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# Glucose exogenous increases biochemical and physiological responses in *Beta vulgaris* L.

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*Key words*: Antioxidant activity, elicitation, glucose, leaf pigments, phenolic compounds, secondary metabolism, yield.

**Abstract: Glucose can act as a bioregulator in plants, influencing physiological and metabolic processes. This investigation aimed to analyses how the application of glucose impacts the photosynthetic rate, growth, and concentration of bioactive compounds with antioxidant potential in beet. The current investigation evaluated the effect of glucose (0, 5, 10, 20, and 30 mmol $L^{-1}$) on beet plants 10 days after transplantation. Glucose elicitation in beet plants increases levels of bioactive compounds, resulting in higher antioxidant potential. In addition to antioxidant benefits, glucose also plays a hormonal role, leading to increases in biomass. These effects are correlated with enhanced photosynthetic efficiency, elevated carbohydrate concentrations, and positive modulation of the plant antioxidant system.**

## 1. Introduction

*Beta vulgaris* L (beet) belonging to the Chenopodiaceae family, is a nutritious vegetable often used in salads and juices. It is an excellent source of nutrients, including vitamins (folic acid, and vitamin A, B1, B2, B6, and C), minerals (Sadowska-Bartosz and Bartosz, 2021), fiber, proteins (Fu *et al.,* 2020 a), and sugar (sucrose) (Tayyab *et al.,* 2023). Additionally, beet is considered a great health food, due to the high levels of bioactive compounds present, such as betalains, polyphenols, and flavonoids (Gorni *et al.,* 2023), which are powerful antioxidants associated with cardiovascular health and the reduction of cancer cells, among other diseases (Chhikara *et al.,* 2019). In addition to its nutritional properties, beets are valued for their diverse culinary applications. It can be consumed raw, grated in salads, cooked, roasted, grilled, and even turned into juices and smoothies.

Beet production faces major challenges due to climate change. Rising



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**Data Availability Statement:**
All relevant data are within the paper and its Supporting Information files.

**Competing Interests:**
The authors declare no conflict of interests.

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temperatures and changes in rainfall patterns affect both its growth, development, and the formation and quality of roots (Tayyab *et al.,* 2023). In addition, water shortages and the emergence of pests and diseases are becoming more frequent, putting productivity at risk (Baryga *et al.,* 2020). To deal with these problems, new techniques are being adopted to improve agricultural practices, making crops more sustainable and stimulating the plant to biosynthesize more bioactive compounds, which results in increased plant resistance to climate changes.

Elicitation has been used as a technique that involves the induction of responses in plants, generally in response to biotic (such as pathogens or herbivores) or abiotic (such as climate variations) stresses (Kandoudi and Németh-Zámboriné, 2022). Elicitors can act at several levels, such as activating plant defence mechanisms. For example, elicitation technique can be used to increase the amount of phytocompounds in plant, improving their functionality, this practice has been applied to increase the yield of secondary metabolites, biological activities (antioxidant properties) and mainly to induce plant growth (Baenas *et al.*, 2019; Siddiqui *et al.*, 2020; Gorni *et al.,* 2023) and can also stimulate cellular signaling pathways, leading to the expression of genes related to defence and strengthening of cellular structure (Sami *et al.,* 2021).

Among the various elicitors, glucose (Glc) has been studied due to its important roles in plants, to increase the biosynthesis and accumulation of secondary metabolites, resulting in increased plant yield (Hennion *et al.,* 2019). Regarding the elicitors that act in secondary metabolism, it is known that Glc is an essential sugar that plays a vital role in plant metabolism, acting as one of the main sources of energy (Saddhe *et al*., 2021). Produced during photosynthesis, it not only feeds plants, but is also essential for their health and growth (Fu *et al.,* 2020 b). In addition to providing energy, Glc is an important component in the formation of several biomolecules necessary for plant development (Wang *et al.,* 2019).

Given this information, some researchers have observed that the application of Glc acted as a growth inducer for plants, which was correlated with increased photosynthesis (Siddiqui *et al.,* 2020), seed germination (Gorni and Polimento, 2023), regulates antioxidant metabolism (Sami and Hayat, 2019), activates key enzymes involved in secondary metabolism (Zahid *et al.,* 2018), increases the biosynthesis of phenols, flavonoids, anthocyanins, and antioxidant activity (Xu *et al.,* 2016; Baenas *et al.,* 2019), gene expression (Rezaee *et al.,* 2018), and alleviates stresses (Sami *et al.,* 2021).

From this perspective, Glc is a substance that promotes plant growth and enhances quality by increasing the biosynthesis of bioactive compounds and reducing ROS concentration (Baenas *et al.*, 2014; Sami and Hayat, 2019). Given this information, the goal is to improve the quality and yield of beet roots through Glc application at different concentrations, aiming for a biostimulant effect. This approach could potentially enhance the growth and development of the crops, increase yield, and improve root quality. Therefore, this investigation aimed to analyses how the application of Glc impacts the photosynthetic rate, growth, and concentration of bioactive compounds with antioxidant potential in beet.

## 2. Materials and Methods

*Experimental location and plant material*

The experiment was conducted using beet plants in pots inside a greenhouse on a wooden bench, kept open sky, covered by shading which provided 50% of solar radiation located at the Escola Superior de Agronomia de Paraguaçu Paulista (ESAPP), Brazil (22°41'76" S, 50°58'33" W). The Köppen classification identifies the region's climate as Aw, the climate data is presented in figure 1. The experiment was conducted between April 19 and June 18, 2024 (60 days).

Fifteen-day-old beet seedlings were transplanted into pots with sandy soil. The soil was analysed and

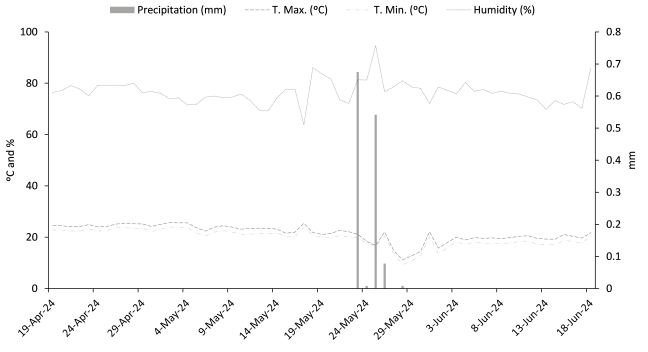


Fig. 1 - Climatic data of Paraguaçu Paulista, São Paulo, during the experimental period (April 19 to June 18, 2024). Source: INMET - Instituto Nacional de Meteorologia.

corrected according to Bulletin 100 (Cantarella *et al.,* 2022). The pots were irrigated by sprinklers twice a day during the experimental period. Every 15 days, cattle manure was applied as coverage fertilization. At 10 days after transplantation, the treatments were applied at concentrations of 0 (control), 5, 10, 20, and 30 m mol $L^{-1}$ of Glc. Each treatment was replicated four times.

All analyses below were performed at the end of the experiment at 60 DAT.

*Pigments analysis and quantification*

Photosynthetic pigments were extracted in 95% ethanol and calculations were performed according to Lichtenthaler (1987). Fresh tissues (0.2 g) were added to 8 mL of 95% ethanol and shaken, leaving them to rest for 24 h at 4°C. After this process, readings were performed at wavelengths of 470, 647 and 663 nm.

*Photosynthetic parameters*

Chlorophyll fluorescence parameters were measured using a portable chlorophyll fluorometer (Opti-Sciences, $F_V/F_M$ Meter). Beet leaves were placed under dark adaptation clips for 30 min prior to measurement to allow complete accumulation of chlorophyll energy. After the adaptation period, measurements of initial fluorescence ($F_0$), maximum fluorescence ($F_m$), variable fluorescence ($F_v$), maximum photochemical efficiency of PSII ($F_v/F_m$), and absorbed energy conversion ($F_v/F_0$).

*Biochemical assessment of enzymes activities and protein*

Frozen samples were extracted in 0.1 M potassium phosphate buffer (pH 7.5) containing EDTA and PVP. The supernatant was utilized for enzyme activity assays, included those SOD (EC 1.15.1.1) was measured by the inhibition of nitroblue tetrazolium (NBT) reduction by superoxide, which is generated by the reaction of riboflavin under light (Giannopolitis and Ries, 1977), CAT (EC 1.11.1.6) is determined by the decomposition of hydrogen peroxide, which can be monitored spectrophotometrically (240 nm) (Azevedo *et al.,* 1998), and APX (EC1.11.1.11) is measured by the decrease in absorbance of ascorbic acid in the presence of $H_2O_2$ (Moldes *et al.*, 2008).

For phenylpropanoid pathway enzymes, leaves were extracted using 0.5 mM Tris-EDTA buffer (pH 8.5) containing EDTA. The supernatant was used to measure the activity of phenylalanine ammonia lyase (PAL, EC 4.3.1.5) following the method of Hyodo *et al.* (1978), and chalcone synthase (CHS, EC 2.3.1.74) according to Moustafa and Wong (1967). The PAL assay was conducted at 40°C for 1 hour in a reaction mixture (1 mL) consisting of 0.5 M Tris-HCl (pH 8.5), enzyme extract, and 30 µM L-phenylalanine. The reaction was stopped in an ice bath for 5 minutes. PAL activity was quantified spectrophotometrically by measuring the concentration of trans-cinnamic acid produced at 290 nm. The CHS assay was conducted at 30°C for 1 hour in a reaction mixture (1 mL) consisting of 5 mM Tris-HCl (pH 7.8), 10 mM KCN, enzyme extract, and chalcone was added to ethylene glycol monomethyl ether. CHS activity was measured by the production of chalcone at 370 nm.

Protein concentration was determined using the Bradford method (1976), where the supernatant was added to the Bradford solution, after 2 minutes the reading was taken at 590 nm, using bovine serum albumin as standard.

*Biochemical assessment of ROS*

To determine ROS, samples were extracted in 0.1% trichloroacetic acid. The supernatant used for the hydrogen peroxide ($H_2O_2$) was evaluated according to Alexieva *et al.* (2001), samples were added to a solution of 0.1 M potassium phosphate buffer (pH 7.5) and 1 M potassium iodide solution. The mixture was incubated on ice for 1 h. The reading was performed at a wavelength of 390 nm. Using $H_2O_2$ standards. Lipid peroxidation was by the malondialdehyde (MDA) method by Heath and Packer (1968), supernatant was mixed with a solution containing 0.5% TBA (thiobarbituric acid) in 20% TCA (trichloroacetic acid). This mixture was incubated at 95°C for 30 min, and the reaction was stopped with an ice bath. The absorbances were 535 nm and 600 nm. Superoxide radical ($O_2^{\cdot-}$) content was evaluated according to Elstner and Heupel (1976), sample was added to a 65 mM potassium phosphate buffer solution (pH 7.8) and a 10 mM hydroxylamine hydrochloride solution and incubated at 25 ºC for 20 min. After incubation, 1% (m/v) sulfanilamide in hydrochloric acid (2.4 N) and 0.02% N-(1-naphthylethylenediamine) dihydrochloride were added. The reaction mixture was homogenized and incubated again at 25°C for 20 min. Finally, ethyl ether was added, the solution was centrifuged at 1500 rpm for 5 min, and the wavelength used was 530 nm.

*Determination of nitrogen compounds*

For extraction, leaf samples were macerated in 80% ethanol solution. The material was macerated and centrifuged, and the supernatant was used for analysis.

Total soluble sugar content was determined according to Dubois *et al.* (1956), sample was mixed with a phenolic reagent (5%) and then sulfuric acid was added. After cooling, the absorbance was read at 390 nm, using glucose as a standard.

Reducing sugar content was determined using Somogyi-Nelson (Bezerra Neto and Barreto, 2004), sample was mixed with the stirred Somogyi reagent and incubated in boiling water for 15 minutes. After cooling on ice, the Nelson reagent was added, mixed and left to stand for 20 minutes. After the addition of water, the absorbance was read at 760 nm, glucose was used as a standard.

Sucrose content was determined according to Van Handel (1968), sample was mixed with a KOH reagent (30%) and then sulfuric acid was added. The mixture was incubated at 100°C for 10 minutes, after cooling the absorbance was read at 490 nm, using sucrose as a standard.

Starch content was determined using anthrone reagent (Sadasivam and Manickam, 1996). Samples were mixed in anthrone reagent and boiled for 10 minutes, the mixture was cooled, and the absorbance read at 650 nm, glucose was used as a standard.

Total amino acids content was determined according to Yemm *et al.* (1955). Samples were mixed in sodium citrate (0.2 M), ninhydrin solution (5%) and KCN (0.2 mM). The mixture was homogenized and heated at 100°C for 20 minutes. After cooling in running water, 60% ethanol was added and the absorbance was read at 570 nm, using methionine as a standard.

*Determination of proline*

Proline was determined by Bates *et al.* (1973). For extraction, leaf samples were macerated in a 5-sulfosalicylic acid solution (3%), the material was macerated and centrifuged, and the supernatant was used for analysis. The sample was mixed with a solution of acid ninhydrin and acetic acid, which was incubated at 100°C for 1 h. Afterwards, the proline was partitioned with the addition of toluene and the mixture was shaken. Absorbance was read at 520 nm, using proline as a standard.

*Biochemical analysis of tuberous root*

Fresh beet root (5 g) was subjected to a washing and peeling process and extracted with a processor for two minutes. After this procedure, the juice was filtered through sieves (2 mm) and stored in a refrigerator before analysis.

*Total soluble solids*

The analysis of total soluble solids was performed using an analogue refractometer, where the samples were centrifuged, and a drop deposited on the refractometer prism and the values were collected. The results were expressed in degrees Brix (°Brix).

*Total polyphenols, flavonoids, anthocyanins, carotenoid, and betalains concentrations*

Polyphenols concentrations was determined according to the Folin-Ciocalteu method and 25% sodium carbonate solution (Stagos *et al.,* 2012), using gallic acid as standard. Flavonoids concentrations were measured according to Yao *et al*. (2013), using 5% $NaNO_2$, 10% $AlCl_3$, and NaOH (1 M), using rutin as standard. Anthocyanins were determined according to the Francis method (1982), in 95% ethanol extraction solution acidified with HCl, the mixture was left to rest for 24 hours at 4°C, protected from light, after which it was filtered and the absorbance read at 535 nm. The carotenoid content was extracted from fresh leaf samples with chloroform/acetone/ethanol (2:1:1, v/v/v) (Sadler *et al*., 1990). For determination of β-carotene, absorbance was measured at wavelengths of 450 nm. Quantification of betalains followed the methodology of Nilsson (1970), root samples were homogenized, and absorbance readings were performed at wavelengths of 480 nm and 540 nm.

*DPPH activity*

DPPH radical scavenging was determined by Blois (1958). Samples were mixed with ethanol, acetate buffer (0.1 M, pH 5.5) and a DPPH solution (0.5 mM) was added. After resting in the dark for 30 minutes, absorbance was read at 517 nm. The results were expressed as a percentage (%).

*Growth and yield parameters*

The measured traits included the number of leaves. Using a caliper, measurements were taken of the root diameter, and length. For shoot, root, and total fresh weight a precision scale was used.

*Experimental design and statistical analysis*

Analysis of the data was performed using Sisvar (Ferreira, 2019). ANOVA was performed on the data to determine the Tukey's test ($p \leq 0.01$). A multivariate analysis of variables was performed for all parameters using SigmaPlot software.

## 3. Results

Beet plants treated with Glc showed significant results in chlorophyll fluorescence (Fig. 2). The $F_0$ values exhibited reductions of 15.6%, 14.9%, 16.48%, and 12.2%, respectively, compared to the control (Fig. 2 a). Increases were observed for $F_m$ by 5.5%, 9.8%, 1.5%, and 4.6% (Fig. 2 b), $F_v$ by 10.8%, 16.1%, 6.0%, and 8.9% (Fig. 2 c), $F_v/F_m$ by 5.1%, 5.36%, 4.5%, and 4.1% (Fig. 2 d), and $F_v/F_0$ by 31.3%, 36.5%, 27.3%, and 24.4% (Fig. 2 e), respectively, compared to untreated plants.

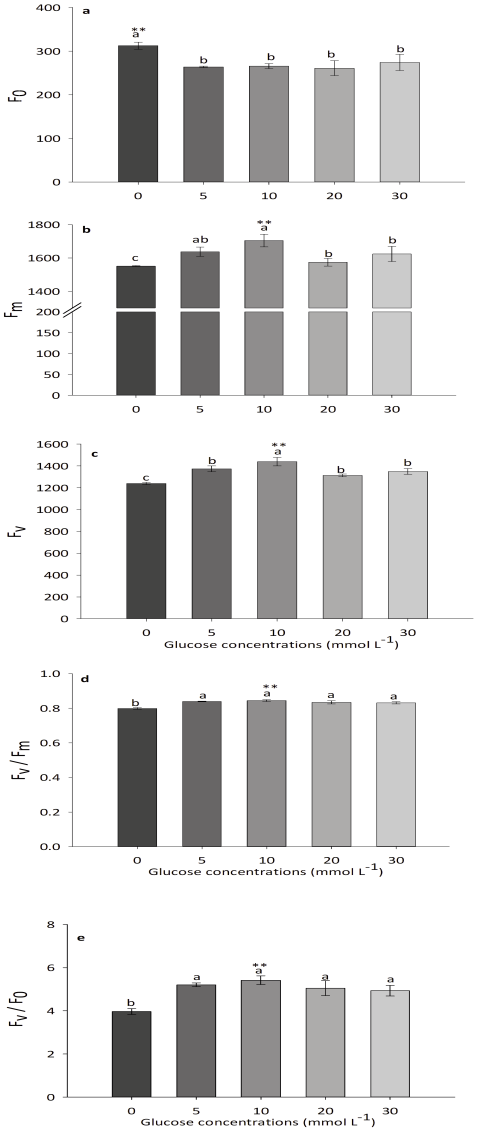


Fig. 2 - *F0* (a), *Fm* (b), *Fv* (c), *Fv/Fm* (d), and *Fv/F0* (e) in beet plants treated with different glucose concentrations. **= significant at a probability level of p≤0.01 using the Tukey's test at 5% probability. Bars represent standard error of the mean; n = 4.

Chlorophyll concentrations treated with 5 and 20 mmol $L^{-1}$ of Glc increased by 47.2% and 20.2% for Chlorophyll *a*, 42.6% and 17.4% for Chlorophyll *b*, 46.2% and 19.6% for Total Chlorophyll, and 39.9% and 13.6% for Carotenoids, respectively, compared to the control. However, when the Glc concentration was 10 and 30 mmol $L^{-1}$, the Chlorophyll a, Chlorophyll b, Total Chlorophyll, and Carotenoids were lower than those obtained without Glc treatment (Fig. 3).

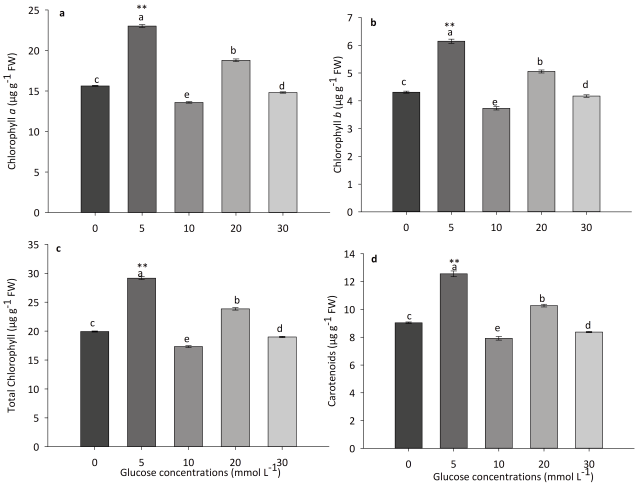


Fig. 3 - Chlorophyll a (a), chlorophyll b (b), total chlorophyll (c) and carotenoids (d) concentrations in beet plants treated with different glucose concentrations. **= significant at a probability level of p≤0.01 using the Tukey's test at 5% probability. Bars represent standard error of the mean; n = 4. FW = fresh weight.

Beet plants treated with Glc showed reductions in MDA concentrations of 82.5%, 31.9%, 40.9%, and 38.9%, respectively, compared to the control (Fig. 4 a). However, $H_2O_2$ concentrations decreased by 27.2% and 13.1%, respectively, in plants treated with 5 and 10 mmol $L^{-1}$ of Glc compared to the control (Fig. 4b), and concentrations of 20 and 30 mmol $L^{-1}$ of Glc, did not differ from the control. As for $O_2^-$ concentrations, there was an increase of 15.3% in plants treated with 20 mmol $L^{-1}$ of Glc, while concentrations of 5 and 10 mmol $L^{-1}$ decreased by 18.1% and 62.2%, respectively, compared to the

control (Fig. 4 c).

Regarding the activity of the SOD enzyme, treatments with 5, 10, and 30 mmol $L^{-1}$ of Glc showed increases of 37.9%, 15.0%, and 12.4%, respectively, compared to the control (Fig. 5 a). However, when the Glc concentration was 20 mmol $L^{-1}$ was lower than those obtained without Glc treatment. For CAT and APX, there was an increase in activity in plants treated with 5, and 10 mmol $L^{-1}$, respectively, compared to the control (Fig. 5 b, 5 c). However, the application of concentrations of 20 and 30 mmol $L^{-1}$ showed lower activity than those obtained without treatment with Glc.

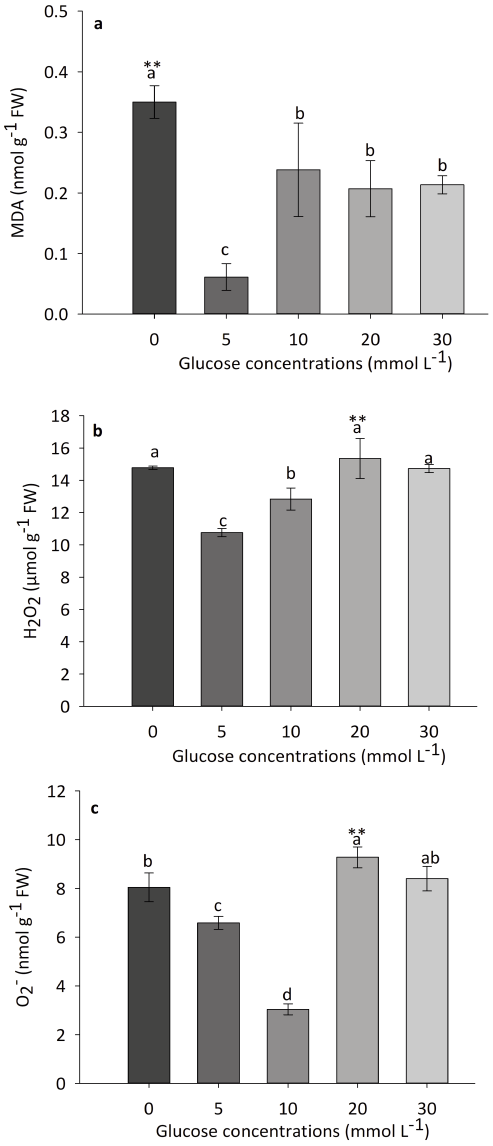


Fig. 4 - MDA (a), $H_2O_2$ (b), and $O_2^-$ (c) concentrations in beet plants treated with different glucose concentrations. **= significant at a probability level of p≤0.01 using the Tukey's test at 5% probability. Bars represent standard error of the mean; n= 4. FW = fresh weight.

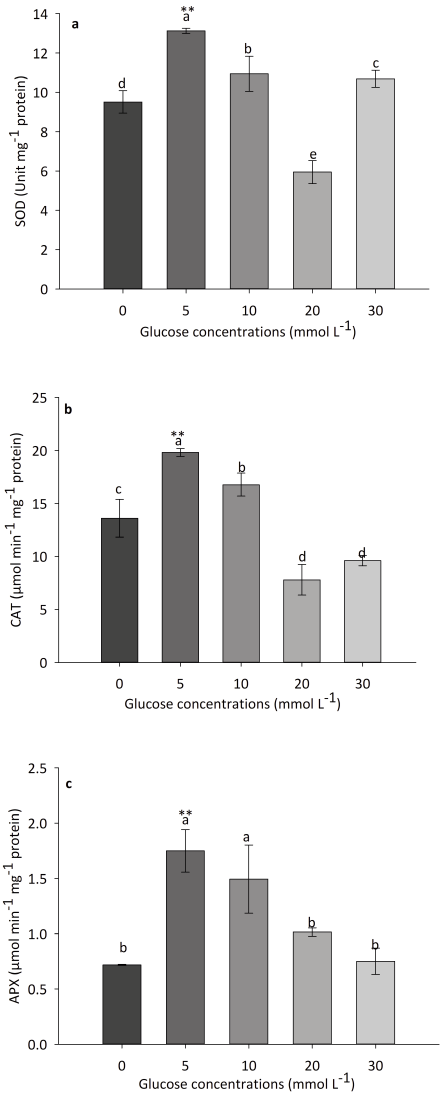


Fig. 5 - Specific activity of SOD (a), CAT (b), and APX (c) in beet plants treated with different glucose concentrations. **: significant at a probability level of p ≤ 0.01 using the Tukey's test at 5% probability. Bars represent standard error of the mean; n = 4.

Total sugar concentrations in plants treated with 10 and 30 mmol $L^{-1}$ of Glc showed increases of 5.4% and 11.3%, respectively, compared to the control (Fig. 6 a). For reducing sugar concentrations, applications of 10, 20, and 30 mmol $L^{-1}$ of Glc increased by 61.6%, 41.1%, and 53.8%, respectively, compared to the control (Fig. 6 b). Sucrose concentrations increased by 14.4% and 32.9%, respectively, in plants treated with 10 and 20 mmol $L^{-1}$ of Glc compared to the control (Fig. 6 c). Starch concentrations were higher by 9.3%, 30.7%, and 18.9%, respectively, in plants treated with 10, 20, and 30 mmol $L^{-1}$ of Glc compared to the control (Fig. 6 d), however, when the Glc concentration was 5 mmol $L^{-1}$

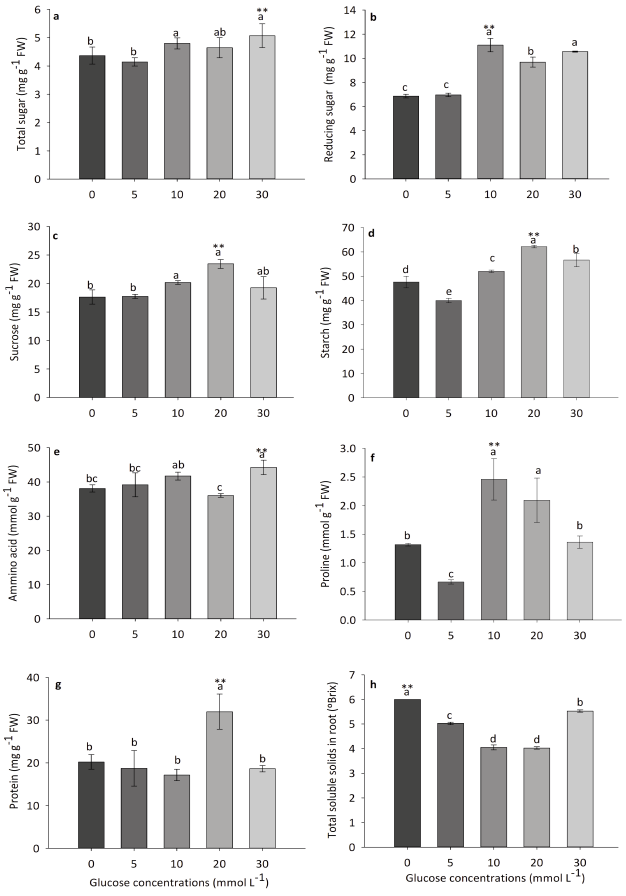


Fig. 6 - Total sugar (a), reducing sugar (b), sucrose (c), starch (d), amino acid (e), proline (f), protein (g), and total soluble solids in root (h) concentrations in beet plants, and total soluble solids in juice (f) treated with different glucose concentrations. **= significant at a probability level of p≤0.01 using the Tukey's test at 5% probability. Bars represent standard error of the mean; n= 4. FW= fresh weight.

was lower than those obtained without Glc treatment. For amino acid concentrations, only plants treated with 30 mmol $L^{-1}$ of Glc showed an increase of 16.1% compared to the control (Fig. 6 e). However, proline concentrations increased by 86.5% and 58.7%, respectively, in plants treated with 10 and 20 mmol $L^{-1}$ of Glc compared to the control (Fig. 6 f), however, when the Glc concentration was 5 mmol $L^{-1}$ was lower than those obtained without Glc treatment. Protein content increased by 26.6% in plants treated with 20 mmol $L^{-1}$ of Glc compared to the control (Fig. 6 g). However, total soluble solids in tuberous roots decreased in all treatments compared to the control (Fig. 6 h).

The PAL enzyme activity showed increases of 30.3%, 48.8%, and 42.0%, respectively, in plants treated with 5, 10, and 30 mmol $L^{-1}$ of Glc compared to the control (Fig. 7 a). However, the CHS enzyme activity increased by 7.9%, and 17.2%, respectively, in plants treated with 10, and 30 mmol $L^{-1}$ of Glc compared to the control (Fig. 7 b). However, the application of concentration of 20 mmol $L^{-1}$ showed lower activity than those obtained without treatment with Glc. The application of glucose at 30 mmol $L^{-1}$ resulted in increases of 21.2% for polyphenols, 34.6% for flavonoids, 22.7% for β-carotene, 26.9% for anthocyanins, and 28.7% for betalains, respectively, compared to the control (Fig. 8 a-e). However, the application of concentrations of 5, 10, and 20 mmol $L^{-1}$ showed lower activity than those obtained without treatment with Glc respectively. However, increases of 27.6%, 99.4%, and 20.4% were observed at concentrations of 5, 20, and 30 mmol $L^{-1}$, respectively, in the antioxidant activity by the DPPH

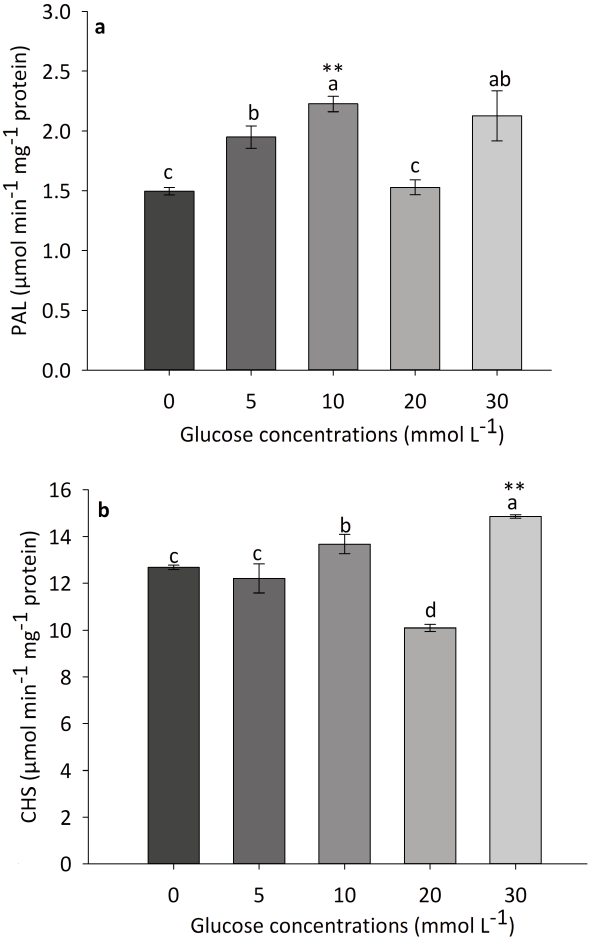


Fig. 7 - Specific activity of phenylalanine ammonia lyase (PAL) (a), chalcone synthase (CHS) (b) in beet plants treated with different glucose concentrations. **= significant at a probability level of p≤0.01 using the Tukey's test at 5% probability. Bars represent standard error of the mean; *n* = 4.

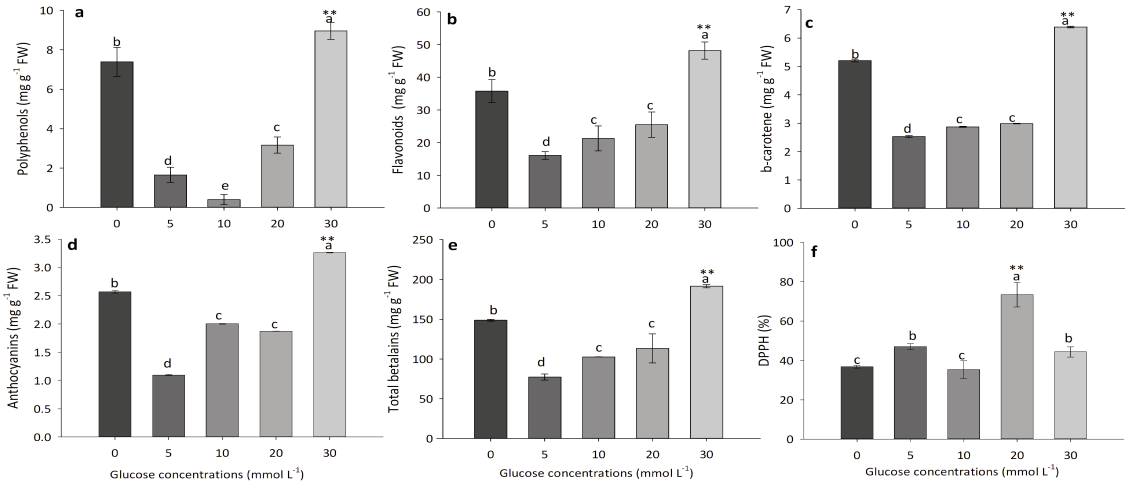


Fig. 8 - Polyphenols (a), flavonoids (b), β-carotene (c), anthocyanins (d), total betalains (e), and DPPH (f) concentrations in beet plants treated with different glucose concentrations. **: significant at a probability level of $p \leq 0.01$ using the Tukey's test at 5% probability. Bars represent standard error of the mean; n = 4. FW = fresh weight.

method compared to the control plants (Fig. 8 f).

For growth analysis, plants treated with 20 mmol $L^{-1}$ of Glc showed increases in leaf fresh weight by 113.2% compared to the control (Fig. 9 a). Root fresh weight increased by 45.9% in plants treated with 10 mmol $L^{-1}$ compared to the control (Fig. 9 b). Total fresh weight increased by 24.6% and 26.6%, respectively, in plants treated with 5 and 20 mmol $L^{-1}$

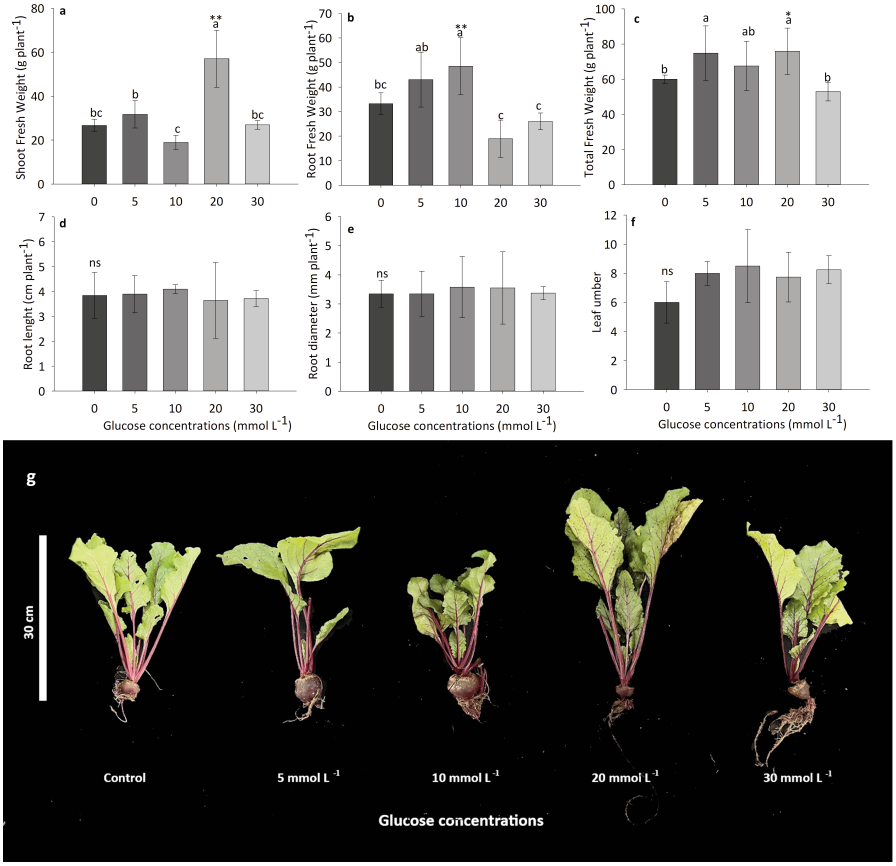


Fig. 9 - Shoot fresh weight (a), root fresh weight (b), total fresh weight (c), root length (d), and root diameter (e), and leaf number (f), and visual growth development (g) in beet plants treated with different glucose concentrations. ** and *= significant at a probability level of $p \leq 0.01$ and $p \leq 0.05$; NS= not significant using the Tukey's test at 5% probability. Bars represent standard error of the mean; n = 4.

of Glc compared to the control (Fig. 9 c). However, root length, root diameter, and number of leaves did not show significant responses to glucose application (Fig. 9 d-f).

PCA was conducted to evaluate the relationship between morpho-physiological traits and Glc treatments (Fig. 10). The variables were depicted along two principal axes, which together explained 57.81% of the total variance (PC1: 32.18%; PC2: 25.63%). This analysis indicated a positive correlation between biometric characteristics and the 20 mmol $L^{-1}$ concentration. In contrast, physiological and biochemical parameters were associated with the 5, 10, and 30 mmol $L^{-1}$ concentrations.

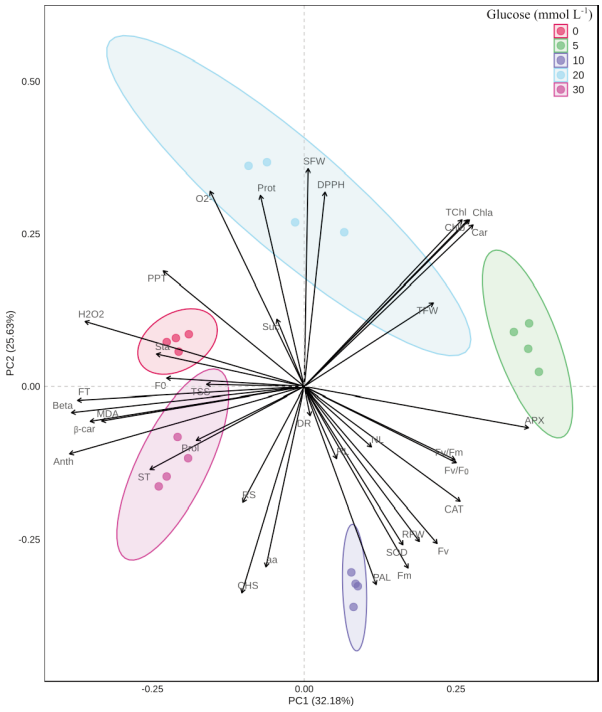


Fig. 1 - PCA of the analyzed parameters from beet plants treated with different glucose concentrations. Minimum fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv), maximum photochemical efficiency (Fv/Fm), variable fluorescence/initial fluorescence (Fv/F0), chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (TChl), carotenoid (CAR), malondialdehyde (MDA), hydrogen peroxide (H2O2), superoxide radical (O2-), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), proline (Pro), amino acids (aa), total sugar (ST), reducing sugar (RS), sucrose (SUC), starch (STA), phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), total flavonoids (FT), total polyphenols (PPT), anthocyanin (Anth), β-carotene (β-car), 2,2-diphenyl-1-picrylhydrazyl (DPPH), total soluble solids (TSS), protein (Prot), root size (SR), root diameter (RD), number of leaves (NL), shoot fresh weight (SFW), root fresh weight (RFW), and total fresh weight (TFW).

## 4. Discussion and Conclusions

In the present study, the data show that chlorophyll fluorescence differed among treatments (Fig. 2). However, chlorophyll fluorescence analysis has been widely used to assess photosynthetic efficiency in plants, as this technique is sensitive to changes in the environment and plant metabolism (Jiang *et al.,* 2012). Based on the results found, Glc can act as an energy substrate for plant metabolic processes, particularly for photosynthesis (Sinha and Roitsch, 2001). With increased Glc availability in the plant, there was consequently an increase in photosynthetic rate, thereby enhancing energy production and improving light absorption efficiency, resulting in higher chlorophyll fluorescence (Wang *et al.,* 2019).

The use of Glc showed positive responses in several crops, such as promoting germination and vegetative growth, inducing greater plant yields (González-Hernández *et al.,* 2020; Gorni and Polimeno, 2023). Although Glc is a byproduct of photosynthesis, its exogenous application can stimulate the synthesis of photosynthetic pigments (Siddiqui *et al.,* 2020), which supports our findings (Fig. 3). Furthermore, studies indicate that elicitation with Glc enhances electron transfer from PSII to PSI, as well as increases ATP and NADPH production, crucial for various biochemical reactions in plants, thereby influencing carbohydrate, lipid, and protein synthesis and regulating biochemical processes (Rineau *et al.,* 2013; Siddiqui *et al.,* 2020). Similar results confirm that Glc application stimulates pigment accumulation in plants, as observed in mustard (*Brassica juncea*) (Sami *et al.,* 2021), triticale (× *Triticosecale Wittmack*) (Wang *et al.,* 2019), and cucumber (*Cucumis sativus*) (Yusuf *et al.,* 2021).

During photosynthesis and other aerobic metabolic processes, plants generate reactive oxygen species (ROS) as part of their normal metabolism and in response to environmental stresses. However, factors such as biotic and abiotic stresses can induce higher ROS production in plants as part of their defense mechanisms, leading to cellular damage and oxidative stress (Das and Roychoudhury, 2014). Our results demonstrated that Glc was effective in reducing ROS accumulation compared to the control (Fig. 4). Considering these findings, Glc elicitation induced an adaptive response in beet plants, increasing the activity of SOD, CAT, and APX (Fig. 5), which correlated with reduced ROS levels,

contributing to a more stable redox balance and fewer ROS-induced cellular damages (Siddiqui *et al.,* 2020). Similar results have shown increased activity of antioxidant enzymes and reduced ROS levels following Glc elicitation in mustard and cucumber plants (Sami *et al.,* 2021; Yusuf *et al.,* 2021).

Glc is a simple sugar that, when applied to plants, can stimulate carbohydrate metabolism, including increased synthesis of other carbohydrates such as sucrose and reducing sugars (Siddiqui *et al.,* 2020). Our results demonstrate that Glc application increased concentrations of total sugars, reducing sugars, sucrose, starch, and proteins in beet plants (Fig. 6). These findings indicate that Glc application was effective, as when absorbed by plants, this substance can be converted into starch as a form of energy storage, resulting in increased availability of carbohydrates and proteins (Siddiqui *et al.,* 2020). Additionally, there were increases in amino acid and proline concentrations (Fig. 6 e-f). The exogenous application of Glc results in a significant increase in amino acid concentration, as Glc serves as a substrate for the synthesis of various compounds, including amino acids. Moreover, exogenous Glc application can induce glutamine synthetase and glutamate dehydrogenase enzymes involved in amino acid synthesis (Forde and Lea, 2007; González-Hernández *et al.,* 2020). Furthermore, Glc can also modulate the enzyme P5CS (pyrroline-5-carboxylate reductase), leading plants to increase proline production as part of their adaptive strategies (Sami and Hayat, 2019).

Glucose application increases PAL and CHS activities in plants, which are responsible for the production of phenolic and flavonoids compounds (Wei *et al.,* 2011). These compounds play crucial roles in defense against biotic and abiotic stresses, flower and fruit pigmentation, and other essential physiological functions for plant development and adaptation to the environment (Baenas *et al.,* 2019). Studies on Glc application in plants have been shown to stimulate the expression of genes encoding enzymes, particularly plant defense enzymes (PAL, CHS, CHI), thereby inducing increases in the concentration of secondary metabolites. This approach is considered more efficient for enhancing the production of desired secondary metabolites and for manipulating biochemical and metabolic pathways (Guo *et al.,* 2011; Zahid *et al.,* 2018). Our results demonstrate that Glc application induced increases in PAL and CHS activity (Fig. 7), as well as an increase in secondary compounds at a concentration of 30 mmol $L^{-1}$ (Fig. 8a-g), resulting in enhanced plant antioxidant activity (Fig. 8h). These compounds have garnered significant interest due to their positive impacts on various fundamental cellular processes, in addition to possessing antioxidant, anti-inflammatory, anticancer, among others (Chen, 2016). However, Glc application stimulated the increase of these compounds, making these plants more valuable for human consumption due to their rich source of bioactive nutrients, promoting a healthy and balanced diet.

The use of Glc can induce improvements in vegetative growth and yield in plants (Sami and Hayat, 2019; Sami *et al.,* 2021). In the present study, the beneficial role of Glc was confirmed due to the increase in fresh shoots, roots, and total weight (Fig. 9 a-c). Exogenous application of Glc promotes plant growth by acting as an energy source for cells, thereby significantly enhancing metabolic activity and positively regulating the expression of genes related to photosynthesis and hormonal signaling pathways (Mao *et al.,* 2018). These effects positively influence carbohydrate metabolism, stimulating the synthesis of reserves such as starch and sucrose, which translates into enhanced plant growth (Wang *et al.,* 2019; Gorni and Polimeno, 2023).

The results of this study show that the application of Glc provides significant improvements in the growth, development and productivity of beetroot, in addition to favoring the accumulation of bioactive compounds. These findings indicate that Glc not only serves as an energy source, but also acts as a stimulator of the plant's physiological responses, increasing its resistance to environmental stresses. The incorporation of Glc into agricultural management practices may therefore represent an effective strategy to optimize beet production, contributing to the nutritional quality and sustainability of crops. Future research may deepen the understanding of the mechanisms underlying these responses, expanding the potential of Glc in agricultural systems. This study underscores the importance of exogenous Glc application not only as an essential nutrient but also as a key modulator of biochemical processes that control plant development. It can thereby optimize agricultural management and crop yield.

Glucose application had a positive impact on the development of beets, stimulating different processes according to the concentration used. At 5

and 10 mmol $L^{-1}$, there was greater plant growth, increased chlorophyll fluorescence and activation of antioxidant enzymes. Higher concentrations (30 mmol $L^{-1}$) favored the production of pigments and bioactive compounds. These results indicate that Glc can act as a metabolic signal, helping the plant grow better and strengthen its defense mechanisms

Although the results of this study are promising, some limitations should be considered. The experiment was carried out in a greenhouse. To advance this line of research, future studies should test the application of glucose under field conditions and evaluate how different environmental factors affect its benefits for beet. Another important factor is the specific stage of the plant at which glucose application is more efficient, in addition to better exploration of the molecular processes involved in this response. These investigations can contribute to making this strategy even more efficient and applicable to real farming systems.

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