Colegio de Postgraduados

Biostimulant Effects of Glycine Betaine in Strawberry Exposed to Salinity Stress

Parra-Robles, Brenda Estefania[1]; Trejo-Téllez, Libia Iris[1,2]; Fernández-Pavía, Yolanda Leticia[2]; Rebollar-Alviter, Ángel[3]; Buendía-Valverde, María de la Luz[2]; Gómez-Merino, Fernando Carlos[1*]

[1] Colegio de Postgraduados Campus Montecillo. Department of Plant Physiology. Carretera Federal México-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264. Mexico.
[2] Colegio de Postgraduados Campus Montecillo. Department of Soil Science. Laboratory of Plant Nutrition. Carretera México-Texcoco km 36.5, Montecillo, Texcoco, State of Mexico, Mexico. C. P. 56264. Mexico.
[3] Universidad Autónoma Chapingo. Centro Regional Universitario del Centro Occidente (CRUCO). Av. Río Grande No. 22, Morelia, Michoacán. C. P. 58195. Mexico.
* Correspondence: fernandg@colpos.mx

ABSTRACT

Objective: To evaluate the biostimulant effects of glycine betaine (GB) in strawberry plants subjected to salt stress.

Design/methodology/approach: A 2×3 factorial experiment was carried out with a completely randomized experimental design where the effects of foliar application of GB (0, 10, 20 mM) were evaluated in strawberry plants exposed to salt stress induced by NaCl (0 and 50 mM). Plant sampling was done 148 days after the establishment of the experiment. The biochemical and nutritional variables were measured. Analysis of variance was performed on the data obtained and the means were compared using the Tukey test ($p \leq 0.05$).

Results: GB application increased chlorophyll concentration, reduced sugar and proline concentration, and increased P uptake. The 20 mM GB dose maintained the K^+/Na^+ ratio under salt stress.

Study limitations/implications: The reproductive phase was not considered in this study; therefore, the effects of GB on fruit yield and quality are not assessed.

Findings/conclusions: Foliar application of GB in strawberry plants subjected to salinity stress is concluded to improve adaptive responses of a biochemical nature by promoting chlorophyll biosynthesis and nutrient concentration.

Keywords: Biostimulation, osmotic stress, strawberry, quality, resistance to abiotic stress.

Citation: Parra-Robles, B. E., Trejo-Téllez, L. I., Fernández-Pavía, Y. L., Rebollar-Alviter, Á., Buendía-Valverde, M. de la L., & Gómez-Merino, F.C. (2024). Biostimulant Effects of Glycine Betaine in Strawberry Exposed to Salinity Stress. *Agro Productividad*. https://doi.org/ 10.32854/agrop.v17i9.3050

Academic Editor: Jorge Cadena Iñiguez
Guest Editor: Juan Franciso Aguirre Medina

Received: August 25, 2024.
Accepted: September 13, 2024.
Published on-line: October 4, 2024.

Agro Productividad, 17(9). September. 2024. pp: 3-9.

INTRODUCTION

Biostimulation is a technology that improves agronomic attributes related to crop productivity and quality; it also provides plants with better mechanisms to face environmental adversities of an abiotic nature, including drought and salinity stress. Glycine betaine (GB) has potential biostimulant effects on various horticultural crops (Adak, 2019). This non-ionic quaternary amine is synthesized in some plants as an adaptive response to stress situations, producing an osmoprotective effect at the cellular level, which is crucial for the protection of cellular structures and the regulation of vital biological processes (Ali *et al.*,

2020). In particular, GB prevents protein denaturation and changes in lipid fluidity, which allows maintaining the structural integrity of the membrane and ensuring its selective permeability (Adak, 2019; Zulfiqar *et al.*, 2022). GB can also enhance the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), as well as stabilize DNA and histones (Liu *et al.*, 2011). It also allows maintaining the functionality of some components of the photosynthetic apparatus in chloroplasts by protecting protein and lipid complexes, thus allowing greater photosynthetic efficiency even under adverse conditions (Sarikhani *et al.*, 2021; Zulfiqar *et al.*, 2022), and intervenes in cell signaling processes mediated by protein kinases that regulate the expression of abiotic stress response genes (Adak, 2019; Zulfiqar *et al.*, 2022).

The use of biostimulants such as GB contributes to sustainable agricultural production in restrictive environments aggravated by the effects of global climate change. Soil and water salinity is an increasingly worrying problem in agriculture, which can be exacerbates by excessive evaporation caused by extreme heat resulting from global climate change (Atta *et al.*, 2023).

With nearly 600 thousand tons of strawberries produced per year, Mexico is ranked as the country with the fourth highest production of this fruit, only after China, the United States, and Egypt (WPR, 2024). In Mexico, the main strawberry producing states are Michoacán, Guanajuato, Baja California, State of Mexico, and Baja California Sur (SIAP, 2021). Given the economic importance of this crop and the growing salinization problem affecting groundwater and soils in our country, there is a need to generate updated and detailed information on the application of biostimulants and the mechanisms they promote in plants to improve tolerance to osmotic stress and help maintain and even improve crop productivity and quality. The objective of this research was to evaluate the effect of foliar application of GB on strawberry plants subjected to salinity stress, in terms of biochemical and nutritional indicators.

MATERIALS AND METHODS

Location and experimental conditions

The experiment was carried out in a greenhouse in the Plant Nutrition area of the Graduate Program in Soil Science of the Colegio de Postgraduados Campus Montecillo, which is located at 19° 29' North latitude, 98° 53' West longitude, at 2250 m.a.s.l. The average temperature during the night was 12 °C, and 27 °C during the day. The relative humidity was 66.95 % and the light intensity during the day was 205 W m^{-2}.

Strawberry plants (*Fragaria×ananassa* Duch.) cv. Aromas were used to perform our study. This cultivar is day-neutral and shows better resistance to diseases (Muramoto, 2003; León-López *et al.*, 2014). The plants were established in 30×30 cm black nursery bags with red tezontle as substrate and watered by a stake-drip system. Irrigation was programmed in six 2-min intervals throughout the day, supplying 30 mL per plant.

Treatment design and experimental design

A 2×3 factorial experiment was conducted with a completely randomized experimental design. The study factors and levels were: A) Foliar glycine betaine (GB) at 0, 10, and

20 mM; and B) Salinity, induced by sodium chloride (NaCl) in the nutrient solution, at 0 and 50 mM.

Preparation and application of glycine betaine (GB)

Glycine betaine (Sigma-Aldrich; Toluca, Mexico) was used, with a betaine concentration of 98% and molecular weight=117.15 g mol^{-1}. GB was dissolved in distilled water with 0.1% Tween® 20 as surfactant; the pH was adjusted to 5.5 using 0.1 N NaOH. GB was applied in two foliar sprays, the first 20 d after the NaCl treatments began and the last 20 d before the end of the experiment.

Preparation and application of the nutrient solution plus NaCl

Fifteen days after transplantation, irrigation was started with Steiner nutrient solution at a 50% macronutrient concentration. One month after transplantation, the macronutrient concentration of the Steiner solution was increased to 100% and NaCl application was started to induce osmotic stress due to salinity. Throughout the experiment, the pH of the nutrient solutions was adjusted to 5.5; the electrical conductivity (EC) was 2.0 dS m^{-1} in the nutrient solution, and 5.5 dS m^{-1} in the solution with 50 mM NaCl. The nutrient solutions described were supplemented with the micronutrient concentrations in mg L^{-1}, as follows: 4.99 Fe, 2.33 Mn, 0.47 Zn, 0.43 B, 0.19 Cu, 0.17 Mo.

Variables evaluated

Chlorophyll concentration. Chlorophyll *a*, *b*, and total concentrations were determined using the modified Harborne method (1973). Absorbance was measured at 470, 652, and 665 nm in a spectrophotometer (BioTek®, Gen5™; Vermont, WI, USA), with 100% methanol used as a blank.

Proline quantification. The methodology of Bates *et al.* (1973) was used with modifications. Readings were performed in a spectrophotometer (VELAB VE-5600UV PC; Mexico City, Mexico) at 520 nm absorbance. Toluene was used as a blank and a calibration curve with a proline concentration of 400 nM mL^{-1} as a base.

Total soluble sugars. These were determined by the method described by Southgate (1976). The samples were read at an absorbance of 600 nm, in a spectrophotometer (BioTek®, Gen5™; Vermont, WI, USA). A 1 μg L^{-1} sucrose stock solution was used in the calibration curve.

Concentrations of N, P, K, and Na. They were quantified in leaves by wet digestion of the dry material with a mixture of HNO_3:$HClO_4$ (Alcántar and Sandoval, 1999). The extracts were read in an inductively coupled plasma optical emission spectrometer instrument (Agilent ICP-OES 725-OES; Santa Clara, CA, USA). N was determined using the micro-Kjeldahl methodology (Bremner, 1965).

Statistical analysis

An analysis of variance was performed to assess the main and interaction effects of the study factors, as well as tests for comparing means (Tukey, $p \leq 0.05$). SAS software was used in both analyses.

RESULTS

Chlorophyll concentration

Chlorophyll a concentration was affected by NaCl and GB (Figure 1), but not by their interaction (data not shown).

Salt stress reduced chlorophyll *a* concentration by 9.6% (Figure 1A), while foliar application of 20 mM GB slightly increased this variable, with no differences from the control (Figure 1B). Chlorophyll *a* absorbs most of the light used in the photosystem II (Roca *et al.*, 2016).

The main effects of the study factors and their interaction were not significant for chlorophyll *b* and total. The chlorophyll *b* concentration values ranged between 0.238 and 0.267 mg g^{-1} of fresh matter, while those of total chlorophyll ranged from 0.642 to 0.793 mg g^{-1} of fresh matter. Chlorophyll *b* is an accessory pigment that complements the absorption of light at short wavelengths, that chlorophyll *a* cannot absorb, and transfers the energy produced (Nobel, 2009; Guidi *et al.*, 2017). The maintenance of the concentrations of this pigment in the treatments with and without salinity denotes the protective function of GB in the protein and lipid complexes of the photosystems (Chen and Murata, 2008).

Sugar concentration

The concentration of total soluble sugars was only affected by the main effects of the study factors (Figure 2). Salinity increased the concentration of sugars by 37.4% (Figure 2A). The increase in reducing sugars (sucrose, fructose) represents a strategy for plant tolerance to stress, since they provide protection to cellular, membrane, and protein structures, and also function as an activator of signaling pathways that generate signals that modify gene expression (Singh *et al.*, 2022). On the contrary, the addition of 10 and 20 mM GB reduced this variable by 33.8 and 49.8 %, respectively (Figure 2B). Under salinity conditions, the application of GB caused the highest values of total soluble sugars, compared to the conditions of absence of salinity (data not shown). However, the differences were not significant.

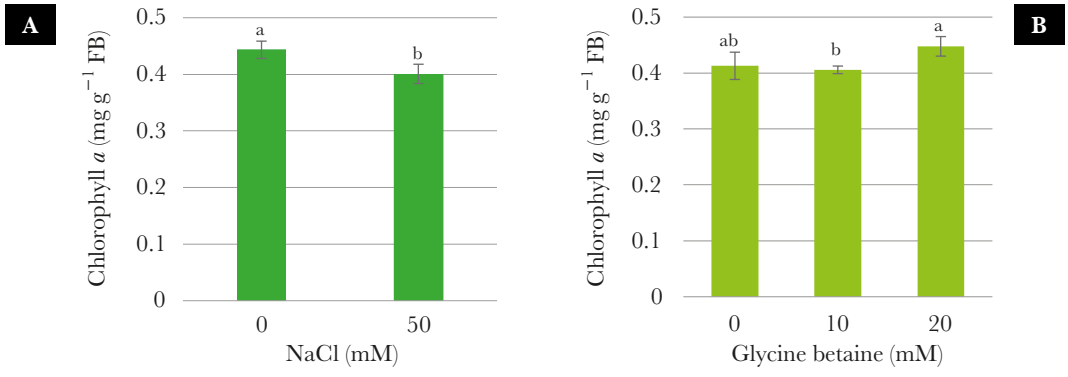

Figure 1. Effect of sodium chloride (A) and glycine betaine (B) on leaf chlorophyll *a* concentration in strawberry plants cv. Aromas. Means ± SD with different letters in each subfigure indicate significant statistical differences (Tukey, $p \leq 0.05$). FB: Fresh biomass.

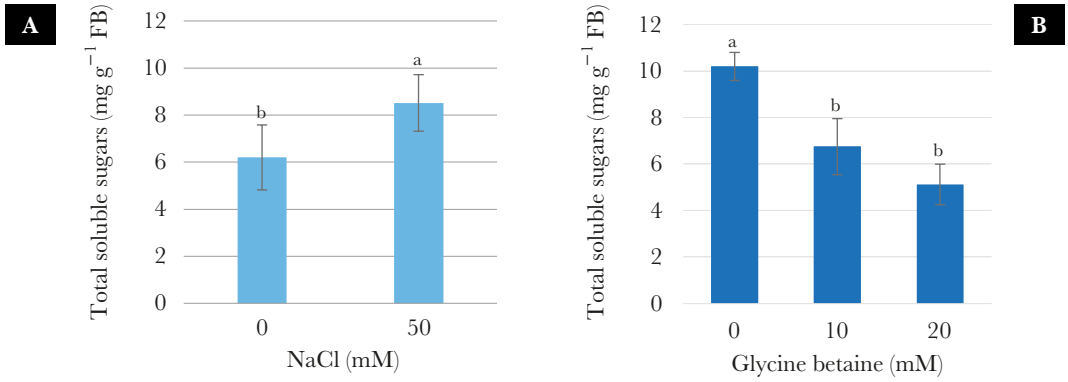

Figure 2. Effect of sodium chloride (A) and glycine betaine (B) on the leaf concentration of total soluble sugars in strawberry plants cv. Aromas. Means ± SD with different letters in each subfigure indicate significant statistical differences (Tukey, $p \leq 0.05$). FB: Fresh biomass.

Proline

The proline concentration was only affected by the NaCl factor, where the addition of a 50 mM dose increased it by 56.5% (Figure 3).

Proline is an osmoprotectant; its synthesis can be stimulated under stress conditions. In strawberry cv. Fern (day neutral) proline concentrations of 16.57 to 33.0 μM g^{-1} are reported and in cv. Camarosa (short day) from 45.0 to 95.5 μM g^{-1} with electrical conductivity levels of 2.0 and 5.0 dS m^{-1}, respectively (Pirlak *et al.*, 2004).

Concentrations of N, P, K, and Na

The leaf N concentrations obtained in this study (15.05-18.90 g kg^{-1} of dry matter), as well as those of P (2.84 to 4.96 g kg^{-1} of dry matter), shown in Table 1, are consistent with those reported by Muramoto (2003). Aguilar-Tlatelpa *et al.* (2019) report leaf K concentrations in the range of 13.3 to 24.3 % for the cultivars Albion, Festival, Jacona, and Zamorana; the values obtained here in the cv. Aromas are much lower (Table 1).

In the absence of salt stress, GB increased leaf N concentration. The addition of 20 mM GB with 50 mM NaCl increased P concentration by 73.8% compared to the treatment without NaCl and without GB (Table 1). N is an important constituent of biomolecules

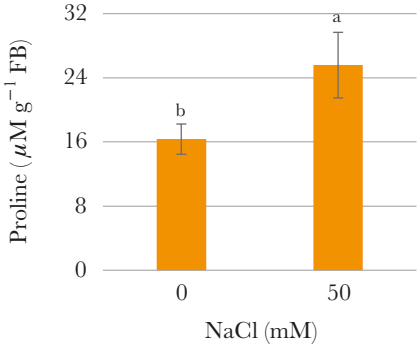

Figure 3. Effect of sodium chloride on leaf proline concentration in strawberry plants cv. Aromas. Means ± SD with different letters in each subfigure indicate significant statistical differences (Tukey, $p \leq 0.05$). FB: Fresh biomass.

Table 1. Effect of glycine betaine (GB) application on the concentration of N, P, K, and Na on leaves of strawberry plants cv. Aromas exposed to salt stress induced by sodium chloride (NaCl).

NaCl (mM)	Glycine betaine (mM)	N	P	K	Na
		g kg^{-1} DB			mg kg^{-1} DB
0	0	15.05±0.67 b	2.84±0.01 d	6.14±0.33 a	597.08±12.33 c
	10	18.90±0.49 a	4.34±0.08 bc	6.13±0.25 a	384.33±9.95 d
	20	17.50±0.48 a	4.43±0.02 b	5.89±0.15 a	316.15±12.23 e
50	0	18.73±0.34 a	4.49±0.02 b	6.04±0.17 a	592.18±8.29 c
	10	18.38±0.21 a	4.18±0.05 c	5.60±0.24 a	1250.78±10.04 a
	20	17.15±0.60 ab	4.96±0.04 a	6.47±0.20 a	664.03±23.96 b

Means ± SD with different letters in each column indicate significant statistical differences (Tukey, $p \leq 0.05$). DB: Dry biomass.

such as chlorophyll, proteins, and nucleic acids, while P is important for energy generation and is involved in the whole cellular metabolism (Alcántar-González *et al.*, 2016).

The leaf concentration of K was not affected by the interaction of the study factors; however, the concentration ratios of this element with respect to Na were not modified in the 50 mM NaCl-20 mM GB treatment with respect to the treatment without NaCl and without GB (Table 1).

Na is not a nutrient in plants, and can be considered a beneficial element. At high concentrations, Na can alter the balance of nutrients in the soil, by affecting the absorption of essential elements such as Ca, K, and Mg. When Na reaches toxic levels for the plant, irreversible damage and death of cells, tissues, and organisms can occur (Lamz *et al.*, 2013; Tarolli *et al.*, 2024).

GB can promote K uptake and reduce Na uptake by stimulating antioxidant activity and the functionality of enzymes such as SOD, CAT, and POD, which are key in ROS detoxification (Ali *et al.*, 2020; Yan *et al.*, 2020). These results were only evident with foliar supply of 20 mM GB, which decreased Na in the presence of salt stress. Although GB did not increase K concentration, it did help maintain stable K levels in plants and thus the K^+/Na^+ ratio.

CONCLUSION

GB increased chlorophyll *a* concentration at 20 mM in plants under salt stress and reduced the concentration of sugars and proline, which appears to be a secondary effect of the decrease in ROS; in addition, it increased N and P uptake, and decreased Na concentration at 20 mM.

REFERENCES

Adak, N. (2019). Effects of glycine betaine concentrations on the agronomic characteristics of strawberry grown under deficit irrigation conditions. *Applied Ecology and Environmental Research, 17*(2):3753-3767.

Aguilar-Tlatelpa, M., Volke-Haller, V. H., Sánchez-García, P., Pérez-Grajales, M., & Fajardo-Franco, M. L. (2019). Concentración y extracción de macronutrimentos en cuatro variedades de fresa. *Revista Mexicana de Ciencias Agrícolas, 10*(6):1287-1299. https://doi.org/10.29312/remexca.v10i6.1552

Alcántar-González, G., & Sandoval-Villa, M. (1999). Manual de análisis químico y de tejido vegetal. Sociedad Mexicana de la Ciencia del Suelo. Publicación especial No. 10. Chapingo, México. 156 p.

Alcántar-González, G., Trejo-Téllez, L. I., & Gómez-Merino, F. C. (2016). Nutrición de cultivos. Segunda edición. Editorial Colegio de Postgraduados Montecillo, Texcoco, Estado de México C. P. 56230. 443 p.

Ali, S., Abbas, Z., Seleiman, M. F., Rizwan, M., YavaŞ, İ., Alhammad, B. A., & Kalderis, D. (2020). Glycine betaine accumulation, significance and interests for heavy metal tolerance in plants. *Plants, 9*(7):896. https://doi.org/10.3390/plants9070896

Atta, K., Shah, M. H., Kundu, S., Ajaharuddin, S. M., Mondal, S., Pal, A., Hossain, A., Pramanik, K., Hembram, A., Ali, M. S., & Pande, C. B. (2023). Potential impacts of climate change on the sustainability of crop production: A case in India. In: Climate Change Impacts in India, Pande, C. B., Moharir, K. N., Negm, A., Eds. Springer International Publishing: Cham, Switzerland. pp. 265-295. https://doi.org/10.1007/978-3-031-42056-6_12

Bates, L., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, 39:205-207. https://doi.org/10.1007/BF00018060

Bremner, J. M. (1965). Organic nitrogen in soils. In: Soil nitrogen, Bartholomew, W. V., Clark, F. E.. Eds., The American Society of Agronomy, Inc.: Madison, WI, USA, Vol. 10, pp. 93-149. https://doi.org/10.2134/agronmonogr10.c3

Chen, T. H., & Murata, N. (2008). Glycine betaine: an effective protectant against abiotic stress in plants. *Trends in Plant Science, 13*(9):499-505. https://doi.org/10.1016/j.tplants.2008.06.007

Guidi, L., Tattini, M., & Landi, M. (2017). How does chloroplast protect chlorophyll against excessive light. In: Chlorophyll, Jacob-Lopez, E., Zepka, L. Q., & Queiroz, M. I., Eds., IntechOpen: Rijeka, Croatia, pp. 21-36. https://doi.org/10.1007/s42994-024-00164-6

Harborne, J. B. (1973). Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall Ltd, London. 279 p.

Lamz-Piedra, A., & González-Cepero, M. C. (2013). La salinidad como problema en la agricultura: la mejora vegetal una solución inmediata. *Cultivos Tropicales, 34*(4):31-42. http://ref.scielo.org/6rykhx

León-López, L., Guzmán-Ortíz, D. L. A., García Berumen, J. A., Chávez Marmolejo, C. G., & Peña-Cabriales, J. J. (2014). Consideraciones para mejorar la competitividad de la región" El Bajío" en la producción nacional de fresa. *Revista Mexicana de Ciencias Agrícolas, 5*(4):673-686. https://doi.org/10.23913/ride.v8i16.371

Liu, J., Wisniewski, M., Droby, S., Vero, S., Tian, S., & Hershkovitz, V. (2011). Glycine betaine improves oxidative stress tolerance and biocontrol efficacy of the antagonistic yeast Cystofilobasidium infirmominiatum. *International Journal of Food Microbiology, 146*(1):76-83. https://doi.org/10.1016/j.ijfoodmicro.2011.02.007

Muramoto, J. (2003). Nutrient analysis of organic strawberries: effect of cultivars and mycorrhizal inoculation. Organic Farming Research Foundation. Santa Cruz, CA, USA. 22 p. https://grants.ofrf.org/system/files/outcomes/muramoto_01f16.pdf

Nobel, H. P. (2009). Physicochemical and Environmental Plant Physiology, Fourth edition, Academic Press: Amsterdam, Netherlands, 582 p.

Pirlak, L., & Eşitken, A. (2004). Effects of salinity on growth, proline and ion accumulation in strawberry plants. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science, 54*(3):189-192. https://doi.org/10.1080/09064710400302 49

Roca, M., Chen, K., & Pérez-Gálvez, A. (2016). Chlorophylls. In: Handbook on natural pigments in food and beverages, Carle R., & Schweiggert, R. M., Eds., Science Direct: Amsterdam, Netherlands, pp. 125-158. https://doi.org/10.1016/B978-0-08-100371-8.00006-3

Sarikhani, H., & Safariyan-Nejad, M. S. (2021). Improving of Winter Cold Hardiness by Glycine Betaine in Strawberry. *International Journal of Horticultural Science and Technology, 8*(4):401-413. https://doi.org/10.22059/ijhst.2020.298478.348

SIAP (Servicio de Información Agroalimentaria y Pesquera). (2021). Datos abiertos Fresa. http://infosiap.siap.gob.mx/gobmx/datosAbiertos.php

Singh, P., Choudhary, K. K., Chaudhary, N., Gupta, S., Sahu, M., Tejaswini, B., & Sarkar, S. (2022). Salt stress resilience in plants mediated through osmolyte accumulation and its crosstalk mechanism with phytohormones. *Frontiers in Plant Science*, 13:1006617. https://doi.org/10.3389/fpls.2022.1006617

Southgate, D. (1976). Determination of food carbohydrates. Applied Science Publications Ltd.: London, United Kingdom: 178 p.

Tarolli, P. J. (2024). Soil salinization in agriculture: Mitigation and adaptation strategies combining nature-based solutions and bioengineering. *iScience, 27*(2). https://doi.org/10.1016/j.isci.2024.108830

WPR (World Population Review). (2024). Strawberry Production by Country 2024. https://worldpopulationreview.com/country-rankings/strawberry-production-by-country

Yan, G., Fan, X., Peng, M., Yin, C., Xiao, Z., & Liang, Y. (2020). Silicon improves rice salinity resistance by alleviating ionic toxicity and osmotic constraint in an organ-specific pattern. *Frontiers in Plant Science*, 11:260. https://doi.org/10.3389/fpls.2020.00260

Zulfiqar, F., Ashraf, M., & Siddique, K. H. (2022). Role of glycine betaine in the thermotolerance of plants. *Agronomy, 12*(2):276. https://doi.org/10.3390/agronomy12020276