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Effect of Ethylmethane Sulphonate on Regeneration and Growth of in Vitro Treated Callus of Dendrocalamus Hamiltonii

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Abstract: ***The present study on the effect of mutagens on regeneration and growth of in vitro grown callus of Dendrocalamushamiltonii was carried out. Developmental characteristics of the EMS treated in vitro raised callus on regeneration media were evaluated. Chemical mutagen treated callus explants were later on transferred and cultured on MS medium supplemented with BAP (1mg/l) + IBA (1mg/l) to record data. The increase in concentration of mutagen gradually decreased the in vitro callus survival and regeneration percentage. The stimulatory effect was observed in lower doses/concentrations of EMS on the length and number of roots and shoots. The study would be beneficial to induce desirable variations in plant growth characteristics of bamboo by the use of mutagens treatment.***
Keywords: Ethyl methane sulphonate, regeneration, in vitro, Dendrocalamushamiltonii and mutagen*.*

I. INTRODUCTION

Dendrocalamushamiltonii is a multipurpose bamboo with many well-known uses, has tremendous scope for use in eco-friendly agro-forestry projects in the hills (up to 1800 m amsl), to bring marginal lands into use. The traditional methods of propagation limit the number of propagules that can be produced, and is both labors intensive and time-consuming. Further, there is also risk involved in bamboo propagation through vegetative means using material from plants of unknown age because of the peculiar behavior of mass flowering and death of the flowered clumps. Therefore, it is imperative to adopt alternative methods for rapid multiplication and tissue culture is one such technique which can fulfill the demand of planting material. Although multiple shoot formation is accomplished with comparative ease, rooting of such shoots is still inconsistent in D. hamiltonii, and only up to 25–30% shows rooting [1]. In this context, plant regeneration via organogenesis is an alternative approach. However tissue culture techniques, combined with a mutagenesis treatment speed up the breeding program. Genetic variability is fundamental to successful breeding programs in vegetatively and sexually propagated plants. This variation can occur naturally or can be induced through mutations, using physical, biological or chemical mutagens and has attracted the interest of plant breeders for many decades. In recent years, the in vitromutagenesis technology has been applied to develop resistant traits and to improve quality. The chemical and physical mutagens are widely used to induce *in vitro* mutations. Among them, ethyl methanesulphonate (EMS) is widely used for mutation induction.

In the plant breeders perspective however, the bottom line, remains ultimately that the genetic variability recovered from regenerated plants should result in a phenotype that is commercially useful. The present work therefore, has been framed out to induce and evaluate variation in callus cultures of D. hamiltonii.

II. MATERIALS AND METHODS

The effect of Ethyl methane sulphonate was studied on the morphological characters of Dendrocalamus hamiltonii. Callus explants were procured from micro propagation of nodal explants. Data were scored in terms of callus survival percentage, callus regeneration percentage, number of roots and shoots, root and shoot length in different mutagen treated plants were examined.

A. Preparation of mutagenic stock solution

The stock solution (0.1%) of EMS was prepared by adding 0.1ml of EMS in 100 ml Phosphate Buffer solution maintained at pH-7. The stock solutions were further diluted to required concentrations using the formula:

S1 V1=S2 V2

Where, S1 = Strength of stock solution.

V1 = Volume of stock solution.

S2 = Strength of desired solution.
V2 = Volume of desired solution.

B. Treatment

The in vitro raised callus was obtained from nodal explants raised on MS medium. These calluses were placed in 100 ml Erlenmeyer flask which contained 50 ml mutagen solution. A range of Ethylmethane sulphonate concentrations 0.01, 0.05, 0.1, 0.2% v/v dissolved in distilled water were used and exposed for 1 and 2 hours on Erlenmeyer flasks in inoculation room under laminar flow at temperature of (25±2ºC). Explants were removed from flasks inside the laminar hood, transferred to MS [2] medium with 2,4D (4 mg/l) for callus multiplication. Cultures were kept in callus multiplication media for 2 weeks.

C. Culturing of mutagen treated callus

Chemical mutagen treated callus explants (1 explant per culture vial of about 50 ml medium) were later on transferred and cultured on MS medium supplemented with BAP (1mg/l) + IBA (1mg/l) to record data in terms of percentage shoot development, shoot number per nodal explants and shoot length. Roots were induced by the use of single node on MS medium fortified with optimized concentration of IBA (1 mg/l).

III. RESULTS

The effects of EMS and its fixed concentrations of 0.01%,0.05%, 0.1% and 0.2% were exposed for 60 and 120 minutes on *in vitro* induction of various biological abnormalities/variations have been studied. The different mutagen treatments administered to callus were inoculated on MS medium incorporating 4.0 mg/l 2, 4-D. Callus cultures were kept in callus multiplication media for two weeks, so as to check their survival capability. After two weeks, the cultures induced callus growth. The morphological variants as well as healthy treated shoots were isolated and transferred to previously standardized MS rooting medium. After the second to third week period shoots have shown root initiation and in the fourth week, they demonstrated well rooted features.
Each induced variation was separately studied regarding the following parameters.

A. Effect of mutagens on survival percentage of callus

The *in vitro* mutagen treated callus were isolated and tested for survival of callus for further growth. Callus cultures raised from in vitro mutagen treatments were removed from the Erlenmeyer flask and transferred to culture flasks with callus multiplication media containing 4.0 mg/l 2,4-D. All the treated cultures were put to acclimatization for further growth and survival analysis.
All the concentrations of EMS produced inhibitory effect on callus survival of D. hamiltonii (Fig 1, Plate 1). The increase in concentration of mutagen gradually decreased the in vitro callus survival percentage. The highest callus survival was obtained in control (71.69%). However after mutagen treatment, maximum callus survival percentage from EMS (67.01%) could be noted at 0.01% concentration for 60 minutes treatment duration. All the concentrations of EMS produced severe inhibitory effects at 120 minutes of treatment duration on in vitro callus survival in D.hamiltoniias compared to the control.

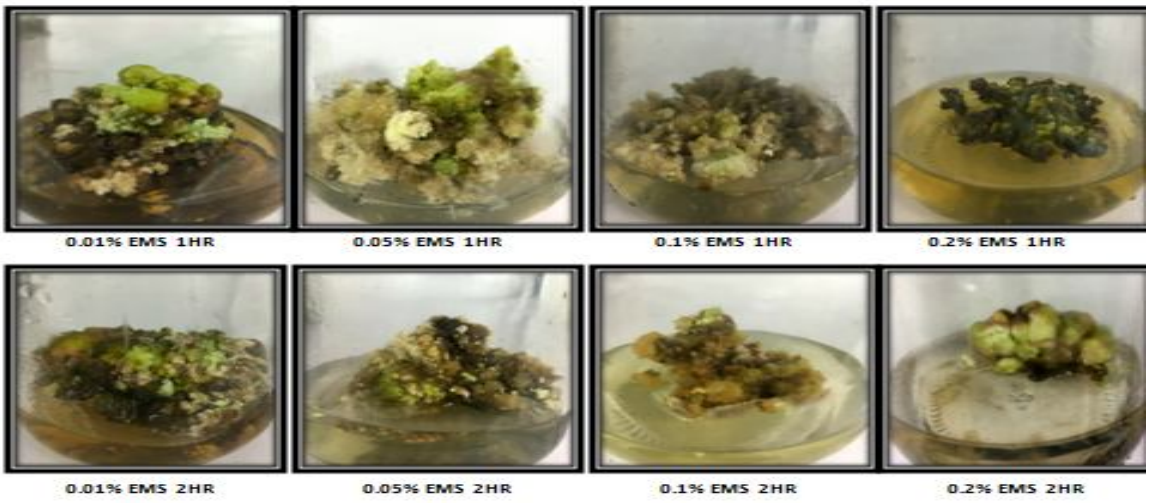
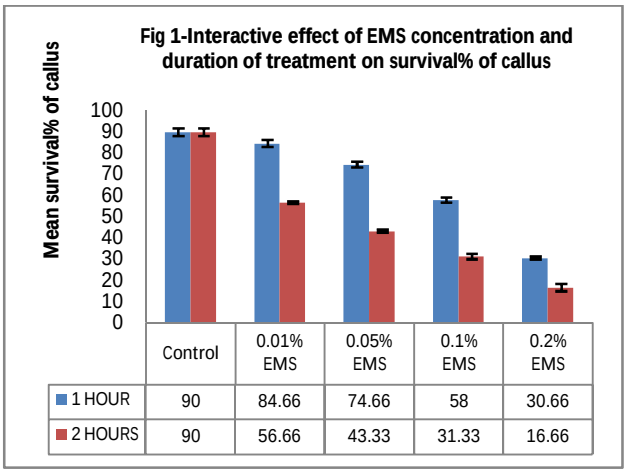


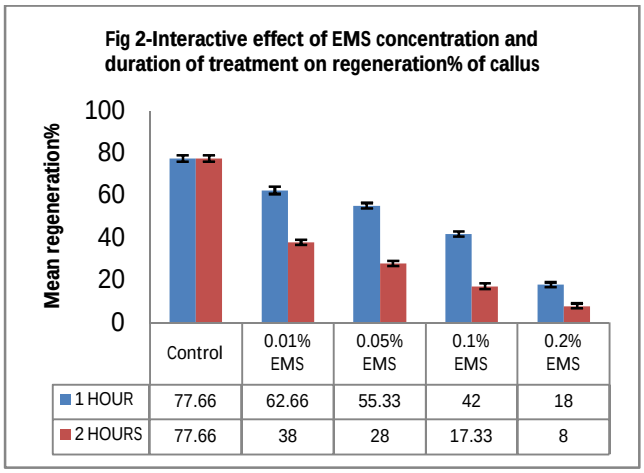
Plate 1- D. hamiltonii callus treated with different concentrations and duration of EMS

B. Effect of mutagens on in vitro callus regeneration percentage

The mutagen treated and untreated control callus were aseptically inoculated on previously standardized *in vitro* callus regeneration MS medium. Cultures were incubated for callus regeneration under controlled artificial conditions in culture room for five to six weeks. Data was recorded for regeneration percentage, after four to five weeks.

All the concentrations of EMS produced inhibitory effect on callus regeneration percentage of D. hamiltonii (Fig 2).The increase in concentration of mutagen gradually decreased the *in vitro* callus regeneration. The highest callus regeneration percentage was obtained in control (61.82%). However maximum germination percentage from EMS (52.34%) could be noted at 0.01% concentration for 60 minutes.





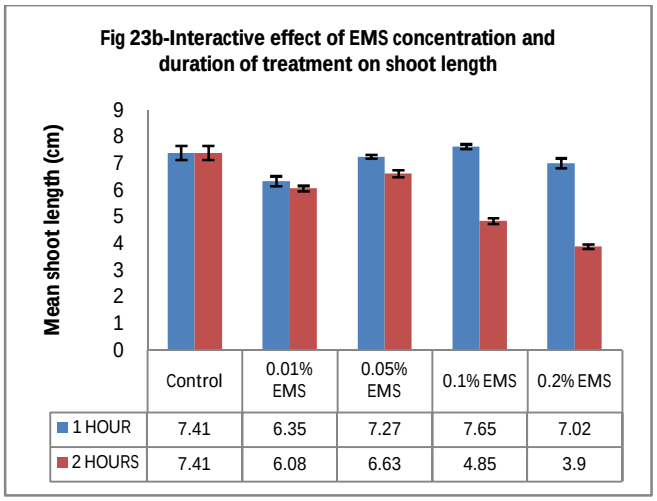
C. Effect of mutagens on shoot length of regenerated plantlets

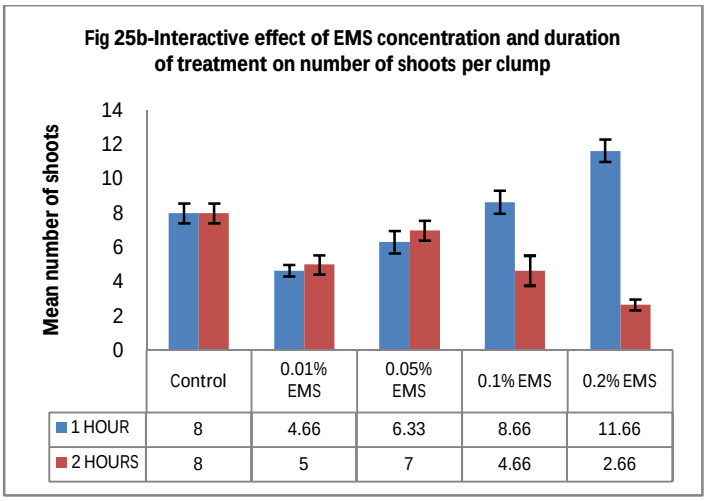
After the *in vitro* treatment of callus, the mutagen treated callus was kept in multiplication media for further growth. Later on after eight to ten weeks treated when callus were transferred in regeneration media containing BAP (1mg/l) + IBA (1mg/l), organogenesis started in regenerating callus. Shoot regeneration in terms of total height of the plantlets was measured and analysed as regards the effect of mutagen for induced variation (Fig 3).

The maximum shoot length from EMS (0.10%) concentration obtained was 7.65±0.09 cm as compared to control (7.41±0.26 cm) . The minimum height of 3.9±0.09 cm was obtained from EMS (0.2%) in 120 minutes duration.

D. Effect of mutagens on number of shoots of regenerated plantelets

The effect of various concentrations of mutagen treated callus on *in vitro* regeneration of number of shoots per shoot has been studied. The results (Fig 4) would reveal that, in vitro derived shoots significantly induced variability from mutagen treatments. The maximum number of leaves (11.66±0.66) could be recorded in 0.2% EMS in 60 minutes treatment while the minimum (2.66±0.33) was in 0.2% EMS in 120 minutes as compared to *in vitro* control 8±0.57.





E. Effect of mutagens on number of roots of regenerated Plantelets

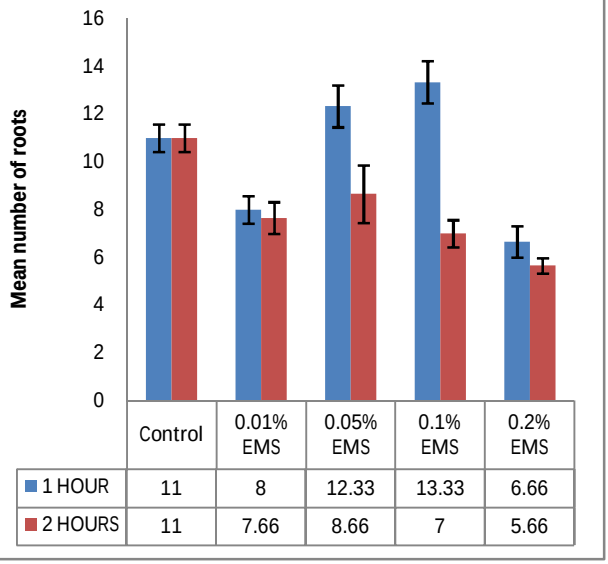
After the in vitro treatment of callus, the mutagen treated callus was kept in multiplication media for further growth. Later on after eight to ten weeks treated when callus were transferred in regeneration media containing BAP (1mg/l) + IBA (1mg/l), organogenesis started in regenerating callus. This regenerating callus were transferred to root regeneration media IBA (1mg/l), measured in terms of root length and number of roots and analyzed as regards the effect of mutagens for induced variation.

The effect of various concentrations of mutagen treated callus on in vitro regeneration of number of roots per clump has been studied (Fig 5). There was a gradual increase in number of roots in EMS treatment for 60 minutes of duration till 0.1% concentration and then a dip at 0.2% EMS (6.66±0.66). In 120 minutes of duration for treatment the increase in concentration of EMS gradually decreased the in vitro root number.

F. Effect of mutagens on root length of regenerated plantlets

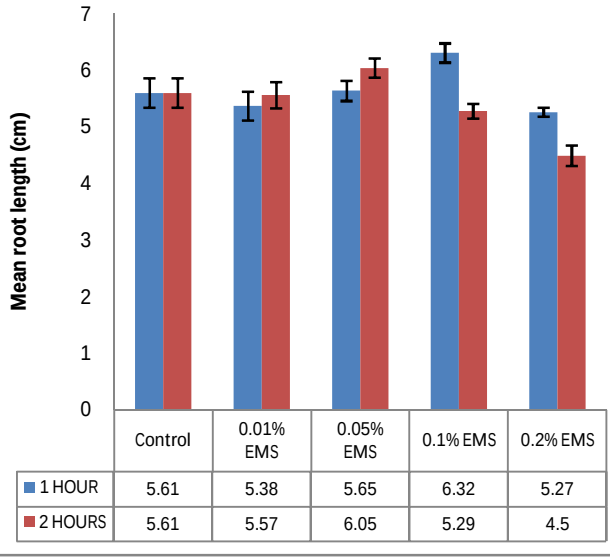
Root regeneration in terms of root length of the plantlets was measured and analyzed as regards the effect of mutagens for induced variation (Fig 6). There was a gradual increase in root length in EMS treatment for 60 minutes of duration till 0.1% concentration and then a dip at 0.2% EMS (5.27±0.08cm). In 120 minutes of duration for treatment the increase in concentration of EMS gradually decreased the *in vitro* root length as compared to control (5.61±0.56 cm).

Fig 26b-Interactive effect of EMS concentration and duration of treatment on number of roots per clump



	Control	0.01% EMS	0.05% EMS	0.1% EMS	0.2% EMS
1 HOUR	11	8	12.33	13.33	6.66
2 HOURS	11	7.66	8.66	7	5.66

Fig 27b-Interactive effect of EMS concentration and duration of treatment on root length



	Control	0.01% EMS	0.05% EMS	0.1% EMS	0.2% EMS
1 HOUR	5.61	5.38	5.65	6.32	5.27
2 HOURS	5.61	5.57	6.05	5.29	4.5

IV. DISCUSSION

All the concentrations of EMS produced inhibitory effect on callus survival and regeneration percentage of D. hamiltonii. The increase in concentration of mutagen gradually decreased the in vitro callus survival and regeneration percentage. Reduction in survival following mutagenic treatment may be due to various factors such as cytogenetic damage and physiological disturbances [3], changes in the metabolic activity of cells [4]disturbances in balance between promoters and inhibitors of growth regulators [5]. Thus biological damage was higher in higher concentrations of mutagens. Our findings are in line with [6] who reported that after treatment of leaf petiole explants by EMS and N-nitrose-N´-ethyl urea (ENU)influenced callus growth of common bean by increase in callus weight at each subculture on a fresh medium. The effect of mutagenic treatment of rice (Oryza sativa) panicles was studied [7] at the uninucleate pollen stage and found that the lowest concentrations of the mutagens stimulated callus induction and its growth. The chemical mutagens mediated reduced survival and regeneration of the calli could be linked to the production of toxic products [8].Dependence between applied doses of gamma irradiation and ENU on cultured maize callus growth and plant regeneration was also obtained [9].

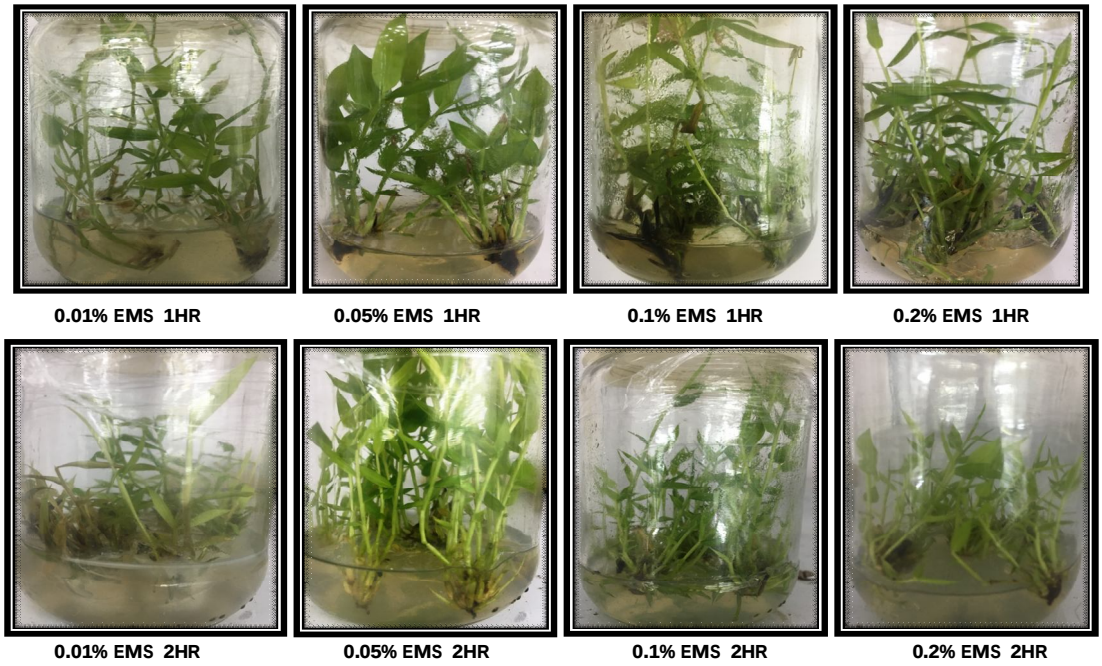


Plate 2-Regenerated plantlets from EMS treated callus of *D .hamiltonii*

Shoot regeneration in terms of total height of the plantlets, number of leaves and shoots was measured and analyzed as regards the effect of mutagen for induced variation. Results revealed that height as well as the number of shoots of plantlets generally decreased with the increasing concentrations of mutagen, but it was found to increase in lower concentrations of EMS over control. Reduction in plant height followed by mutagenic treatments was also observed in many species such as Trigonella [10], Helianthus [11],Capsicum [12],Vigna[13],Triticum[14],Capsicum[15]etc.It was [16]also reported that callusing which was achieved from nodal stem segment explants of D. sanderiana treated with various concentrations of EMS (0.2-1.6cm3m-3) on MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D 1.5 gm-3) showed a high regeneration potential and an increase in number of shoots and significant difference in shoot length was noticed when compared to control and sometimes started to be uncountable due to rosette of micro shoots with stunted shoot growth similarly. The stimulatory effect was observed in lower doses/concentrations of gamma rays and EMS on the length ofroot, shoot and seedling. The hypothetic origin of these stimulations by irradiation and EMS treatments was due to in cell division rates as well as an activation of growth hormone e.g., auxin[17],[18].

The inhibitory effect of mutagens on the length of plantlets was evident from the decrease in length of roots with increasing concentration of mutagens however the stimulatory effect was observed in lower concentrations of EMS on the root number and length of roots. The reason for decrease in root length may be due to the presence of some mitotic inhibitors in meristmatic regions. Contrastingly in EMS the increase in length and number may be due to increase in the length and diameter of main root, number and size of lateral roots. The increased root biomass has also been reported in Vignaby hypoxanthine treatment [19]and in Cichoriumby DMS [20].Moreover EMS in low concentration acts as a growth regulator, thus affecting the root length and number. Gamma rays and EMS was drastically reduced the length of root, shoot, seedling and vigour index in J. curcasat higher doses / concentrations. Similar observations were made by several workers in sunflower [21],[22]. The reduction in length of root and shoot was attributed to the effects of mutagens on the physiological system [23] such a reduction in length of root and shoot arising out of mutagenic treatments was previously reported in crop plants[24],[25],[26].

V. CONCLUSION

Through the present research work, it is believed that the in vitro standardized protocol would prove useful for in vitro mutagenesis directed towards development of desirable bamboo traits. The present experimental research work has concluded that in vitro induced mutation in D. hamiltonii provides possibilities of altering one or few traits under the controlled conditions. It also reduces the chances of loss of mutant by sub culturing.

VI. ACKNOWLEDGEMENT

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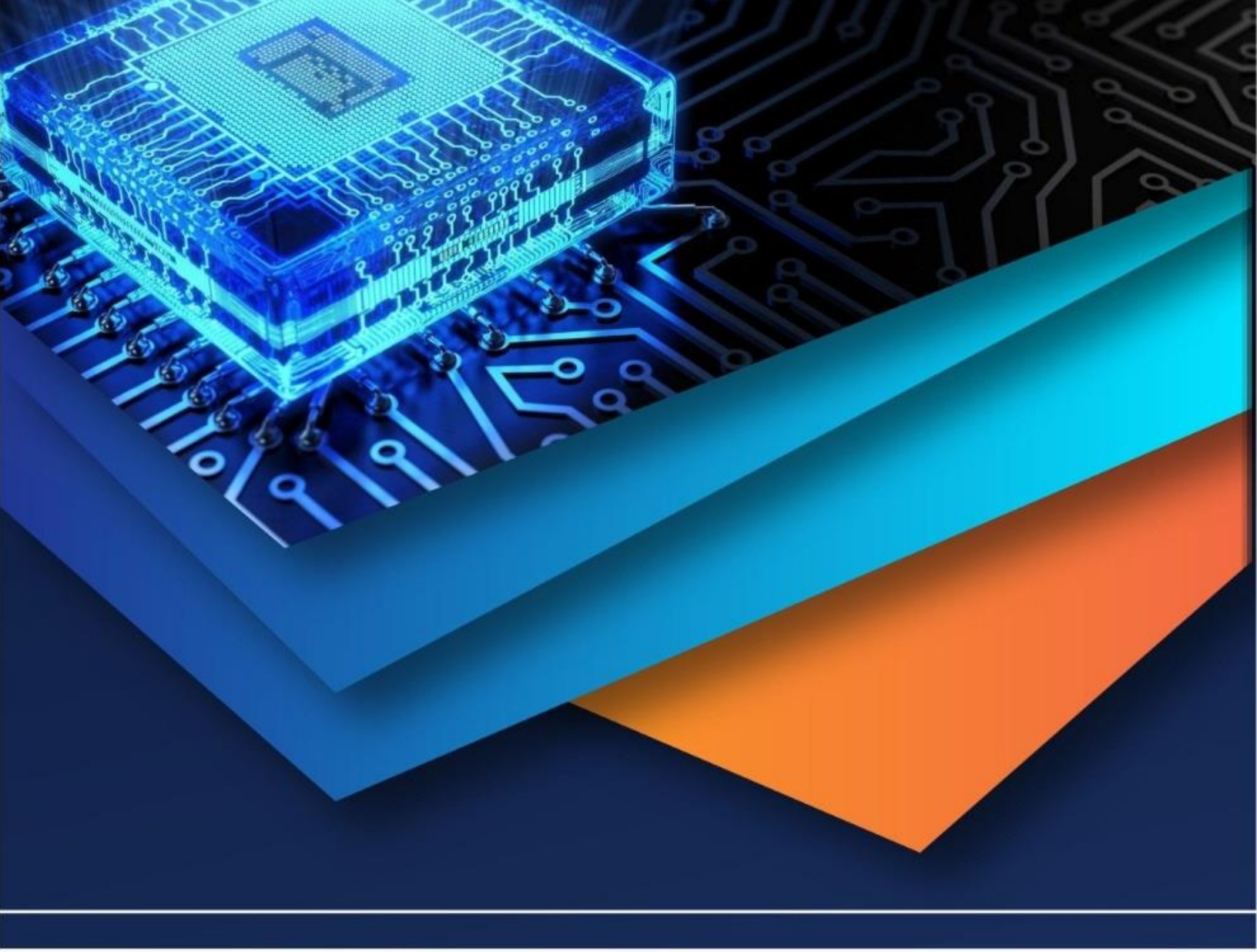

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