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Research Article

Pre-Sowing Seed Treatment with Glucose Boosts the Photosynthetic Efficiency, Metabolic Enzymes and Antioxidant Defense System to increase the Growth of *Abelmoschus esculentus* (L.) Moench

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ABSTRACT

Glucose (Glc) accumulates in leaves as minor product, synthesized during photosynthesis and is famous to control a wide array of plant physiological processes. Hence, is required to assess the role of Glc, when it is supplied exogenously. Seeds of Abelmoschus esculentus (L.) Moench were soaked in different concentrations of Glc (0, 20, 30 and 40 mM) for 4, 8, or 12hrs and later sown in pots with soil and farmyard manure (6:1). Sampling was done at 45-day stage of growth to assess the various biological and biochemical parameters. Pre-sowing treatment of Glc enhanced the growth and metabolism of plants in the following order: 30 mM> 20 mM> 40 mM> 0 mM. Glc promoted gaseous exchange parameters including net photosynthetic rate. It also augmented the activities of enzymes of carbon and nitrogen metabolism. Moreover, it boosts the defence system by increasing activities of antioxidant enzymes along with proline content. Maximum response was noted in the plants emerging from the seeds soaked in 30 mM of Glc for 4hrs. The elevation in photosynthetic efficiency and antioxidant generation proved beneficial in improving the growth of the plant.

1. Introduction

Plants synthesize carbohydrates during photosynthesis through the assimilation of atmospheric carbon dioxide and the release of molecular oxygen [1]. Among these carbohydrates, sugars act not only as energy sources but also as crucial signaling molecules that regulate a wide range of metabolic, physiological, and developmental processes in plants [2, 3]. Glucose (Glc), synthesized as a primary photosynthetic product, is rapidly converted into other carbohydrates or stored in the vacuole of photosynthetic cells, where it functions as both a metabolic substrate and a signaling component [4, 5]. Accumulating evidence indicates that Glc plays a pivotal role in seed germination, root growth and orientation, floral induction, and senescence, mainly through interaction with hormonal and redox signaling pathways [3, 6]. Moreover, Glc has been reported to enhance cell division, chlorophyll biosynthesis, and the rate of photosynthesis, thereby improving overall plant growth and productivity [7, 8]. Exogenous or endogenous increases in Glc levels elevate glucose and fructose contents in leaves, contributing to improved osmotic balance and metabolic activity under varying environmental conditions [9, 10]. In addition, Glc plays a protective role against oxidative stress by restricting the excessive generation of reactive oxygen species (ROS) and by stimulating antioxidant defense systems, including both enzymatic and non-enzymatic components [11-13].

Abelmoschus esculentus (L.) Moench, commonly known as okra, is a widely consumed and economically important horticultural crop in India and other tropical and subtropical

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regions. Okra is a fast-growing annual vegetable cultivated primarily during warm seasons and is characterized by indeterminate growth habit and continuous flowering, traits that are highly sensitive to nutrient availability and environmental conditions [14, 15]. Nutritionally, okra is a valuable source of carbohydrates, dietary fiber, proteins, minerals, and vitamins, contributing significantly to food and nutritional security [16, 17]. Previous reports indicate that 100 g of fresh okra pods contain approximately 89.6% moisture and are rich in essential nutrients such as potassium, calcium, magnesium, phosphorus, and vitamin C [18]. India is recognized as the largest producer of okra globally, contributing nearly 70% of the total world production [19]. The average nutritive value of *A. esculentus* surpasses that of several commonly consumed vegetables, including tomato, brinjal, and most cucurbits [20]. In addition, okra seeds contain appreciable amounts of oil (18–20%) and crude protein (20–23%), enhancing its agro-economic importance. Since okra fruiting extends over a prolonged period, a continuous and balanced supply of nutrients and growth-regulating substances is essential for maximizing yield and pod quality [21].

The intensive and indiscriminate use of agrochemicals in modern agriculture has emerged as a major global concern due to its adverse environmental and health-related consequences. Excessive application of chemical fertilizers contributes to soil degradation, loss of soil microbial diversity, nutrient leaching, and contamination of water bodies through runoff, ultimately threatening ecosystem sustainability and human health [22, 23]. It has been estimated that nearly 40–70% of applied nitrogen, 80–90% of phosphorus, and 50–70% of potassium are lost to the environment, rendering a substantial fraction of fertilizers unavailable to plants [24, 25]. Under such circumstances, the development of alternative and sustainable strategies aimed at reducing fertilizer dependency while maintaining crop productivity has become imperative. Eco-friendly approaches, including the use of biological stimulants, natural growth regulators, and plant-derived signaling molecules, offer promising avenues for mitigating the negative impacts of agrochemical inputs and promoting sustainable horticultural production systems.

Glc is an eco-friendly, naturally occurring photosynthetic product and also act as an osmo-protectant in plants. Its exogenous application to plants is gaining much interest of research community to increase growth and productivity of horticultural crops. Our previous findings, suggests that Glc when applied as foliar spray improves the growth and physiology of mustard plant [26]. Therefore, the present study was conducted with different mode of application i.e., seed soaking to examine the effects of Glc on growth and physiology of a different crop i.e., okra.

2. Materials and Methods

2.1. Preparation of Glc solution

The requisite amount of Glc was dissolved in double distilled water (DDW) to prepare a 1 M of stock solution which was diluted to 20, 30 or 40 mM using DDW.

2.2. Biological material

Seeds of *Abelmoschus esculentus* var. Sanjana were obtained from National Seed Corporation Ltd, New Delhi, India. Seeds which were uniform in size and colour were selected for soaking.

2.3. Experimental design and treatments

Seeds were soaked in varying concentrations of Glc (0, 20, 30 or 40 mM) for 4, 8, and 12hrs and subsequently sown in pots. The experiment was carried out during the month of May-June in the net house of Department of Botany, Aligarh Muslim University, Aligarh, India. Seeds soaked in DDW were used as control. 5 replicates for each treatment and 3 plants per pot were maintained. The sampling of plants was done at 45-day stage of growth to evaluate growth, physiological and biochemical parameters.

2.4. Growth markers

One plant from each pot was selected randomly to assess growth parameters. The length of root and shoot were assessed using a meter scale whereas weight was assessed using electronic balance. Subsequently, the samples were dried in hot air oven (70°C for 3-4 days) to evaluate the dry mass.

2.5. Leaf area of plant

Leaf area was determined using leaf area meter (AM 350, ADC Bio Scientific Ltd. Global House, Geddings Road, Hoddesdon, Herts, EN11 0NT, UK) and was expressed in cm^2.

2.6. SPAD chl

SPAD value was measured using SPAD chl meter (SPAD-502; Konica, Minolta sensing, Inc., Japan).

2.7. Compound microscopy of stomata

Compound microscopic analysis of stomata was performed using epidermal leaf peels following the procedure described in our previous study [27]. The prepared epidermal peels were carefully mounted on glass slides and examined under a compound microscope. A calibrated micrometre scale was used to accurately measure the stomatal aperture.

2.8. Leaf gaseous exchange

Leaf gaseous exchange parameters were measured using an infrared gas analyzer (IRGA; LI-COR 6400, LI-COR Inc., Lincoln, NE, USA). The parameters recorded included (i) net photosynthetic rate (PN), (ii) stomatal conductance (Gs), (iii) internal CO_2 concentration (Ci), and (iv) transpiration rate (E). During all measurements, the air temperature, relative humidity, CO_2 concentration, and photosynthetic photon flux density (PPFD) were maintained at 25 °C, 85%, 600 µmol mol^{-1}, and 800 µmol m^{-2} s^{-1}, respectively.

2.9. Enzymes of carbon and nitrogen metabolism

Nitrate reductase (NR) activity was determined following the procedure of Jaworski (1971) with minor modifications [28]. Fresh leaf samples (200 mg) were cut into small pieces and transferred to plastic vials containing phosphate buffer and potassium nitrate solution. To each vial, 5% isopropanol was added to facilitate tissue permeability. The vials were then incubated at 30 °C for 2 h. After incubation, the reaction mixture was transferred to test tubes, and sulphanilamide solution followed by NED–HCl was added. The mixture was allowed to stand for 20 min for color development. Finally, the reaction mixture was diluted with double-distilled water (DDW), and the absorbance was recorded at 540 nm using a spectrophotometer.

Carbonic anhydrase (CA) activity was determined following the method of Dwivedi and Randhawa [29]. Fresh

leaf samples were cut into small pieces and immersed in 0.2 M cysteine HCl solution, followed by incubation at 4 °C for 20 min. After blotting excess solution, the leaf pieces were transferred to phosphate buffer (pH 6.8). Subsequently, 0.2 M sodium bicarbonate solution and 0.002% bromothymol blue were added to the test tubes. The reaction mixture was gently shaken and incubated at 4 °C for 20 min. Thereafter, the mixture was titrated against 0.05 N HCl using methyl red as an indicator. The volume of HCl required to achieve the development of a light purple color was recorded and used to calculate CA activity.

2.10. Antioxidants analysis

Superoxide dismutase (SOD) activity was assayed according to the method of Kono [30]. The reaction mixture consisted of 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 µM nitroblue tetrazolium (NBT), 2 µM riboflavin, 0.1 mM EDTA, and the enzyme extract. The reaction was initiated by exposing the mixture to 15 W fluorescent light for 10 min. The absorbance was then recorded at 560 nm using a spectrophotometer.

Catalase (CAT) activity was determined following the method of Chance and Maehly [31]. The reaction mixture contained 3 mL of phosphate buffer (pH 6.8), 0.1 M hydrogen peroxide (H_2O_2), and the enzyme extract. The mixture was incubated at 25 °C for 1 min, after which the reaction was terminated by adding 10 mL of 2% H_2SO_4. The residual H_2O_2 was titrated against 0.1 N $KMnO_4$ until a stable purple color persisted for at least 15 s.

Peroxidase (POX) activity was also measured according to Chance and Maehly [31]. The reaction mixture comprised pyrogallol–phosphate buffer, 1% H_2O_2, and the enzyme extract. The change in absorbance was monitored at 420 nm at 20 s intervals for 3 min using a spectrophotometer.

Proline content was estimated following the method of Bates et al. [32]. Fresh plant samples were homogenized in 3% sulphosalicylic acid and centrifuged at 10,000 × g for 10 min. The supernatant was collected, and 5 mL of sulphosalicylic acid along with 2 mL each of glacial acetic acid and ninhydrin reagent were added. The reaction mixture was heated for 1 h, after which it was placed in an ice bath to terminate the reaction. Subsequently, toluene was added, leading to the formation of a chromophore-containing toluene layer. The absorbance of this layer was measured at 520 nm using a spectrophotometer.

2.11. Statistical analysis

SPSS 17.0 for Windows (SPSS, Chicago, IL, USA) was used statistical analysis. Analysis of variance (*ANOVA*) was performed to determine the least significance difference (*LSD*) between treatments with a level of significance at $P \leq 0.05$.

3. Results and Discussion

Glc synthesized via photosynthesis regulates numerous aspects during plant growth and development hence, could be regarded as a “growth hormone”. In the present study, Glc was supplied exogenously and its response was studied in *Abelmoschus esculentus.* Plants emerged from the seeds soaked in Glc showed higher growth as compared to the control (Fig. 1–3). Growth of the plants increased in presence of Glc in a concentration dependent manner however, 30 mM Glc was found most effective at a soaking duration of 4hrs (Fig. 1–3). The respective increase in root length, shoot length, root fresh mass, root dry mass, shoot fresh mass, shoot dry mass and leaf area were 27.6, 32.2, 24.9, 25%, 28.4%, 30% and 28.5% over their respective control.

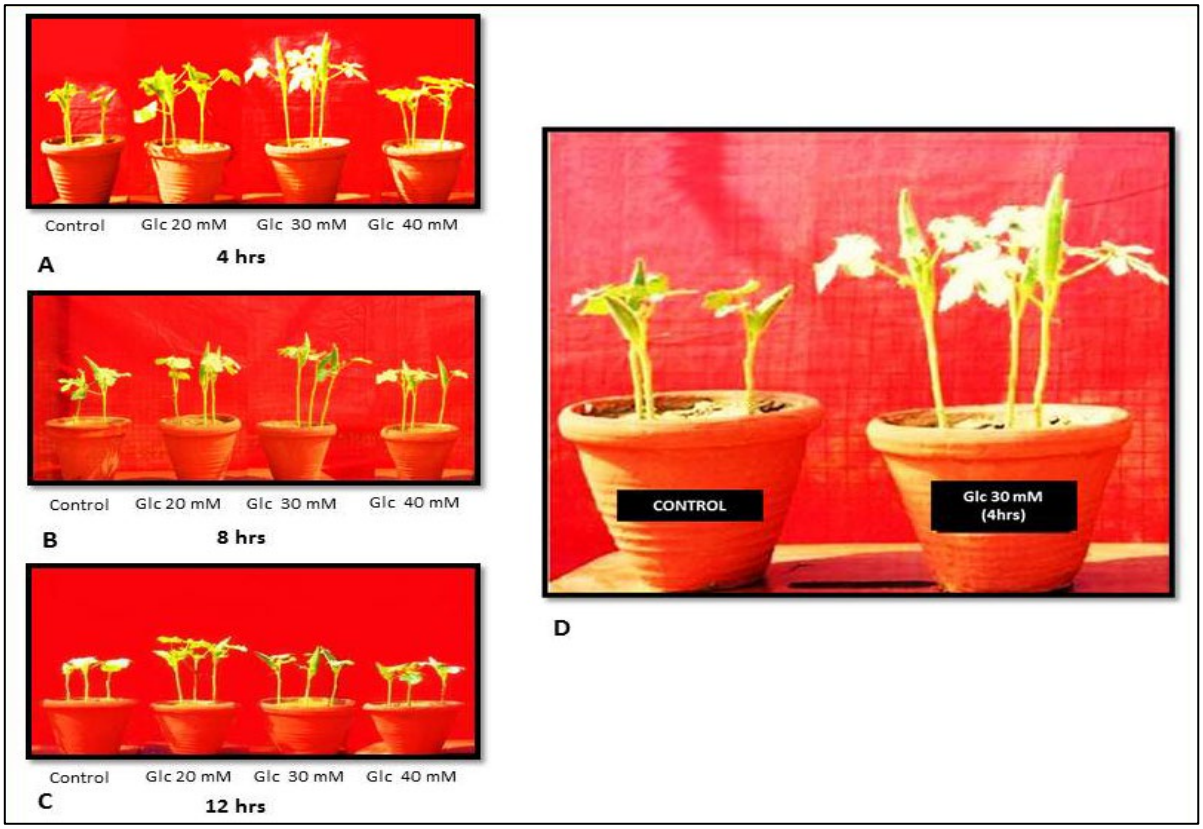
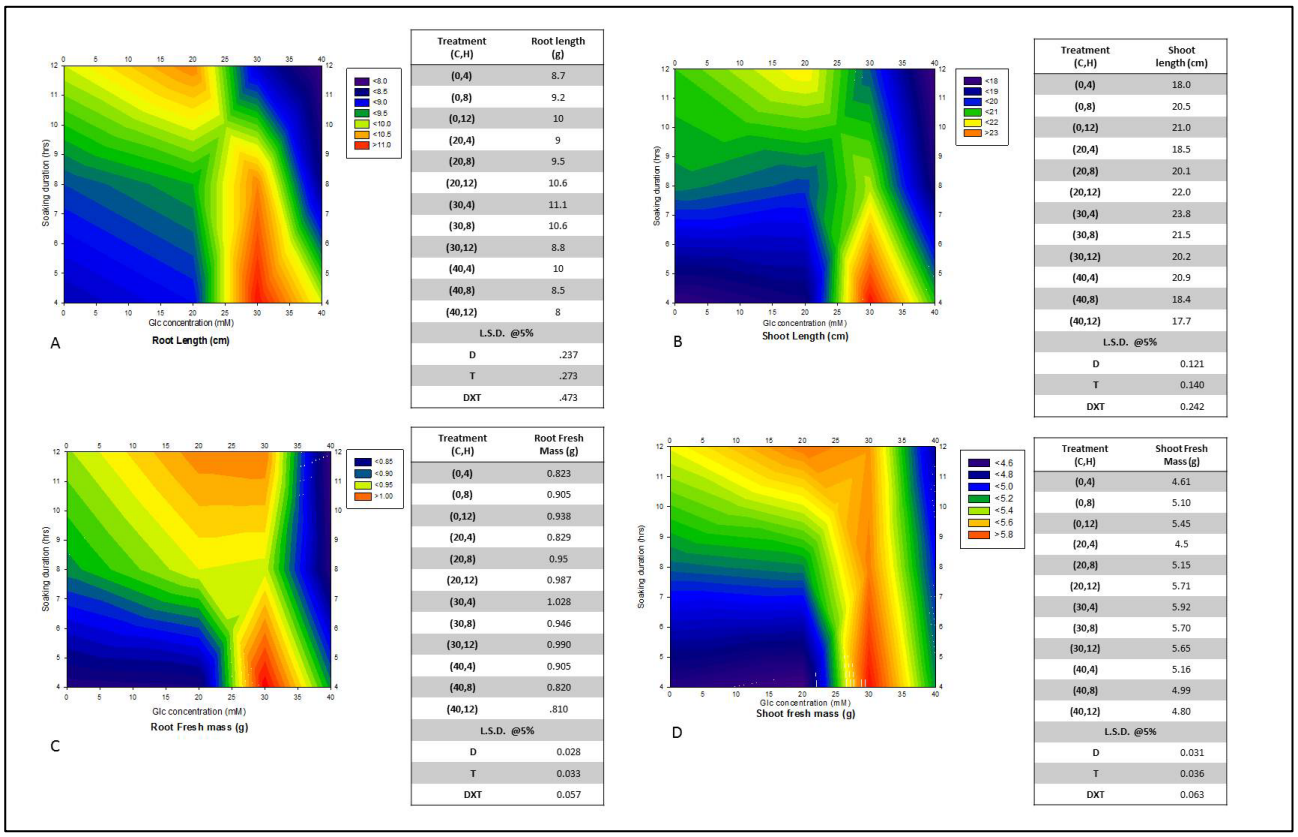


Fig. 1. **(A), (B)** and **(C)** shows the effect of different concentrations of glucose for 4, 8 and 12 hrs soaking duration, respectively on the growth of *Abelmoschus esculentus* at 45 DAS in comparison with their respective DDW treated control. **(D)** Shows the comparison between control and Glc 30mM (DAS- days after sowing; DDW- double distilled water)



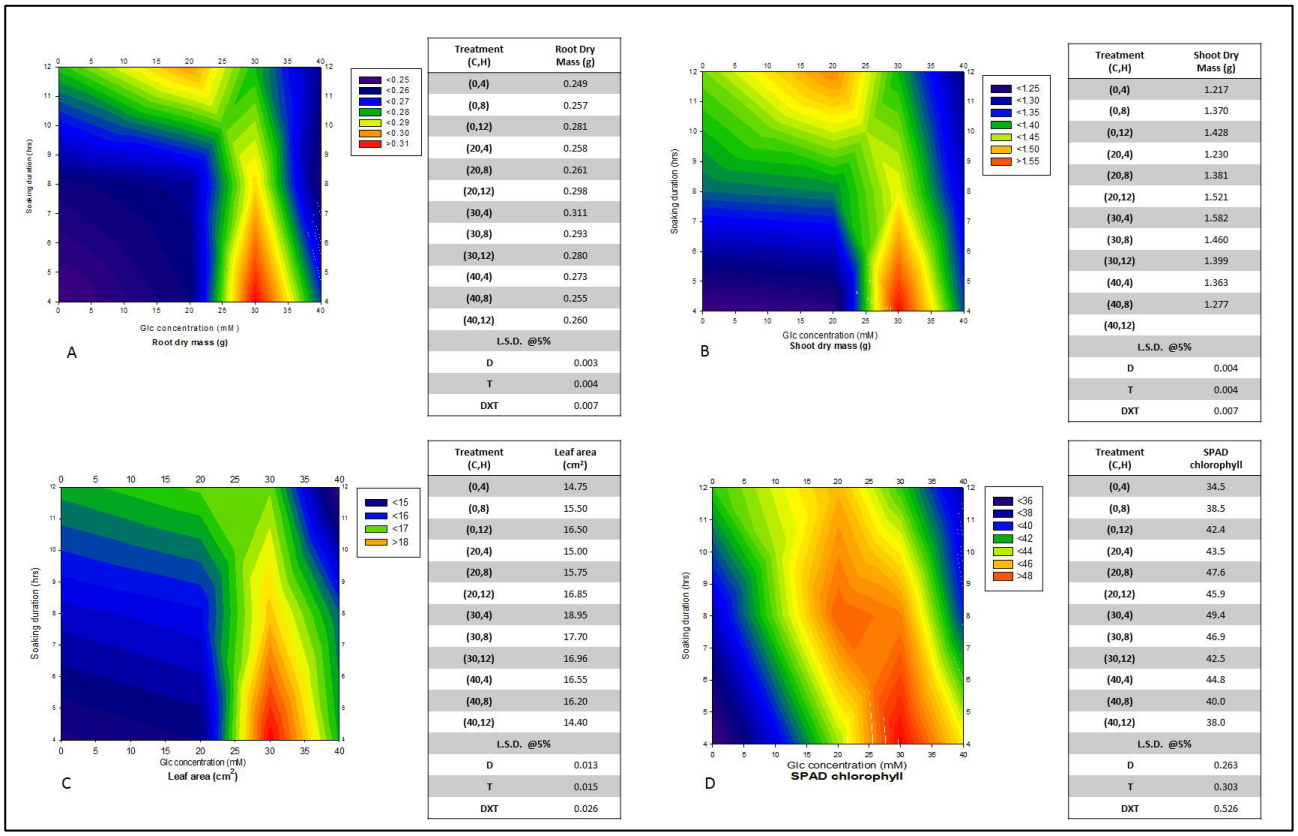
Treatment (C,H)	Root length (g)
(0,4)	8.7
(0,8)	9.2
(0,12)	10
(20,4)	9
(20,8)	9.5
(20,12)	10.6
(30,4)	11.1
(30,8)	10.6
(30,12)	8.8
(40,4)	10
(40,8)	8.5
(40,12)	8
L.S.D. @5%	
D	.237
T	.273
DXT	.473

Treatment (C,H)	Shoot length (cm)
(0,4)	18.0
(0,8)	20.5
(0,12)	21.0
(20,4)	18.5
(20,8)	20.1
(20,12)	22.0
(30,4)	23.8
(30,8)	21.5
(30,12)	20.2
(40,4)	20.9
(40,8)	18.4
(40,12)	17.7
L.S.D. @5%	
D	0.121
T	0.140
DXT	0.242

Treatment (C,H)	Root Fresh Mass (g)
(0,4)	0.823
(0,8)	0.905
(0,12)	0.938
(20,4)	0.829
(20,8)	0.95
(20,12)	0.987
(30,4)	1.028
(30,8)	0.946
(30,12)	0.990
(40,4)	0.905
(40,8)	0.820
(40,12)	.810
L.S.D. @5%	
D	0.028
T	0.033
DXT	0.057

Treatment (C,H)	Shoot Fresh Mass (g)
(0,4)	4.61
(0,8)	5.10
(0,12)	5.45
(20,4)	4.5
(20,8)	5.15
(20,12)	5.71
(30,4)	5.92
(30,8)	5.70
(30,12)	5.65
(40,4)	5.16
(40,8)	4.99
(40,12)	4.80
L.S.D. @5%	
D	0.031
T	0.036
DXT	0.063

Fig. 2. Contour plots exhibiting interactive effects between soaking duration and glucose concentration on root length (**A**), shoot length (**B**), root fresh mass (**C**) and shoot fresh mass (**D**) of *Abelmoschus esculentus* at 45 day stage of growth. All the data are the mean of five replicates ($n = 5$). (*L.S.D.* - least significant difference; D- duration; T- treatment)



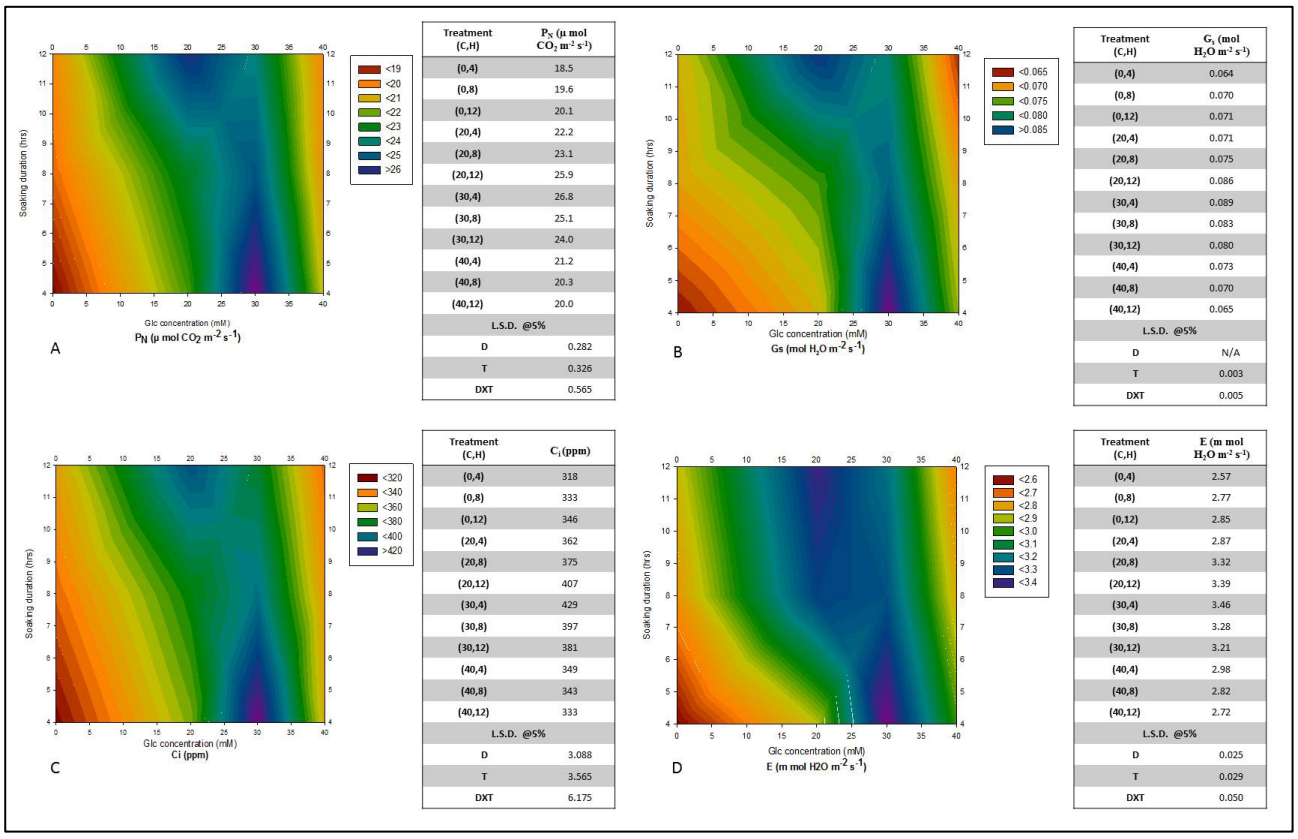
Treatment (C,H)	Root Dry Mass (g)
(0,4)	0.249
(0,8)	0.257
(0,12)	0.281
(20,4)	0.258
(20,8)	0.261
(20,12)	0.298
(30,4)	0.311
(30,8)	0.293
(30,12)	0.280
(40,4)	0.273
(40,8)	0.255
(40,12)	0.260
L.S.D. @5%	
D	0.003
T	0.004
DXT	0.007

Treatment (C,H)	Shoot Dry Mass (g)
(0,4)	1.217
(0,8)	1.370
(0,12)	1.428
(20,4)	1.230
(20,8)	1.381
(20,12)	1.521
(30,4)	1.582
(30,8)	1.460
(30,12)	1.399
(40,4)	1.363
(40,8)	1.277
(40,12)	
L.S.D. @5%	
D	0.004
T	0.004
DXT	0.007

Treatment (C,H)	Leaf area (cm^2)
(0,4)	14.75
(0,8)	15.50
(0,12)	16.50
(20,4)	15.00
(20,8)	15.75
(20,12)	16.85
(30,4)	18.95
(30,8)	17.70
(30,12)	16.96
(40,4)	16.55
(40,8)	16.20
(40,12)	14.40
L.S.D. @5%	
D	0.013
T	0.015
DXT	0.026

Treatment (C,H)	SPAD chlorophyll
(0,4)	34.5
(0,8)	38.5
(0,12)	42.4
(20,4)	43.5
(20,8)	47.6
(20,12)	45.9
(30,4)	49.4
(30,8)	46.9
(30,12)	42.5
(40,4)	44.8
(40,8)	40.0
(40,12)	38.0
L.S.D. @5%	
D	0.263
T	0.303
DXT	0.526

Fig. 3. Contour plots exhibiting interactive effects between soaking duration and glucose concentration on root dry mass (**A**), shoot dry mass (**B**), leaf area (C) and SPAD chlorophyll (D) of *Abelmoschus esculentus* at 45 day stage of growth. All the data are the mean of five replicates ($n = 5$). (*L.S.D.*- least significant difference; D- duration; T- treatment)



Treatment (C,H)	P_N (μ mol CO_2 m^{-2} s^{-1})
(0,4)	18.5
(0,8)	19.6
(0,12)	20.1
(20,4)	22.2
(20,8)	23.1
(20,12)	25.9
(30,4)	26.8
(30,8)	25.1
(30,12)	24.0
(40,4)	21.2
(40,8)	20.3
(40,12)	20.0
L.S.D. @5%	
D	0.282
T	0.326
DXT	0.565

Treatment (C,H)	G_s (mol H_2O m^{-2} s^{-1})
(0,4)	0.064
(0,8)	0.070
(0,12)	0.071
(20,4)	0.071
(20,8)	0.075
(20,12)	0.086
(30,4)	0.089
(30,8)	0.083
(30,12)	0.080
(40,4)	0.073
(40,8)	0.070
(40,12)	0.065
L.S.D. @5%	
D	N/A
T	0.003
DXT	0.005

Treatment (C,H)	C_i (ppm)
(0,4)	318
(0,8)	333
(0,12)	346
(20,4)	362
(20,8)	375
(20,12)	407
(30,4)	429
(30,8)	397
(30,12)	381
(40,4)	349
(40,8)	343
(40,12)	333
L.S.D. @5%	
D	3.088
T	3.565
DXT	6.175

Treatment (C,H)	E (m mol H_2O m^{-2} s^{-1})
(0,4)	2.57
(0,8)	2.77
(0,12)	2.85
(20,4)	2.87
(20,8)	3.32
(20,12)	3.39
(30,4)	3.46
(30,8)	3.28
(30,12)	3.21
(40,4)	2.98
(40,8)	2.82
(40,12)	2.72
L.S.D. @5%	
D	0.025
T	0.029
DXT	0.050

Fig. 4. Contour plots exhibiting interactive effects between soaking duration and glucose concentration on net photosynthetic rate, P_N (**A**), stomatal conductance, G_s (**B**), intercellular CO_2 concentration, *Ci* (**C**) and transpiration rate, *E* (**D**) of *Abelmoschus esculentus* at 45 day stage of growth. All the data are the mean of five replicates ($n = 5$). (*L.S.D.* - least significant difference; D- duration; T- treatment)

The order of response for Glc concentration were 30>20>40>0 mM and duration of soaking were 4>8>12 hrs. Glc mediated enhancement of plant growth in terms of higher leaf area, length as well as fresh and dry mass of plant could be ascribed to the multidimensional action of Glc on growth and development of plant [33-35]. The findings are in accordance with those of Singh et al. [36] where Glc promoted root growth and development. Similarly, a rise in plant fresh and dry mass upon Glc application has also been reported [37]. The response depends on the concentration of Glc and duration of soaking. The results share similar trend with that of Mao et al. [38], where growth of *Vitis venifera* L. plantlets increased in a concentration-dependent manner in presence of Glc. In the present study also, the increase in growth was in the following order: Glc 30> Glc 20> Glc 40> Glc 0 mM. The observed trend might depend on ABA content as higher concentration of Glc lowers down the decline in endogenous ABA whereas low concentration of Glc releases ABA-induced inhibitory effects on plant growth [3]. Furthermore, there is a wide collection of genes which is controlled by Glc such as growth, metabolism, transport, development and adaptive response against stress [10, 39].

One of the important markers for photosynthesis estimation is chl content and rise in chl value would influence the photosynthesis. It was observed that the level of SPAD chl (Fig. 3D) and all the photosynthetic attributes (Fig. 4) increased in the presence of Glc in all the soaking duration. Where, 30 mM of Glc and 4hrs soaking duration was proved best and increased the SPAD value (43.2%), P_N (44.9%), *Ci* (34.9%), *Gs* (39.1%) and *E* (34.6%) at 45 DAS over their respective control. Moreover, Glc improved the stomatal pore size that enhanced CO_2 diffusion inside the leaf via stomata (Fig. 5) and as a consequence *Ci* also increased (Fig. 4 and 5).

The present findings strongly corroborate earlier reports by Sami and Hayat [40], who demonstrated that foliar application of glucose (Glc) significantly enhanced chlorophyll content, stomatal conductance (Gs), and stomatal aperture in *Brassica juncea* (L.) Czern and Coss across different developmental stages. In addition to improvements in photosynthetic attributes, the activities of key carbon and nitrogen assimilatory enzymes, namely carbonic anhydrase (CA) and nitrate reductase (NR), were markedly elevated in plants raised from Glc-soaked seeds. In the current study, the maximum enhancement in CA and NR activities was recorded in plants treated with 30 mM Glc for 4 h, showing increases of 24.8% and 19.6%, respectively, over the corresponding control (Fig. 6A–B). These results suggest that Glc plays a decisive role in coordinating carbon and nitrogen metabolism to support improved plant performance.

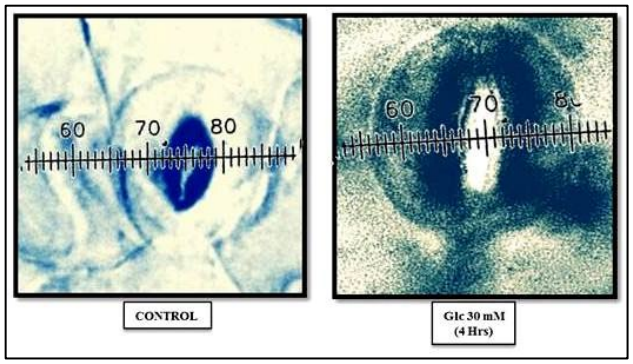
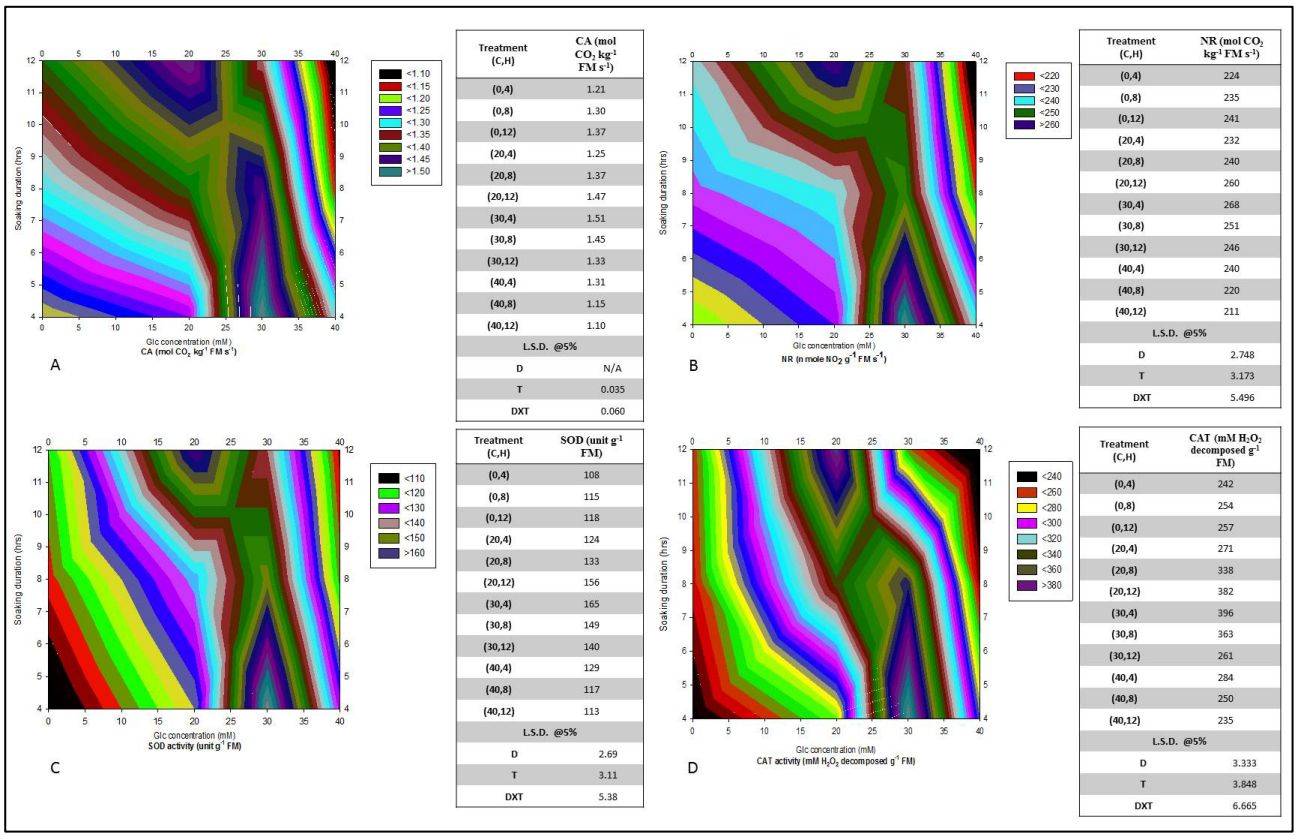


Fig. 5. Compound microscope images of stomata: Response of stomatal aperture of 45 days old *Abelmoschus esculentus* leaves to the treatment was studied using compound microscope. **(A)** represents DDW treated control and **(B)** glucose (30 mM) at 4hr soaking duration at 40X. (DDW- double distil water)



Treatment (C,H)	CA (mol CO_2 kg^{-1} FM s^{-1})
(0,4)	1.21
(0,8)	1.30
(0,12)	1.37
(20,4)	1.25
(20,8)	1.37
(20,12)	1.47
(30,4)	1.51
(30,8)	1.45
(30,12)	1.33
(40,4)	1.31
(40,8)	1.15
(40,12)	1.10
L.S.D. @5%	
D	N/A
T	0.035
DXT	0.060

Treatment (C,H)	NR (mol CO_2 kg^{-1} FM s^{-1})
(0,4)	224
(0,8)	235
(0,12)	241
(20,4)	232
(20,8)	240
(20,12)	260
(30,4)	268
(30,8)	251
(30,12)	246
(40,4)	240
(40,8)	220
(40,12)	211
L.S.D. @5%	
D	2.748
T	3.173
DXT	5.496

Treatment (C,H)	SOD (unit g^{-1} FM)
(0,4)	108
(0,8)	115
(0,12)	118
(20,4)	124
(20,8)	133
(20,12)	156
(30,4)	165
(30,8)	149
(30,12)	140
(40,4)	129
(40,8)	117
(40,12)	113
L.S.D. @5%	
D	2.69
T	3.11
DXT	5.38

Treatment (C,H)	CAT (mM H_2O_2 decomposed g^{-1} FM)
(0,4)	242
(0,8)	254
(0,12)	257
(20,4)	271
(20,8)	338
(20,12)	382
(30,4)	396
(30,8)	363
(30,12)	261
(40,4)	284
(40,8)	250
(40,12)	235
L.S.D. @5%	
D	3.333
T	3.848
DXT	6.665

Fig. 6. Contour plots exhibiting interactive effects between soaking duration and glucose concentration on carbonic anhydrase activity (**A**), nitrate reductase (**B**), superoxide dismutase, *SOD* (**C**) and catalase, *CAT* (**D**) of *Abelmoschus esculentus* at 45 day stage of growth. All the data are the mean of five replicates ($n = 5$). (*L.S.D.* - least significant difference; D- duration and T- treatment)

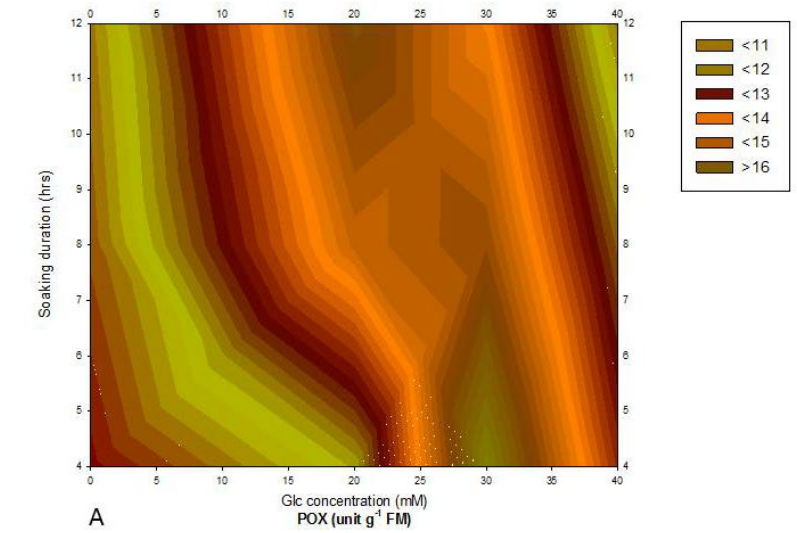
It is well established that nitrate (NO_3^-) availability and accumulation act as primary inducers of NR activity. Glucose influences NR activity either directly or indirectly by serving as a major source of reducing power required for nitrate reduction and by providing carbon skeletons essential for NR synthesis [26, 40]. In line with the present observations, Aslam and Oaks [41] reported that Glc supplementation enhanced the accumulation of metabolically active nitrate in corn roots. Since leaf NR activity is widely regarded as a reliable indicator of plant nitrogen status, the Glc-induced rise in NR activity observed in this study (Fig. 6B) reflects improved nitrogen assimilation, further supporting the findings of Sami and Hayat [40].

The enhanced NR activity under Glc treatment may also be linked to the observed increase in transpiration rate (E), which facilitates greater nitrogen uptake by roots through mass flow. Elevated NR activity is likely to stimulate amino acid synthesis, particularly proline, which plays a crucial role in osmoprotection, redox balance, and stress tolerance, thereby contributing to improved plant growth. Glucose serves as a central metabolic fuel, providing ATP and NADPH via respiration and the oxidative pentose phosphate pathway, respectively. These energy equivalents are critical for proline biosynthesis from glutamate and α-ketoglutarate. Therefore, it can be inferred that NR activity, together with the availability of ATP and NADPH, operates in a coordinated manner to promote enhanced proline accumulation in okra leaves (Fig. 7B).

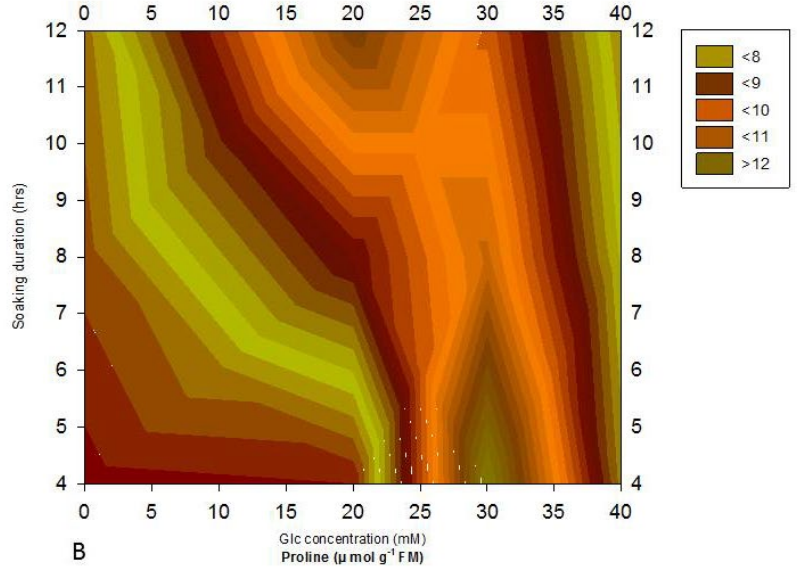
Furthermore, the rise in intercellular CO_2 concentration (Ci) coupled with increased CA activity (Fig. 6A), which facilitates the rapid interconversion of CO_2 and bicarbonate and enhances the availability of CO_2 to Rubisco, resulted in a significant improvement in net photosynthetic rate (PN). Similar enhancements in PN following Glc supplementation have been reported by Jiang et al. [11] in watermelon leaves. The cumulative effect of improved photosynthetic efficiency ultimately translated into greater biomass accumulation (Fig. 3), a finding that is consistent with the work of Huang et al. [37], who observed increased biomass production in cucumber following Glc application. Collectively, these results underline the pivotal role of Glc in integrating carbon and nitrogen metabolism, leading to improved physiological performance and growth in okra.

Reactive oxygen species (ROS) are continuously generated within plant cells as by-products of electron transport processes during photosynthesis and respiration. Excessive ROS accumulation leads to photoinhibition of photosystem II (PSII) and photosystem I (PSI), thereby severely restricting plant growth and productivity [27]. Superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH) are among the major ROS produced in plants, and their excessive accumulation can cause oxidative damage to cellular membranes, proteins, and nucleic acids [42].

To counteract ROS-induced oxidative stress, plants operate an efficient antioxidant defense system that functions concurrently with ROS generation. This defense machinery comprises enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX), as well as non-enzymatic osmoprotectants like proline [43]. These antioxidants detoxify ROS and mitigate their harmful effects, thereby maintaining cellular redox homeostasis. In the present study, the activities of SOD, CAT, POX, and the accumulation of proline increased significantly across all glucose (Glc) concentrations and soaking durations (Figs. 6C–D; 7A). Notably, plants raised from seeds soaked in 30 mM Glc for 4 h exhibited the highest enhancement, with increases of 52.8%, 63.6%, 64.9%, and 72.3% for SOD, CAT, POX, and proline, respectively, at 45 DAS compared to the control.



Treatment (C,H)	POX (unit g^{-1} FM)
(0,4)	10.12
(0,8)	10.67
(0,12)	10.94
(20,4)	11.63
(20,8)	14.67
(20,12)	15.68
(30,4)	16.69
(30,8)	15.18
(30,12)	14.16
(40,4)	13.15
(40,8)	12.14
(40,12)	10.92
L.S.D. @5%	
D	0.028
T	0.033
DXT	0.057



Treatment (C,H)	Proline (µ mol g^{-1} FM)
(0,4)	7.10
(0,8)	7.50
(0,12)	7.73
(20,4)	7.20
(20,8)	9.23
(20,12)	11.36
(30,4)	12.24
(30,8)	10.65
(30,12)	9.94
(40,4)	8.52
(40,8)	0.820
(40,12)	.810
L.S.D. @5%	
D	0.033
T	0.039
DXT	0.067

Fig. 7. Contour plots exhibiting interactive effects between soaking duration and glucose concentration on peroxidase, POX (**A**) and proline (B) of *Abelmoschus esculentus* at 45 day stage of growth. All the data are the mean of five replicates ($n = 5$). (*L.S.D.* - least significant difference; D-duration

The observed enhancement in antioxidant enzyme activities and proline content may be attributed to Glc-mediated upregulation of genes involved in antioxidant metabolism and to increased activity of pyrroline-5-carboxylate synthetase, a key enzyme in proline biosynthesis [42]. Interestingly, sugars in plants are known to exhibit a dual role: on one hand, they can promote ROS generation through respiratory metabolism, while on the other hand, they participate in ROS detoxification via the oxidative pentose phosphate pathway, which supplies reducing equivalents in the form of NADPH [13, 44]. Furthermore, sugars such as Glc and fructose, particularly those associated with membrane interactions, have been recognized as effective ROS scavengers, contributing directly to oxidative stress tolerance. Collectively, these findings emphasize the central role of Glc in reinforcing the antioxidant defense system and enhancing redox stability, thereby supporting improved growth and stress resilience in plants.

4. Conclusions

Supplementation of Glc increased the *Gs* as well as pore size which increased the CO_2 entry into the leaf. Rise in CO_2 concentration inside the leaf cells accompanied by CA promoted CO_2 fixation and lead to an increment in P_N. Moreover, the wide stomatal aperture also promoted the E which might have enhanced the absorption of water and mineral nutrients required for synthesis of different biomolecules. The increased in the activity of NR contributed to accumulation of amino acid like proline. Moreover, Glc released the ROS-mediated break on photosystems by enhancing activity of antioxidant enzymes. Hence, the enhanced photosynthetic efficiency, metabolic enzymes and antioxidant status resulted in better growth and development of plant. These Glc-generated effects were dose and duration dependent where 30 mM and 4 hrs soaking duration proved best in bringing out the response. This gives a clear indication that maximum response occurs up to a certain level and duration of Glc supplementation (30 mM and 4 hrs in this

study) and beyond that the effects start to fade. This decline in effect at higher concentration could be an outcome of Glc-mediated feedback mechanism which should be verified further using molecular tools and techniques.

Author Contributions

Conceptualization, S.H.; investigation, H.S., K.B.M.A.; resources, M.F., M.F.B.; writing-original draft preparation, H.S., K.B.M.A.; writing-review and editing, P.A., M.F., and T.A.B.; visualization, S.H. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest.

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