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Stress Responses of Sugar Beet to Different Drought Regimes

Lama Loho[1], Nandita Jena[2], Saroj Kumar Mohanty[3]
[1,2,3]Dept. of Crop Physiology, Institute of Agricultural Sciences, SOA Deemed to be University, Bhubaneswar, INDIA

Abstract: Water deficit (drought) stress is a major environmental factor limiting the production of crops. To evaluate the tolerance performances, a pot experiment was conducted using tropical sugar beet under different regimes of drought stress duration of 5, 10, 15 and 20days (T1, T2, T3, T4)with day temperature of 36-40°C. After subjected to various stress durations all plants were re-watered regularly till harvest. Significant reduction observed in plant fresh and dry weight, shoot fresh weight and dry weight, root fresh weight and dry weight and shoot: root ratio over control. Reduction in photosynthesis expressed in terms of decrease in Chl a, Chl b, and total chlorophyll in leaves of sugar beet was noticed significantly. Proline which acted as osmo-protectant and induced tolerance, gradually increased with increase of stress duration of 5days (2.024µg.g^{-1}) to 20days (7.398µg.g^{-1}). Total soluble carbohydrate contents in stressed plants also increased against control, where starch accumulation increased up to 15days stress and then declined significantly at 20days of water withholding showing its mobilization towards root growth and higher value of adaptability. The reduction in growth performance somehow recovered after re-watering of the stressed plants which is observed at harvest. This stress impact is managed to some extent in their tuber yield values from moderate to severe stress treated plants i.e.T2(25.817t.ha^{-1}) and T4(13.918t.ha^{-1}). At moderate stress, sugar beet plants showed tolerance and adaptation by triggering accumulation in roots. Hence it may be concluded that sugar beet crop is relatively tolerant to drought, but is very sensitive during growing stage.

Keyword: Drought, Stress, Tolerance, Tuber yield, Proline

I. INTRODUCTION

Impact of water deficit (drought) stress on crop plants is a major cause of limiting agricultural productivity, upon long term or repeated drought durations [1]. During recent years drought stress has become a prominent plant growth limiting factor globally. Metabolic responses of plants to water deficit can not only be stress-induced disfunctions but also undergoes adaptive changes that reflect its metabolic regulatory mechanisms. Osmotic adjustment is an active mechanism in response to water stress, maintains the turgor potential and hence plant growth [2].

Sugar beet (*Beta vulgaris* L.) an important biennial crop, usually cultivated for commercial sugar production, can be grown in a wide range of climatic conditions, where water availability play a major cause of yield loss in connection with weather status and water management goal [3]. Water deficit condition imposes modifications in morphological, physiological and biochemical characteristics of sugar beet in response to changing climatic conditions.

Changes in sugar beet composition under drought may be affected by stress severity, duration of stress and regeneration processes. Metabolic regulation of sugar beet to drought stress induce the accumulation of specific solutes in the plant tissues, make major contribution to osmotic potential of the cell sap [4] and shown a tolerance mechanism under water scarcity [5]. Proline accumulation in plant tissues is a clear marker for environmental stresses specially drought stress. Free proline is a key metabolite which accumulates in sugar beet when exposed to drought [6]. Change in its concentration in tissues is an indicator of stress, which affect sugar accumulation and crop yield. Severe drought stress induced a significant increase of proline concentrations in both shoot and root, and shoot soluble sugar [7]. However, at moderate stress, in some cases stress may increase sugar beet root quality and potentiality of recovery [8].

Water deficit leads reduction in starch accumulation in several crops [9] and accumulation of total soluble carbohydrates in the plant, which can be used to avoid the adverse effect of water stress [10]. Primary starch accumulation and mobilization are the emerging important factors within plant responses [11],where under temporary drought, Weeding et al. (2018)[12] reported changes in several metabolites including starch, also declined in leaves than normal watered plants.

Drought stress negatively affects plant growth, development, physiological and metabolic processes that may cause serious metabolic disorders which result increase in root:shoot ratio, decrease in leaf area and shoot fresh weight [13], [7] ; reduction in dry weight of the whole plant, water potential, Chl content and LAI [14], [15].

In water deficient soil, root growth is much less affected than shoot growth and there is a decrease in shoot: root ratio in response to withholding water up to 9days [16], [17]. However chlorophyll is easily affected by abiotic stresses. It induces change in the amount of photosynthetic pigment in plant leaves [18], leads reduction in starch accumulation and photosynthetic efficiency in several crops [9]. It is suggested that leaf chlorophyll is one of the significant indicator in determining yield [19]. Both chl a and chl b are much prone to drought stress conditions which variously altered chlorophyll contents. This reduction in chlorophyll content may occur due to stress-induced impairment in pigment biosynthesis pathways or in pigment degradation [20], [21].
The aim of the present study was to evaluate the tolerance ability of tropical sugar beet (LS-6), imposed to various drought durations in relation to yield, some physiological traits, biochemical components and its recovery ability under water deficit conditions.

II. MATERIALS AND METHODS

The experiment was conducted to determine the effect of water stress on sugar beet during January to May 2021 with a maximum and minimum temperature of 42.8 to 11^{o}C, and RH of 42.8 to 11.2%. Sugar beet seeds (LS-6),a tropical variety, were planted in pots with 25 kg of fertile soil. Normal doses of fertilizer as well as need base application of pesticides and insecticides along with other cultural practices were applied. The experiment was designed with 3 replications in CRD. Stress treatment was given to sugar beet plants at 50 DAP once for a duration of 5, 10, 15 and 20 days as T1, T2, T3 & T4 respectively by with-holding irrigation and control (C) with normal regular irrigation. After the stress treatment, all the plants of all treatments were regularly re-watered up to crop maturity. Impacts of water stress on different physiological and bio-chemical parameters were studied. Parameters like proline, total soluble carbohydrate, starch, chl a, chl b, and total chlorophyll in leaves during treatment and total soluble sugar and starch in tuber at harvest was estimated. Besides these yield parameters like whole plant fresh weight, tuber fresh weight, whole plant dry weight, shoot dry weight, tuber dry weight, shoot:root fresh weight during treatment and whole plant fresh weight, tuber fresh weight and tuber dry weight at harvest were recorded. Proline content in leaves was determined by ninhydrin method taking OD at 520nm as explained by Bates et al. (1973)[22]. Total soluble carbohydrate was estimated by anthrone method [23] and starch content was determined by the standard procedure of Hedge and Hofreitor (1962)[24] in leaves after respective stress treatments. Total soluble carbohydrate and starch percentage also estimated in tubers by following same method as in leaves. Starch estimation was mostly done by dry weight basis. Chl a, Chl b, and total chlorophyll content in leaves were estimated following the procedure of Arnon, (1949)[25]. Yield parameters in sugar beet due to water stress was studied taking weight of whole plant fresh weight, tuber fresh weight, tuber dry weight of randomly selected 3 tubers from each replications of all the treatments at harvest. Total yield t.ha^{-1} was calculated based on total plants per hectare area. Fresh weight of root, shoot and whole plant during stress treatment was also recorded in similar manner. Corresponding dry weight was determined putting the chopped samples in hot air oven at 60^{o}C for 72 hours. Statistical analysis was done using SPSS which indicated the statistically significant difference between treatments at $p<0.05$ and taking mean of three replications and 9 numbers of plants from each treatment along with respective control.

III. RESULTS

A. Proline

The accumulation of proline in leaves is a useful indicator of stress in sugar beet. During various stress imposition from 5 days to 20 days, the rate of proline increased continuously to reach maximum value 7.398 µg.g^{-1} at T4 (20 days of stress) and minimum at T1(5 days of stress) of 2.024 µg.g^{-1} against control 1.974µg.g^{-1} and 1.242µg.g^{-1} respectively. Significant increase of proline content also recorded in plants of all the treatments, as shown in Table I.

B. Total soluble carbohydrates (TSC)

More accumulation of total soluble carbohydrate in leaves of sugar beet also reported from 5 days (T1) up to 15 days stress (T3) as 11.454% to 20.887%, then declined at 20 days of stress (T4) showing its utilization towards cellular maintenance and root development. Increase in TSC % is observed at all the stress imposed over control. Percentage increase of TSC was found the highest value 92.755% at 15 days stress (T3) over their controls, but decreased in accumulation due to severe stress at T4 i.e. 20 days of stress, as shown in Table I.

C. Starch

Starch accumulation in leaves gradually increased with increase in stress period (5.679 to 11.672% at T1 to T3 respectively) but decreased against respective control showing stress effects under water scarcity. But adverse effect of stress reported in 20 days stressed plants (T4) which was 4.667% against control of 13.696%, as shown in Table I.

Table I Proline (µg.G^{-1}), Total Soluble Carbohydrate (%), Starch (%) Content in Sugar Beet Leaves under Drought Stress Conditions.

Treatments	Proline µg.g^{-1}	Total soluble carbohydrate % (fresh wt)	Starch % (dry wt)
T1 (5 days S)	2.024± 0.00	11.454± 0.03	5.679± 0.04
Control	1.242± 0.01	10.589± 0.03	15.211± 0.04
T2 (10 days S)	4.411± 0.02	13.581± 0.12	10.226± 0.05
Control	1.567± 0.02	10.905± 0.02	21.142± 0.02
T3 (15 days S)	6.559± 0.07	20.887± 0.09	11.672± 0.04
Control	1.873± 0.01	10.836± 0.04	25.724± 0.02
T4 (20 days S)	7.398± 0.09	13.581± 0.07	4.667± 0.07
Control	1.974± 0.05	10.905± 0.01	13.696± 0.06

All values in the table are averages of 9 plants from 3 replicates (n=9),± standard errors and significant according to Fisher's t- test ($P<0.05$)

D. Tuber TSC and Starch % at Harvest

Total soluble carbohydrate deposit in roots was reduced at harvest, usually occurred in the plants subjected to 15 days and 20 days of drought stress along with high summer temperature showing its growth and stress recovery ability against control (Table 2). Starch content in sugar beet tubers also exhibited the same trend as TSC but its accumulations in roots reduced against control at all stress durations. Starch percentages were more in roots of 10 days stressed plants (T2, 14.561%) than other stress treated plants showing its tolerance activity, as shown in Table II.

Table II Total Soluble Carbohydrate % & Starch % in Sugar Beet Tubers at Harvest.

Treatments	Total soluble carbohydrate % (fresh wt basis)	Starch % (dry wt basis)
Control	9.571	15.058
T1(5 days S)	7.486	10.142
T2(10 days S)	8.613	14.561
T3(15 days S)	7.667	12.492
T4(20 days S)	4.367	10.375
C.D.(0.05)	0.341	0.258
SE(m)	0.124	0.091
C.V.(%)	1.917	1.282

E. *Yield Parameters*

Whole plant fresh weight, tuber fresh weight and tuber dry weight obtained were showing same response at harvest, subjected to different stress periods during growing phase. A very limited stress effect on whole plant and tuber fresh weight (336.310g.plant^{-1} and 249.587g.plant^{-1}) was observed in T1 (5 days stressed plants) in comparison with normal watering plants, control (372.282g.plant^{-1} and 276.536 g.plant^{-1} respectively) (Table 3). The effect was significant in 15 days and 20 days stressed plants, whereas the drastic changes of both parameters occurred in T4 (20 days stressed plants) as 186.468 g.plant^{-1} and 139.182g.plant^{-1} respectively. Tuber dry weight also indicated the trend as yield parameters. Near about 50% yield loss was observed in 20 days water stress (13.918t.ha^{-1}) against control (27.653t.ha^{-1}), as shown in Table III.

Table III Analysis of Yield Parameters of Sugar Beet at Harvest (Tuber Fresh Wt / Plant, Tuber Dry Wt / Plant and Whole Plant Fresh Wt.

Treatments	Yield g.plant^{-1}			Yield t.ha^{-1}
	Whole plant fresh weight	Tuber fresh weight	Tuber dry weight	
Control	372.282	276.536	66.355	27.653
T1(5 days S)	336.310	249.587	59.991	24.958
T2(10 days S)	348.384	258.173	61.886	25.817
T3(15 days S)	243.233	180.352	43.214	18.035
T4(20 days S)	186.468	139.182	33.359	13.918

Whole plant fresh weights significantly decreased in different stress during treatment against corresponding control. In control the fresh weight increased with increase of growing period up to 15 days but declined at 20 days. The whole plant dry weight showed the same trend in all the treatments, as shown in Table IV.

Table IV Whole Plant Fresh Wt (g) and Dry Wt (g) of Sugar Beet During Various Stress Treatments.

Treatments	Whole plant fresh weight (g)	Whole plant dry weight (g)
T1(5 days S)	113.15 ±1.73	11.401±0.06
Control	218.489±0.98	20.310±0.32
T2(10 days S)	88.046±0.97	12.023±0.09
Control	259.961±2.66	27.247±0.11
T3(15 days S)	75.977±0.73	14.535±0.09
Control	349.473±0.84	44.295±0.34
T4(20 days S)	67.521±0.76	17.482±0.07
Control	279.001±1.12	34.070±0.12

All values in the table are averages of 9 plants from 3 replicates (n=9), ± standard errors and significant according to Fisher's t- test ($P<0.05$).The whole plant dry weight, shoot dry weight and root dry weight showed the same trend as above in all the cases during treatment. The shoot: root ratio was highest (8.035) in 5 day stress (T1) and lowest (2.685) in 20 day stress treatment, as shown in Table V.

Table V Dry Weight (Whole Plant, Shoot and Root) and Shoot:Root Ratio of Sugar Beet During Various Stresses

Treatments	Whole plant Dry Weight	Shoot Dry Weight	Root Dry Weight	Shoot : Root (Fresh Weight)
T1 (5 days S)	11.401	10.133	1.268	8.035
Control	20.310	17.076S	3.234	4.982
T2 (10 days S)	11.999	9.501	2.498	4.885
Control	27.247	23.737	3.690	6.769
T3 (15 days S)	14.535	10.831	3.704	3.262
Control	44.295	33.271	11.024	3.022
T4 (20 days S)	17.482	12.794	4.634	2.685
Control	34.070	25.928	8.142	2.508

F. Chlorophyll content in leaves (Chl a, Chl b & total Chl)

Leaf pigment contents are also affected by drought stress. Chl a, Chl b, and total chlorophyll significantly decreased under drought conditions compared to their respective controls. Plants under 20 days of drought stress (T4) showed most reduction in Chl a, Chl b and total Chl i.e 4.226mg.g^{-1} , 1.096 mg.g^{-1} and 5.323 mg.g^{-1} respectively against their controls, which were recorded having significantly higher i.e. 8.356 mg.g^{-1}, 4.055 mg.g^{-1} and 12.411mg.g^{-1} respectively. However under 5 days and 10 days of stress, the pigment contents were less affected than gradual increase of stress durations, as presented in Fig. 1.

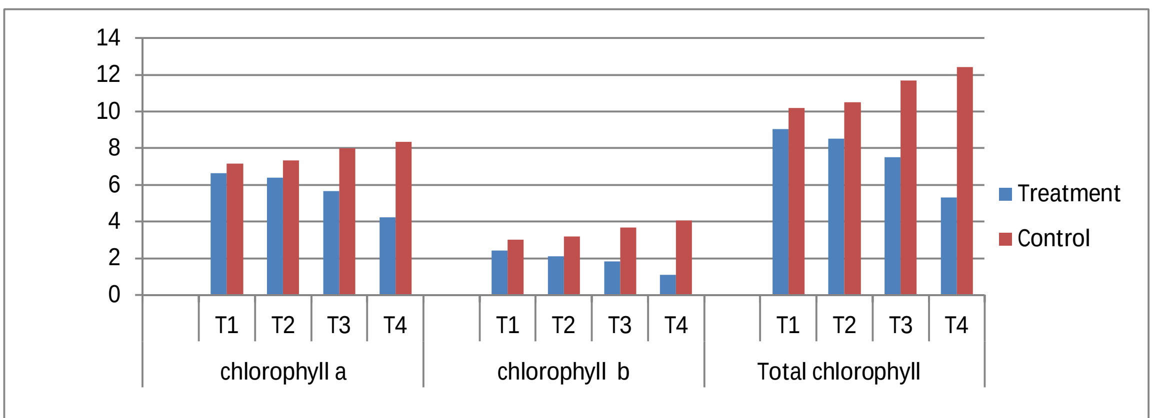

Fig. 1 Chlorophyll (Chl A, Chl B & Total Chl) Content (Mg.G^{-1}) in Leaves of Sugar Beet During Various Stresses.

IV. DISCUSSION

In the present investigation, the response of sugar beet crop to different drought durations is evaluated, basing on plant growth, physiological traits, biochemical components and yield characters. Under stress conditions, plants only adopted to 10 days stress, showing recovery by cellular adjustments than other treatments. During water deficit condition when the plant is subjected to oxidative stress resulted serious metabolic disorders [26]. To combat oxidative stresses plant accumulates different osmolytes like proline, ascorbic acid, betaine, carbohydrates, and polyamines by maintaining their water relations [27], [28]. Proline is considered as the most important organic solute, can play a key role to regulate ROS, hydroxyl radicals to develop resistance in plants under severe drought stress [10], [29]. Long drought stress induced a significant increase in proline concentrations of both shoot and roots and also in shoot total soluble sugar concentration in sugar beet [7]. The same trend also observed in this experiment as proline concentration obtained a higher value under long duration of water withholding up to 20 days (7.398µg.g^{-1}), expressed its degree of stress tolerance, where a low rate of accumulation resulted at 5 days stress (2.024µg,g^{-1}) showing resistance to the mild stress. Accumulation of total soluble carbohydrates in the plant can be used to avoid the adverse effect of water stress [10]. Enhancing of total soluble carbohydrate (TSC) and increasing in proline content in sugar beet, have also been investigated by Ghaffari et.al 2020 [30]. TSC % in sugar beet leaves increased when the stress imposed up to 15 days and reduced at 20 days, expressing its utilization to combat severe stress. These results are matched with a strong correlation which was described in previous researches between drought stress and the accumulation of these substances [31].

It also has been revealed that drought stress leads to a change in biochemical content in the sugar beet as reduction in starch accumulation. When sugar beet growing older, starch accumulation is gradually less under long stress periods than normal watering plants and the evidence showed its utilization towards cellular adjustments under stress. Sugar beet has no self- regulatory mechanisms to promote sugar accumulation in roots which was also found in the TSC and starch content reduction at higher stress durations of 20 days of drought, but is dependent upon external stimuli from the climatic factors to a great extent [32].

Leaf pigment contents such as, chl a, chl b, and total chlorophyll were also affected by drought stress [13], which was evidenced from this drought experiment, showing significant reduction ($P<0.05$) in all treatments under water withholding than normal watering plants (control). Low photosynthetic rate is a common issue under drought due to the reduction in green pigments synthesis [33] which causes impairment in plant growth and yield [34]. Water withholding reduced plant growth, dry matter accumulation, and final yield when imposed at successive growth stages, which was partially compensated by an increase in the fraction of assimilate partitioned to storage. Never the less, its response was most severe when stress affected foliar development. Water deficiency during early growing stages is the main cause of potential yield loss in sugar beet production [35], which was more pronounced at 15 days and 20 days of stress. In this experiment under stress conditions, plants only adopted to 10 days stress duration, showing recovery by cellular adjustments than other treatments. Drought limits water flow into the plant, which reduces movement of water, nutrients and photosynthates within the plant [36]. Yield and quality of any crop is a result of complex set of interactions, occurring during growth and development of crop plants due to genetic, environmental, and agronomic factors which influence sugar beet yield [36] and its harvesting time. After plant emergence, the growth and development activities are largely influenced by atmosphere temperature and crop nutrition. Temperature more than 30°C retards yield and quality of sugar beet [37], which was also observed throughout the period of stress application and yield results at harvest, with respective drought treatments. Planting of tropical sugar beet at different dates significantly affected tuber yield (t.ha^{-1}). Gradually, the time of planting of sugar beet at any location is decided by the sowing area's prevailing temperature [38]. The present experiment was conducted during late winter to summer days from Jan to May with high climatic temperatures of maximum day temp.34.4°C - 40°C during stress application and 35°C – 42°C till harvest. In the present study this climatic impact also influenced the root yield of sugar beet at harvest.

V. CONCLUSION

From the findings of the present investigation it may be concluded that the drought stress effects are very much pronounced in plants of all treatments in support with climatic temperatures during the experimental period. Drought imposed at earliest stage of plant development reduced physiological activities more severe than other periods of stress applied. Less impact is observed in 5 days and 10 days of stress against normal watering plants. Though the stress impacts are clearly visible but plants resumed their normal physiological activity after re-watering and showing less impact on their yield. Sugar beet plants exhibited osmotic adjustment through accumulation of total soluble carbohydrates, proline, and starch in leaves which increased antioxidant activities to show tolerance over various degrees of drought stress. Stress also affects accumulation of biomolecules like TSC and starch in the storage root (tuber) of sugar beet. Starch % is observed promising in tubers of ten days stress treated plants, evidenced their stress tolerance and enhanced stress management capacity.

Variations seen in parameters because of ongoing degrees of stress impact and ability of stress recovery. Severe water shortage influenced both root yield and biomass, depending on drought duration, planting time and harvesting time and the overall max. climate temperature of 29-42^{o}C throughout the experimental period that adversely affect root accumulation and productivity. Performing average photosynthetic rates during drought and rapid recovery after re-watering, it can be concluded that the tropical sugar beet LS-6 variety is a drought sensitive but also a promising moderately tolerant cultivar.

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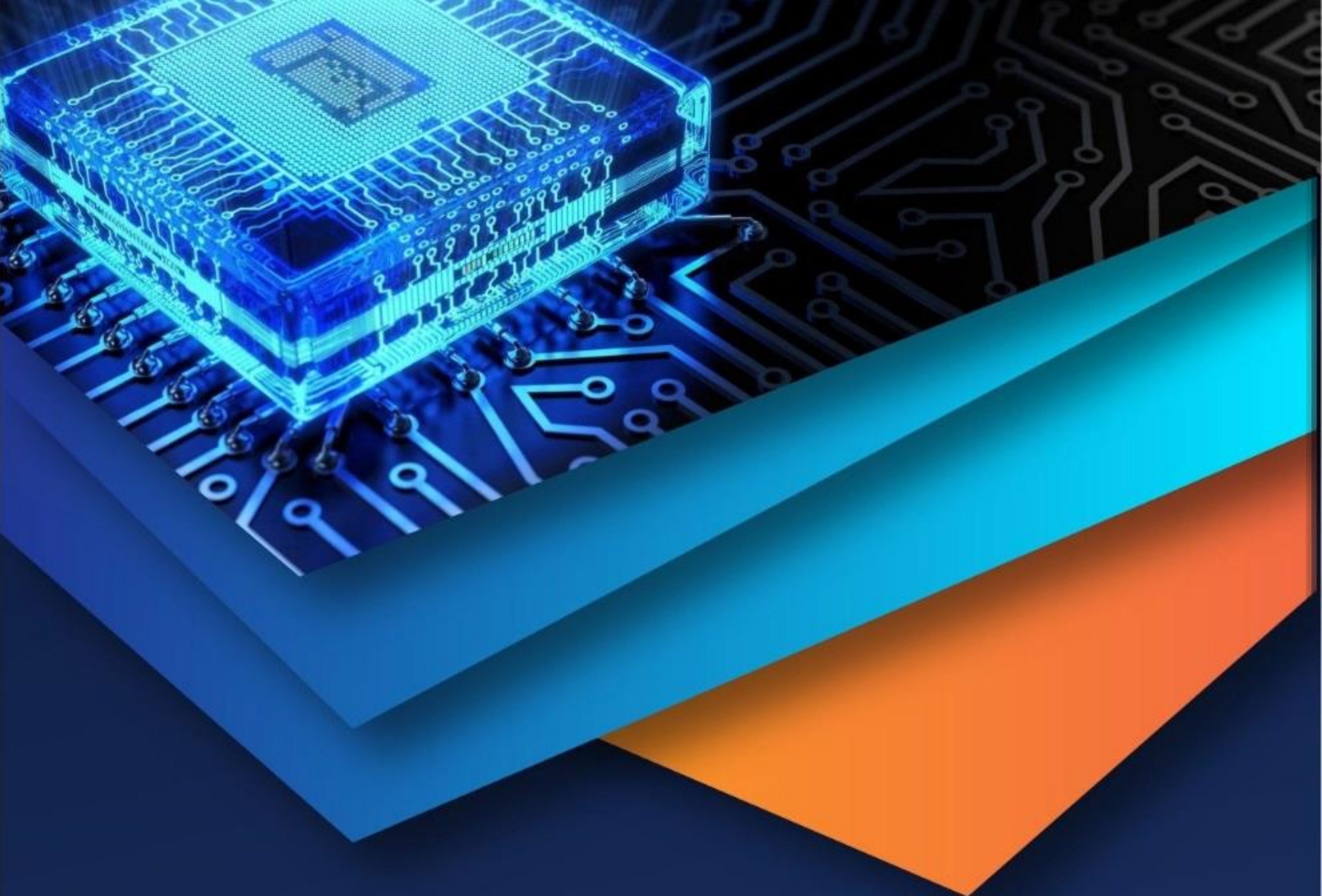

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