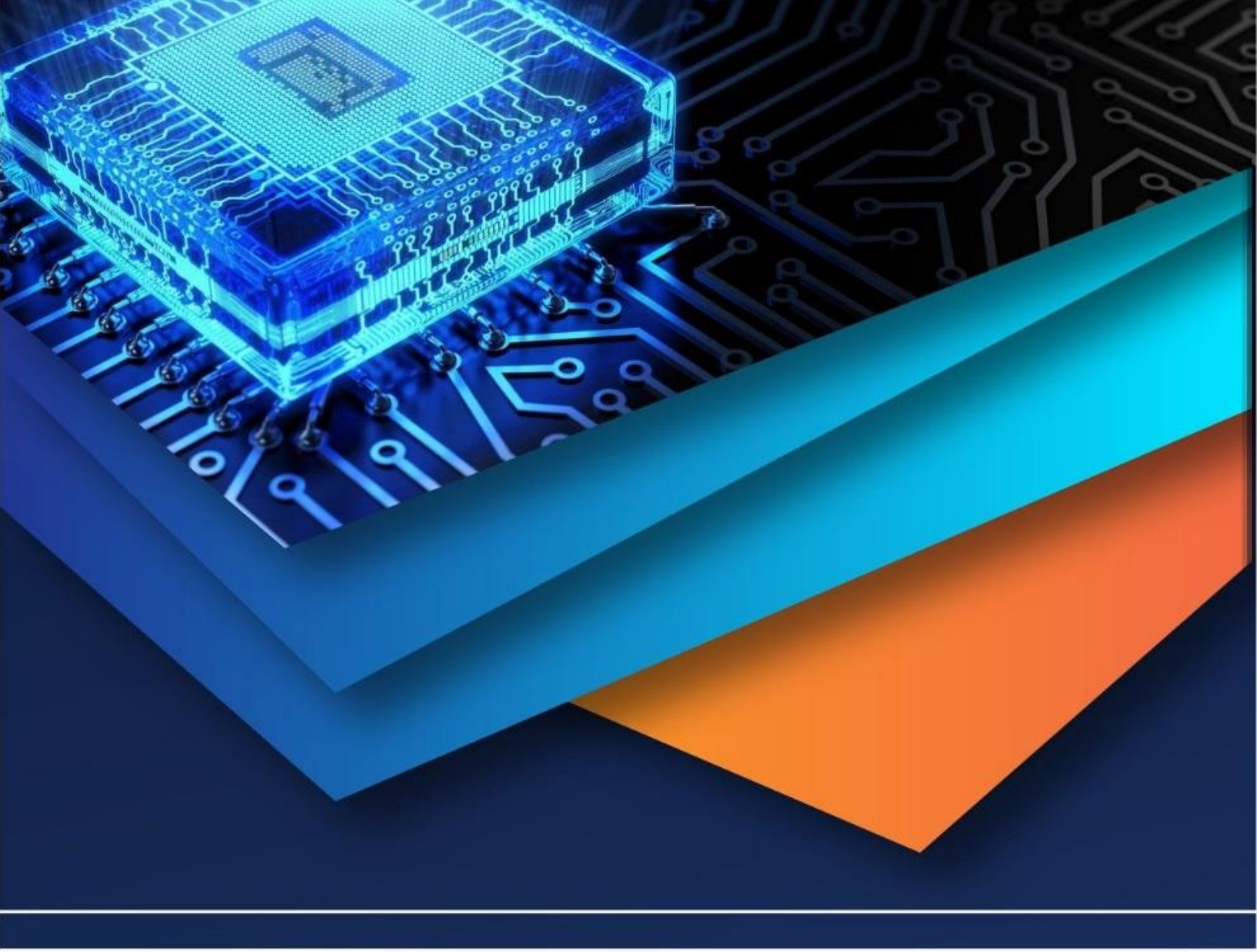

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