Stimulation Effects of Glutamic and 5-Aminolevulinic Acids On Photosynthetic Pigments, Physio-biochemical Constituents, Antioxidant Activity, and Yield of Peanut

Ibrahim Mohamed El-Metwally 1 · Mervat Shamoon Sadak 1 · Hani Saber Saudy 2

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Abstract
Soil not only represents the main supporter for root growth, but also is the supplier of water and nutrients. However, several soils, i.e. sandy soils, do not adequately fulfill the plant growth requirements of the environmental resources. Therefore, it is necessary to compensate, even partially, the lack of these required resources for better plant growth and development. Amino acids could introduce a substantial solution in this respect. Therefore, two field experiments under field conditions were carried out to investigate the effect of glutamic (GLA) and 5-aminolevulinic (ALA) acids on photosynthesis pigments, oxidative defense indicators as well as yield and seed quality of peanut. Three concentrations of glutamic acid (10, 20 and 40 mg L⁻¹, denoted GLA10, GLA20, and GLA40, respectively) and three concentrations of 5-aminolevulinic acid, (10, 20 and 40 mg L⁻¹, abbreviated to ALA10, ALA20, and ALA40, respectively), in addition to a check treatment (tap water) were applied. Treatments were arranged in a randomized complete block design with three replicates. Findings exhibited potentiality of GLA20 treatment for recording the highest values of chlorophyll a, chlorophyll b, chlorophyll a/b, carotenoids and total pigments compared to the other treatments. The increases in indole acetic acid, phenolics and free amino acids were 68.1, 58.9 and 19.6% as well as 64.6, 51.2 and 17.7%, due to application of GLA20 and ALA20, respectively. Substantial improvements in pod yield ha⁻¹, oil %, flavonoids and antioxidant activity were obtained with GLA20 or ALA20. In conclusion, since glutamic or 5-aminolevulinic acids at concentration of 20 mg L⁻¹ showed promotive effect on physiological and biochemical status of peanut, such amino acids should be adopted as a promising practice in peanut cultivations.

Keywords Antioxidants · Glutamate · Hormonal precursors · Oilseeds · Plant pigments · Reactive oxygen species

Hani Saber Saudy
hani_saudy@agr.asu.edu.eg

1 Botany Department, Agricultural and Biological Research Institute, National Research Centre, El-Behos St. Dokki, P.O. Box 12622-Dokki, Cairo, Egypt

2 Agronomy Department, Faculty of Agriculture, Ain Shams University, P.O. Box 68-Hadayek Shoubra, 11241 Cairo, Egypt
Stimulierende Wirkung von Glutaminsäure und 5-Aminolävulinsäure auf photosynthetische Pigmente, physio-biochemische Bestandteile, antioxidative Aktivität und Ertrag von Erdnüssen

Zusammenfassung
Der Boden ist nicht nur der Hauptträger des Wurzelwachstums, sondern auch der Lieferant von Wasser und Nährstoffen. Einige Böden, z. B. Sandböden, erfüllen jedoch die Anforderungen des Pflanzenwachstums an die Umweltressourcen nicht in ausreichendem Maße. Daher ist es notwendig, den Mangel an diesen erforderlichen Ressourcen für ein besseres Pflanzenwachstum und eine bessere Entwicklung auszugleichen – auch nur teilweise. Aminosäuren könnten in dieser Hinsicht eine Lösung darstellen. Daher wurden zwei Feldversuche unter Freilandbedingungen durchgeführt, um die Wirkung von Glutaminsäure (GLA) und 5-Aminolävulinsäure (ALA) auf die Photosynthesepigmente, die Indikatoren für den oxidativen Schutz sowie den Ertrag und die Samenqualität von Erdnüssen zu untersuchen. Drei Konzentrationen von Glutaminsäure (10, 20 und 40 mg L⁻¹, bezeichnet als GLA10, GLA20 bzw. GLA40) und drei Konzentrationen von 5-Aminolävulinsäure (10, 20 und 40 mg L⁻¹, abgekürzt als ALA10, ALA20 bzw. ALA40) wurden zusätzlich zu einer Kontrollbehandlung (Leitungswasser) angewendet. Die Behandlungen wurden in einem randomisierten vollständigen Blockversuch mit drei Wiederholungen angeordnet. Die Ergebnisse zeigten, dass die GLA20-Behandlung im Vergleich zu den anderen Behandlungen die höchsten Werte für Chlorophyll a, Chlorophyll b, Chlorophyll a/b, Carotinoide und Gesamtpigmente aufwies. Wiederholungen angeordnet. Die Ergebnisse zeigten, dass die GLA20-Behandlung im Vergleich zu den anderen Behandlungen die höchsten Werte für Chlorophyll a, Chlorophyll b, Chlorophyll a/b, Carotinoide und Gesamtpigmente aufwies. Der Anstieg der Indolessigsäure, der Phenole und der freien Aminosäuren betrug 68,1, 58,9 und 19,6 % bzw. 64,6, 51,2 und lungen die höchsten Werte für Chlorophyll a, Chlorophyll b, Chlorophyll a/b, Carotinoide und Gesamtpigmente aufwies. Die Behandlungen wurden in einem randomisierten vollständigen Blockversuch mit drei Wiederholungen angeordnet. Die Ergebnisse zeigten, dass die GLA20-Behandlung im Vergleich zu den anderen Behandlungen die höchsten Werte für Chlorophyll a, Chlorophyll b, Chlorophyll a/b, Carotinoide und Gesamtpigmente aufwies. Der Anstieg der Indolessigsäure, der Phenole und der freien Aminosäuren betrug 68,1, 58,9 und 19,6 % bzw. 64,6, 51,2 und 17,7 % durch die Anwendung von GLA20 und ALA20. Mit GLA20 bzw. ALA20 wurden erhebliche Verbesserungen des Schotenertrags pro ha, des Ölanteils, der Flavonoide und der antioxidativen Aktivität erzielt. Da Glutaminsäure oder 5-Aminolävulinsäure in einer Konzentration von 20 mg L⁻¹ eine positive Wirkung auf den physiologischen und biochemischen Status der Erdnuss zeigten, sollten diese Aminosäuren im Erdnussanbau eingesetzt werden.

Schlüsselwörter Antioxidantien · Glutamat · Hormonelle Vorstufen · Ölsaaten · Pflanzenpigmente · Reaktive Sauerstoffspezies

Introduction
Under natural conditions, many crops are vulnerable to a variety of stresses, such as drought, salinity, heavy metals, and disease (Shahid et al. 2017; Saudy and El-Metwally 2019; Abd–Elrahman et al. 2022). Crops cultivated in marginal or sandy soils, especially in arid and semi-arid regions (El-Metwally et al. 2022) could subject to unfavorable edaphic/atmospheric conditions, causing yield losses (Saudy et al. 2020a; Mubarak et al. 2021; Salem et al. 2021, 2022). Oilseed crops are the third most significant crops after cereals and legumes (Lafarga 2021). The seeds of oil crops are the main source of edible oils used directly in human nutrition or in several chemical industries. Among the edible oilseeds, peanut (Arachis hypogaea L.) seeds are used as a chief source of vegetable oils all over the world (Krishna et al. 2015). Peanut is an annual crop widely cultivated in equatorial and sub-equatorial climatic zones. Under such conditions crop tolerance should be enhanced to face the probable stressful impacts (El-Metwally et al. 2021; El-Metwally and Saudy 2021; Saudy et al. 2021; El-Bially et al. 2022b).

Amino acids as osmolytes participate in plant stress responses, sharing in regulation of ion transport, the stomatal opening and detoxification mechanisms (Rai 2002). Moreover, amino acids have several significant biological tasks in plant cells involving boosting nutrient uptake, translocation and metabolism, vitamin biosynthesis, growth biostimulation, creating higher tolerance to environmental stresses as well as synthesis and production of aminochelate fertilizers (Sharma and Dietz 2006; Souri and Hatamian 2019, Bakry et al. 2020). Glutamic acid is one of the most important amino acids in plants and has a major role in the biosynthesis of proline and other nitrogen-containing compounds (Okumoto et al. 2016). Several studies have pointed out the positive effect of glutamic acid application on photosynthetic activity and leaf functionality assessed through the chlorophyll fluorescence measurement (Lv et al. 2009; Fabbrin et al. 2013; Röder et al. 2018). Glutamic acid application had a positive effect also under stressful conditions, reducing physiological damage by enhancing the activity of antioxidant enzymes (Lee et al. 2017). Generally, the exogenous application of amino acids has been shown to enhance growth and productivity in many crops (Cao et al. 2010; Amin et al. 2011; Khan et al. 2012; Mohammadipour and Souri 2019a; Fahimi et al. 2016; Saudy et al. 2020b).

5-aminolevulinic acid as a plant growth regulator is responsible for numerous biological activities in plants. Biosynthesis of chlorophyll, and cytochrome is mainly dependent on 5-aminolevulinic acid (Ali et al. 2015; An et al. 2016). Enhanced chlorophyll content could contribute to the increases in leaf net photosynthetic rate by 5-aminolevulinic acid that may promote the light harvesting capacity (Youssef and Awad 2008). 5-aminolevulinic acid enhanced
photosynthesis (Wang et al. 2004, 2018), primary root elongation (An et al. 2019), and plant biomass accumulation (Nunkaew et al. 2014). Moreover, 5-aminolevulinic acid has a substantial role in plant response to abiotic stress (Wu et al. 2019). In this respect, application of 5-aminolevulinic acid enhanced plant tolerance to various abiotic stresses, such as heat stress (Zhang et al. 2012), salinity (Naeem et al. 2012) and water deficit stress (Liu et al. 2011). Glutamic acid is considered a precursor of 5-aminolevulinic acid, in plants, 5-aminolevulinic acid is synthesized from glutamate and appears to be highly regulated; this reaction requires a glutamyl-tRNA intermediate as well as ATP and NADPH cofactors (Beale 1990).

However, how 5-aminolevulinic acid and glutamic acid application and their effective concentration may influence peanut growth and productivity and seed quality are not well documented. Therefore, the objective of this study was to evaluate the physiological and biochemical effects of 5-aminolevulinic acid and glutamic acid on peanut yield and seed quality under sandy soil conditions.

### Material and Methods

#### Trial Site Description

Along two summer growing seasons of 2019 and 2020 (from May to October), the current study was conducted under field conditions at the research station of National Research Centre, El Nubaria District, Egypt (30°31’N, 30°18’E; 21 m above sea level). Soil of the experimental site was sandy and its mechanical and chemical properties (Table 1) were determined according to Jackson (1973). The study location belongs to arid regions with no rainfall and hot dry in summer. The averages of air temperature, wind speed, relative humidity and insolation incident were 29.9 and 30.4 °C, 3.02 and 3.04 m sec⁻¹, 52.7 and 54.6% and 29.1 and 30.3 MJ m⁻² day⁻¹ in 2019 and 2020 seasons, respectively. The preceding cultivated crop was wheat in both seasons.

#### Treatments and Practices

The experiment included three concentrations of glutamic acid, GLA (10, 20 and 40 mg L⁻¹, denoted GLA10, GLA20, and GLA40, respectively) and three concentrations of 5-aminolevulinic acid, ALA (10, 20 and 40 mg L⁻¹, abreviated to ALA10, ALA20, and ALA40, respectively), in addition to a check treatment (tap water). Treatments were arranged in a randomized complete block design with three replicates. At 30 and 45 days after sowing (DAS), the aqueous solutions of each glutamic and 5-aminolevulinic acids were separately sprayed. The spray solution of amino acids was applied by a knapsack sprayer had one nozzle with using 480 L water ha⁻¹ as a solvent/carrier.

Prior to seeding, ordinary single super phosphate at a rate of 350.0 kg ha⁻¹ was broadcasted and incorporated into soil. The experimental unit area was 10.5 m²; including five ridges, 3.5 m length and 0.6 m width. On the 27th and 31st of May in 2019 and 2020, respectively, peanut cultivar Giza-6 seeds (3–4 seeds per hill) were inoculated with the specific Rhizobium strain and immediately sown, 0.25 m apart on both sides of the ridge. At 25 DAS, plants were thinned to one plant per hill, followed by fertilizing 150.0 kg ha⁻¹ of ammonium nitrate (33.5% N). Moreover, plants received 150.0 kg ha⁻¹ of potassium sulphate (48.0% K₂O) at 35 DAS.

### Assessments

#### Physiological Parameters

At 60 DAS, the top leaf of peanut plant was isolated to estimate the physiological parameters. Photosynthetic pigments expressed in chlorophyll a and b and carotenoids contents in fresh plant were estimated using the method of Lichtenthaler and Buschmann (2001). Indole acetic acid (IAA) content was extracted and analyzed by the method of Larsen et al. (1962). Phenolics content was analyzed according to Daniil and George (1972). Free amino acid content was extracted as described by Vartainan et al. (1992). and determined with the ninhydrin reagent method (Yemm and Cocking 1955).

#### Agronomic Traits

At harvest (on 3rd and 5th October in 2019 and 2020, respectively), plants of the central two ridges were collected to estimate pods number and weight plant⁻¹, seed index and pod yield ha⁻¹.

### Table 1  Soil physico-chemical properties of El-Nubaria region

| Particle size distribution (%) | Texture class | Chemical properties |
|--------------------------------|---------------|---------------------|
| Coarse sand | Fine sand | Clay + silt | Organic matter (%) | pH | EC (dS m⁻¹) | CaCO₃ (%) |
| 55.1 | 40.5 | 4.4 | Sandy | 0.35 | 8.6 | 0.32 | 3.55 |

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Biochemical Parameters

In peanut seeds, oil content was determined by using Soxhlet extraction apparatus using petroleum ether as a solvent and then the seed oil percentage was calculated on dry weight basis according to AOAC (2012). Flavonoids contents using aluminum chloride colorimetric assay (Ordoñez et al. 2006) and antioxidant activity (DPPH %) according to Gyamfi et al. (1999) were estimated.

Data Analysis

Prior to analysis of variance (ANOVA), the collected data were subjected to homogeneity test (Levene 1960) and Anderson-Darling normality test (Scholz and Stephens 1987). Since the outputs proved that the homogeneity and normality of the data are satisfied for running further a one-way ANOVA for the data of the two seasons was performed according to Casella (2008), using Costat software program, Version 6.303 (2004). Means separation was performed only when the F-test indicated significant ($P \leq 0.05$) differences among the treatments using Duncan’s multiple range test.

Table 2 Photosynthesis pigments of peanut response to glutamic and 5-aminolevulinic acids application in 2019 and 2020 seasons

| Variable          | Chlorophyll (mg 100 g⁻¹ f wt.) | Carotenoids (mg 100 g⁻¹ f wt.) | Total pigments (mg 100 g⁻¹ f wt.) |
|-------------------|---------------------------------|---------------------------------|-----------------------------------|
|                   | Chlorophyll a                    | Chlorophyll b                   | a/b                               |                                 |
| Season of 2019    |                                 |                                 |                                   |                                 |
| GLA10             | 927.8 ± 2.5d                     | 689.4 ± 1.7bc                   | 1.34 ± 2.62 cd                    | 320.1 ± 0.8e                    | 1937.3 ± 3.3e                   |
| GLA20             | 1230.8 ± 3.7a                    | 767.0 ± 3.9a                    | 1.61 ± 0.08a                      | 385.3 ± 0.1a                    | 2416.5 ± 10.5a                  |
| GLA40             | 982.1 ± 1.0c                     | 768.4 ± 2.8a                    | 1.27 ± 0.01d                      | 350.4 ± 1.0c                    | 2101.0 ± 2.9c                   |
| ALA10             | 904.5 ± 4.6e                     | 656.5 ± 1.1 cd                  | 1.37 ± 0.01c                      | 307.1 ± 3.1f                    | 1868.1 ± 8.9f                   |
| ALA20             | 1096.0 ± 1.3b                    | 727.1 ± 3.2ab                   | 1.50 ± 0.01b                      | 359.8 ± 0.8b                    | 2182.9 ± 3.7b                   |
| ALA40             | 981.4 ± 2.3c                     | 717.1 ± 2.5b                    | 1.36 ± 0.01cd                     | 339.1 ± 3.7d                    | 2037.6 ± 8.6d                   |
| Check treatment   | 857.9 ± 2.5f                     | 627.4 ± 2.3d                    | 1.36 ± 9.39cd                     | 284.3 ± 2.2g                    | 1769.6 ± 2.6g                   |
| Season of 2020    |                                 |                                 |                                   |                                 |
| GLA10             | 912.9 ± 9.1d                     | 680.8 ± 6.0c                    | 1.34 ± 0.01c                      | 321.9 ± 2.2cd                   | 1915.6 ± 15.9d                  |
| GLA20             | 1229.1 ± 2.0a                    | 790.1 ± 11.5a                   | 1.55 ± 0.02b                      | 377.0 ± 7.2a                    | 2396.2 ± 4.6a                   |
| GLA40             | 1154.5 ± 8.6b                    | 739.5 ± 9.8b                    | 1.56 ± 0.03b                      | 346.1 ± 2.6b                    | 2240.1 ± 4.9b                   |
| ALA10             | 879.0 ± 11.4d                    | 648.0 ± 5.8d                    | 1.35 ± 0.01c                      | 312.3 ± 1.7d                    | 1839.4 ± 16.4c                  |
| ALA20             | 1196.9 ± 7.1a                    | 727.0 ± 12.1b                   | 1.64 ± 0.03a                      | 355.2 ± 2.8b                    | 2279.2 ± 7.4b                   |
| ALA40             | 1110.4 ± 20.9e                   | 692.9 ± 4.3c                    | 1.60 ± 0.02ab                     | 332.8 ± 1.7c                    | 2136.1 ± 25.2c                  |
| Check treatment   | 863.8 ± 18.5e                    | 635.8 ± 7.4d                    | 1.35 ± 0.04c                      | 282.2 ± 5.9e                    | 1781.9 ± 15.6f                  |

GLA10, GLA20, and GLA40 as well as ALA10, ALA20, and ALA40 are exogenous applications of glutamic acid and 5-aminolevulinic acid at concentrations of 10, 20 and 40 mg L⁻¹, respectively. Different letters within columns indicates that there are significant differences at 0.05 level of probability.

Results

Photosynthesis Pigments

Significant differences in photosynthesis pigments were obtained owing to application of different levels of glutamic and 5-aminolevulinic acids in 2019 and 2020 seasons (Table 2). In 2019 season, glutamic acid at a rate of 20 mg L⁻¹ showed the highest values of chlorophyll a, chlorophyll b, chlorophyll a/b, carotenoids and total pigments. Also, the differences between glutamic acid at rate of 20 and 40 mg L⁻¹ and 5-aminolevulinic acid at a rate of 40 mg L⁻¹ in chlorophyll b were not significant. In 2020 season, glutamic acid at a rate of 20 mg L⁻¹ recoded also the maximum values of all photosynthesis pigment traits, except chlorophyll a/b. ALA20 was similar to GLA20 for achieving the maximum value of chlorophyll a. Chlorophyll a/b showed the highest values with ALA20 along with ALA40.

Biochemical Constituents of Peanut Leaves

Both of GLA20 and ALA20 in 2019 and 2020 seasons, in addition to GLA40 (in 2019 season for free amino acids) showed enhancement effects on indole acetic acid, phenolics and free amino acids (Table 3). As averages of the two seasons, the increases in indole acetic acid, phenolics and free amino acids were 68.1, 58.9 and 19.6% as well as 64.6, 51.2 and 17.7%, due to application of GLA20 and ALA20 respectively.
Table 3  Indole acetic acid, phenolics and free amino acids of peanut response to glutamic and 5-aminolevulinic acids application in 2019 and 2020 seasons

| Variable | Indole acetic acid (µg 100 g⁻¹ f wt.) | Phenolics (mg 100 g⁻¹ f wt.) | Free amino acids (mg 100 g⁻¹ d wt.) |
|----------|-------------------------------------|-------------------------------|-------------------------------------|
| Season of 2019 | | | |
| GLA10    | 50.5 ± 0.6bc                     | 91.0 ± 0.9d                   | 262.1 ± 0.7b                        |
| GLA20    | 64.5 ± 0.8a                      | 115.4 ± 1.8a                  | 284.4 ± 0.6a                        |
| GLA40    | 47.1 ± 0.7c                      | 104.6 ± 1.0b                  | 279.6 ± 0.5a                        |
| ALA10    | 51.1 ± 3.1bc                     | 100.4 ± 1.1c                  | 258.2 ± 0.1b                        |
| ALA20    | 62.9 ± 0.3a                      | 104.9 ± 0.9b                  | 278.7 ± 8.9a                        |
| ALA40    | 54.5 ± 1.2b                      | 99.2 ± 0.3c                   | 256.9 ± 1.9b                        |
| Check treatment | 37.2 ± 0.8d                     | 72.0 ± 0.9c                   | 238.1 ± 1.4c                        |
| Season of 2020 | | | |
| GLA10    | 50.5 ± 0.9b                      | 90.7 ± 1.4c                   | 259.7 ± 1.8c                        |
| GLA20    | 64.8 ± 1.1a                      | 114.5 ± 2.2a                  | 282.5 ± 1.6a                        |
| GLA40    | 53.2 ± 1.4b                      | 107.4 ± 0.9b                  | 272.0 ± 1.6b                        |
| ALA10    | 49.9 ± 1.5b                      | 89.6 ± 1.1c                   | 253.9 ± 1.0d                        |
| ALA20    | 63.7 ± 0.7a                      | 113.9 ± 1.6a                  | 279.1 ± 0.9a                        |
| ALA40    | 51.8 ± 1.8b                      | 105.2 ± 1.1b                  | 270.7 ± 1.5b                        |
| Check treatment | 39.8 ± 1.2c                     | 72.7 ± 1.5d                   | 235.9 ± 2.7e                        |

GLA10, GLA20, and GLA40 as well as ALA10, ALA20, and ALA40 are exogenous applications of glutamic acid and 5-aminolevulinic acid at concentrations of 10, 20 and 40 mg L⁻¹, respectively. Different letters within columns indicates that there are significant differences at 0.05 level of probability

Table 4  Branches and pods number plant⁻¹ and pod yield of peanut response to glutamic and 5-aminolevulinic acids application in 2019 and 2020 seasons

| Variable | Branches number plant⁻¹ | Pods number plant⁻¹ | Pod yield (t ha⁻¹) |
|----------|--------------------------|---------------------|------------------|
| Season of 2019 | | | |
| GLA10    | 10.00 ± 0.57b           | 37.00 ± 1.15c       | 51.43 ± 0.81d    |
| GLA20    | 13.33 ± 0.66a           | 46.66 ± 2.18a       | 64.36 ± 0.64a    |
| GLA40    | 8.66 ± 0.33bc           | 43.00 ± 0.57b       | 58.36 ± 0.75b    |
| ALA10    | 8.66 ± 0.33bc           | 33.00 ± 0.57d       | 50.03 ± 0.81de   |
| ALA20    | 8.33 ± 0.33c            | 42.33 ± 0.33b       | 61.03 ± 1.48b    |
| ALA40    | 7.66 ± 0.33cd           | 38.66 ± 0.88c       | 55.26 ± 1.01c    |
| Check treatment | 6.66 ± 0.33d           | 22.00 ± 0.57e       | 48.40 ± 0.55e    |
| Season of 2020 | | | |
| GLA10    | 11.33 ± 0.33b           | 37.00 ± 2.08de      | 50.33 ± 0.87c    |
| GLA20    | 14.00 ± 0.57a           | 48.33 ± 1.20ab      | 63.86 ± 0.72a    |
| GLA40    | 12.66 ± 0.88ab          | 44.66 ± 0.88bc      | 58.30 ± 1.33b    |
| ALA10    | 10.66 ± 0.33b           | 34.33 ± 1.45e       | 49.13 ± 0.82c    |
| ALA20    | 11.33 ± 0.33b           | 49.33 ± 1.45a       | 62.06 ± 0.73a    |
| ALA40    | 10.66 ± 0.33b           | 41.33 ± 2.02cd      | 55.70 ± 0.92b    |
| Check treatment | 8.33 ± 0.88c           | 22.66 ± 1.20f       | 47.16 ± 1.27c    |

GLA10, GLA20, and GLA40 as well as ALA10, ALA20, and ALA40 are exogenous applications of glutamic acid and 5-aminolevulinic acid at concentrations of 10, 20 and 40 mg L⁻¹, respectively. Different letters within columns indicates that there are significant differences at 0.05 level of probability

Agronomic Traits

As presented in Table 4, the peanut agronomic traits expressed in branches number plant⁻¹, pods number plant⁻¹ and pod yield ha⁻¹ statistically responded to glutamic and 5-aminolevulinic acids in 2019 and 2020 seasons. In this respect, only GLA20 was the effective treatment for enhancing the agronomic traits in the first season. However, in the second season, the differences between GLA20 and GLA40 (for branches number plant⁻¹) as well as GLA20 and ALA20 (for pods number plant⁻¹ and pod yield ha⁻¹) were not significant.
Biochemical Constituents of Peanut Seeds

Oil % (Fig. 1), flavonoids (Fig. 2) and antioxidant activity (Fig. 3) of peanut seeds significantly responded to glutamic and 5-aminolevulinic acids in 2019 and 2020 seasons. In both seasons, application of GLA20 had the potential to increase oil %, flavonoids and antioxidant activity by about 1.10, 1.75 and 1.20 times, respectively, over the check treatment. ALA20 and ALA40 treatments markedly equaled GLA20 for antioxidant activity in the second season.

Discussion

Physiologically, various reactive oxygen species (ROS), such as superoxide radical (O$_2^\cdot$), hydroxyl free radical (OH$^\cdot$), singlet oxygen ($^1$O$_2$) and hydrogen peroxide (H$_2$O$_2$), are continuously produced as a natural byproducts of plant cellular metabolism (Mahalingam and Fedoroff 2003; Mittler et al. 2004). The presence of ROS may influence cell membrane properties and give rise to oxidative damage to proteins, lipids, nucleic acids, and carbohydrates, redox imbalance, peroxidation of plasmalemma, DNA mutation, protein denaturation and ultimately cell death (Gill and Tuteja 2010; Sharma et al. 2012). Moreover, ROS lead to the inactivation of proteins and inhibit the activity of multiple enzymes involved in metabolic pathways, and result in the oxidation of other macromolecules including lipids and DNA (Hossain et al. 2014). Therefore, plant cells should be equipped with an antioxidant defense mechanism to detoxify the harmful effects of ROS.

Foliar application of amino acids is one of the recent agricultural approaches to improve plant growth, yield and quality properties (Sadak et al. 2015; Souri and Hatamian 2019; Saudy et al. 2020b, Sadak and Ramadan 2021). Thus, lately, application of amino acids to plants, particularly under adverse environmental conditions, became a pivotal action in agricultural practices (Ma et al. 2017; Souri et al. 2017). In this context, results of the current research displayed the importance of exogenous spray of amino acids, i.e. glutamic and 5-aminolevulinic acids as a modern strategy for improving the plant pigments, yield and quality of peanut. In this regard, most studied traits exhibited increases with low amino acid concentration (10 mg L$^{-1}$), especially for glutamic acid, greater than the check treatment. Moreover, increasing the concentration up to 20 mg L$^{-1}$ whether
for glutamic acid or 5-aminolevulinic acid led to substantial increase in chlorophylls and carotenoids (Table 2). While, higher concentration of both amino acids, i.e. 40 mgL⁻¹ showed less effect comparing to 20 mgL⁻¹. An increase in leaf pigments owing to application of amino acids has also been reported in previous researches (Fahimi et al. 2016; Mohammadipour and Souri 2019b). Higher chlorophyll content of leaves obtained with amino acids supply could be due to their promotive effect on chlorophyll biosynthesis, synchronizing with decrease in chlorophyll degeneration (Souri et al. 2017; Fahimi et al. 2016). The improvements in plant growth, biomass, and photosynthetic machinery might have resulted from decreased the production electrolyte leakage and ROS caused by the addition of glutamic acid (Farid et al. 2020). Owing to their hormone-like activity and acting in signal transduction, amino acids serve as prophylactic agents against stress conditions and can also enhance the control of stomata and gene expression toward better plant growth (Svennerstam et al. 2008; Souri 2016). Among plant growth regulators, ALA is an essential biosynthetic precursor of all tetrapyrrole compounds (chlorophyll, heme, and vitamin B12) (Senge et al., 2014), which have promotive effects on plant growth and plant biomass (Fu et al. 2014; Xu et al. 2015). Exogenous ALA markedly improved carotenoid biosynthesis by upregulating the gene expression levels of geranylgeranyl diphosphate synthase, phytoene synthase 1, phytoene desaturase, and lycopene b-cyclase (Wang et al. 2021). Accordingly, exogenous application of amino acids can increase chlorophyll biosynthesis and photosynthetic rates resulting in improved plant growth particularly under adverse climatic conditions (Shams et al. 2016). Amino acids not only enhanced photosynthetic pigments of peanut but also the defense response indicators. Herein, all applied concentrations of glutamic acid or 5-aminolevulinic acid surpassed the check treatment for increasing indole acetic acid, phenols and free amino acids (Table 3). Exogenous supply of ALA increased the contents of soluble sugars, soluble proteins, total free amino acids, and ascorbic acid (vitamin C) as well as eleven kinds of amino acid components (Zhang et al. 2012; Wang et al. 2021). Also, improvement in root and shoot growth, and leaf pigmentation and vitamin C content were mainly observed in low to moderate levels of glutamine (Noroozlo et al. 2019). It should be explained that ascorbic acid could regulate plant growth (El-Bially et al. 2018, 2022a) through the interaction with phytohormones (Pastori et al. 2003). Ascorbic acid as an antioxidant may act as an alternative electron donor of photosystem II (PSII) in photosynthesis process, where the electron transfer is inhibited due to the inactivation of oxygen-evolving complex (Gururani et al. 2012). Ascorbic acid as an alternative PSII electron donor can languish the processes of photoinactivation and minimize the ROS activity in the photosynthetic thylakoid membranes, and thus minimize the damage to the entire photosynthetic apparatus (Venkatesh and Park 2014). Consequently, the improvements in peanut growth due to ALA application were obtained.

In addition to proline, other amino acids could act as osmoprotectant agents in plants under water deficit stress (Zulfiqar et al. 2020; Makhlouf et al. 2022), and thus play a significant role in improving the relative water content of plant tissues (Teixeira et al. 2020; Alfsoea-Simon et al. 2020). Enhancement in leaf relative water content with ALA application may reduce stomatal limitation to gas exchanges, thereby also contributing to the increases in leaf net photosynthetic rate (Youssef and Awad 2008). Since glutamic and 5-aminolevulinic acids have the potentiality to induce the synthesis of photosynthesis pigments, indole acetic acid, phenolics and free amino acids, branching and pod yield as well as seed quality were improved. Amino acids can act as hormone precursors and they can contribute to regulate carbon and nitrogen metabolisms and to promote nitrogen assimilation (Calvo et al. 2014; Colla and Rouphael 2015; Bulgari et al. 2019). Findings of the current study also proved the superiority of glutamic acid for promoting the synthesis of photosynthesis pigments (Table 2). Moreover, the overall response of peanut to exogenous application of glutamic was better than those of 5-aminolevulinic in agronomic traits (Table 4) and seed quality (Figs. 1, 2 and 3). This observation may correlate with the fact that glutamic acid is the source of ALA synthesis (Czarnecki and Grimm 2012).

Conclusion

Findings indicate that the application of amino acids as growth stimulants has practical implications in the production of oil crops such as peanut. Application of glutamic acid or 5-aminolevulinic acid under normal or stressful circumstances had beneficial changes on peanut crop expressed in improvements of photopigments and antioxidant defense mechanisms. Therefore, application of such amino acids in peanut field is considered a promising practice for raising yield and quality particularly under arid zones. It is interesting to note that glutamic acid or 5-aminolevulinic acid were more effective with using the concentration of 20 mgL⁻¹ for better growth and yield than lower or higher concentrations.

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**Conflict of interest** I.M. El-Metwally, M.S. Sadak and H.S. Saudy declare that they have no competing interests.

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Hani Saber Saudy was born in Giza, Egypt, in 1973. He is a professor of Agronomy Department, Faculty of Agriculture, Ain Shams University, Egypt and a specialist in Field Crop Production (Crop Physiology and Management). He is interested in the enhancement of the physiological status of oil crop under stressful conditions.