



## Article

# Seed Priming with Exogenous Amino Acids Improves Germination Rates and Enhances Photosynthetic Pigments of Onion Seedlings (*Allium cepa* L.)

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**Abstract:** As a biostimulant, amino acids play crucial roles in enhancing plant growth and development. These roles, combined with the ability to be approved for organic usage, make amino acids a good choice for sustainable farming systems. This work investigates the effect of onion seed priming with different exogenous amino acids, specifically the impact of seed priming in enhancing a diverse range of morpho-physiological responses of onion seedlings. Here, we primed onion seeds (Cultivar Giza 6) with ten exogenous amino acids. Based on the growth parameters of onion seedlings, data showed that glutamine significantly improved the most studied parameters. Germination percentage (GP) ranged from 85% in Methionine (Met) to 98.5% in Proline (Pro) and Tryptophan (Try), with 10% over the control treatment. Glutamine (Glu) enhanced the vigor index (VI) of onion, giving the seeds a high ability to produce normal seedlings. The most extended root system ( $\geq 3.3$  cm) was obtained from Glu, Glycine (Gly), Pro, and Try treatments. The maximum shoot length was obtained from treatments (Glu and Try) with more than 60% over control. Priming onion seeds with amino acids (AAs) increased chlorophyll contents compared with non-primed seeds. Glutamine and Threonine (Thr) had the highest results (122 and 127  $\mu\text{g/g}$  fresh weight, respectively), while the Glu treatment registered the highest Carotene contents with 50% over the control treatment. Furthermore, the data illustrate that the principal component analysis-1 (PCA1) indicates 67.2% variability, and PCA2 indicates 14.8% variability. Strong positive correlations were observed between germination percentage, root length, shoot length, dry matter, chlorophyll a, and carotene. The study concluded that the primed onion seeds by glutamine, proline, and tryptophan had the best germination rates.

**Keywords:** biostimulants; vegetable crops; vigor index; chlorophyll; carotene; PCA



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## 1. Introduction

According to the European Council of biostimulants Industry (EBIC), Plant biostimulants are defined as products that stimulate the nutritional processes of plants independently of the nutrient content of the product, with the objective of promoting the efficiency of nutrients usage in the plant, their quality properties, or increasing nutrient uptake that is immobilized in the soil or rhizosphere [1]. These substances have different chemical families, such as humic acids, hydrolyzed proteins, algae extracts [2], chitosan biopolymers, inorganic compounds, and beneficial microbes [3].

New approaches have been introduced for improving agricultural production sustainability and resilience by applying natural products such as biostimulants that are appraised for being eco-friendly, cost-effective, and enhancing the productivity and quality of cultivated

crops [4]. Biostimulants stimulate plant growth by increasing plant metabolism, germination rates, photosynthesis process, nutrient absorption, and improving productivity [5].

Amino acids (AAs) have various beneficial effects on plant growth and development by their structure as protein units, which play a vital role in glutamine biosynthesis [6] and plant hormones [7]. AAs are a source of organic nitrogen and structural molecules that unite to produce proteins. Additionally, AAs are functional molecules related to several physiological processes in internal plants. Also, they have an influential nutritional role during the germination of plant proteins and the synthesis of phytohormones [8]. Moreover, the exogenous uptake of free amino acids gave beneficial properties, such as enhancing photosynthesis, forming coenzymes [9], and supporting plant organisms against environmental stresses [10]. Increasing protein content in plants has been influenced by applying amino acids [11].

Germination is a critical stage in a plant's life cycle and is the first step toward successful plant establishment, especially under unfavorable conditions. Seed priming, a non-distractive and cheap, effective technique, is a pre-sowing treatment that allows partial rehydration without leading to radicle emergence. Priming can hasten and synchronize the germination rate and improve the productivity of vegetable crops in a sustainable approach [12]. Priming can potentially support rapid seed germination and ensuing growth [13]. The most frequently applied priming techniques are hydro-priming. Priming activates processes associated with the early phases of germination. Priming is a procedure by which plants can be set to the best tolerance for future stresses, either of the same ("stress memory") or of a different ("cross-adaptation") type [14].

As a germination promoter strategy, seed priming has a positive effect on photosynthetic pigments by protecting the degradation of chlorophylls or increasing pigments concentrations [15–17] and improving the concentration of carotenoids [18–20] in optimal and suboptimal conditions. Seed priming is generally applied for synchronized seedling growth and stable crop productivity.

Nowadays, priming has emerged as an effective tool for agriculture sustainability because seed priming can serve as a cost-effective tool, environment-friendly substances, and a pragmatic approach for adjusting global food security through sustainable innovation [21]. Sustainable agricultural processes that may enhance plant growth and increase crop productivity represent a priority in current agricultural systems [22]. The seed priming technique enhances initial germination [23,24], improves its synchronization, and promotes plant growth [25]. This technique has been applied to the main vegetable crops, such as carrots, onion, celery, lettuce, pepper, and tomato [24].

The priming technique mechanism (hydration) is how seed priming improves germination and encourages starting pre-germination metabolic processes. Optimal seed priming positively reflects crop development and uniformity, making farming more profitable [26]. At the imbibition phase, mechanical and biochemical changes initiate, such as embryo enlargement, respiration, protein synthesis, and DNA repair [27–29]. These processes promote cell elongation [30]. Utilizing the application of amino acid components based on biostimulation in agricultural practice is becoming increasingly necessary, as it sustains farming systems thanks to the role that AAs play in the main processes of plants, which have an impact on the plant's development and crop quality [31]. These AAs are often applied as free acids or through sources of botanical or animal origin that have a sufficient concentration of amino acids [32].

Onion seeds are naturally less viable and more hygroscopic than most traditional vegetables [33,34]. Storage of onion seeds in inappropriate conditions may lead to seed deterioration, which causes reduced viability and vigor and decreases seed quality [35,36]. Seed deterioration is a natural process involving tissue metabolism and physical changes in seeds during storage [37,38]. These changes reduce germination rates which cause abnormal seedling emergence and growth. Deteriorated seeds can be recovered using the seed priming technique [24].

Most studies investigated products that contained amino acids, and there is little information regarding the single impact of these amino acids [39,40]. In this context, priming amino acids via seed can improve plant development since these substances act as signals of various beneficial physiological processes [41,42]. Based on the abovementioned, this study was conducted to answer the hypothesis of the relation between priming onion seeds with exogenous amino acids and germination improvement. For this purpose, ten exogenous amino acids were primed onion seeds, and the response of phenotypical criteria and photosynthetic pigments was assessed.

## 2. Materials and Methods

### 2.1. Experimental Design and Treatments

A laboratory germination test was conducted for 14 days for onion (*Allium cepa* L.) seeds [43], with the “Giza 6 cultivar” as a model. The seeds were harvested from the experimental farm, Sohag University, Egypt, during the 2022 season, well-dried, and stored in a paper package until usage. Ten exogenous amino acids (Sigma-Aldrich, St. Louis, USA) were applied (Proline [Pro]; Methionine [Met]; Tryptophan [Try]; Arginine [Arg]; Alanine [Ala]; Glycine [Gly]; Glutamine [Glu]; Threonine [Thr]; Lysine [Lyc]; Phenylalanine [Phe]; in addition to distilled water (Ctl). Each amino acid was applied at 0.5 g/L concentration [44–47]. Twenty seeds were primed with different amino acids and distilled water (control), placed in Petri dishes on two layers of filter paper, moistened with 10 mL of solution, and kept at the laboratory at  $25 \pm 2$  °C temperature and 60% relative humidity.

### 2.2. Data Collection and Calculation

Growth characteristics: fresh weight (FW, mg); dry matter (DM, mg), which was determined by drying the fresh seedlings in the drying oven until constant weight, relative water contents (RWC, %), which were calculated as follows:  $RWC, \% = (FW - DM)/FW$  [48], shoot height (SL, cm), and root length (RL, cm) were measured at 7 and 14 days after starting priming as described by ref. [48]. Germination was registered daily up to 7 days after starting the experiment. The following data were registered: final germinated percentage (FGP) was calculated according to Kader, 2005 [49] as follows:

$$(FGP) = \frac{\text{Germinated seeds}}{\text{total seeds}} \times 100$$

Germination energy (GE) was calculated for the first Four days [50] as follows:

$$GE = \frac{\text{Germinated seeds}}{\text{Total seeds}} \times 100$$

The germination Rate index (GRI) was expressed in (seed/day) and calculated by the following equation:

$$GRI = \Sigma (Gt/Dt)$$

Gt refers to the number of germinated seeds on day t, and Dt represents time corresponding to Gt in days. For measuring the rate of germination time-spread per day, mean germination time (MGT) was calculated as follows:

$$MGT = \left[ \frac{\Sigma(D \times N)}{\Sigma N} \right]$$

Whereas D is the number of days from the start of the trial and N is the number of seeds germinated on day D. Vigor index (VI) was also calculated for all treatments as follows:  $VI = \text{length (root + hypocotyle)} \times GP$ .

From the eighth day, seedlings were exposed to artificial blue light for 8 h daily. Blue light is considered the most effectively utilized wavelength for plant photosynthesis due to the optimum absorption spectra of the pigments ranging between 400–500 nm [51]. Measuring chlorophylls and carotene contents ( $\mu\text{g g}^{-1}$  FW) was conducted spectrophotometrically

(Spectrophotometer-KФK-3-01, Moscow, Russia) at 14 days of aged, germinated shoots. 0.2 g of fresh shoots tissues were ground in 90% acetone. Absorptions were taken at 663 nm and 644 nm for chlorophylls and 452.5 nm for carotenoids [52], and the following equations were applied for final calculations [53]:

$$\text{Chla} = 10.3 \times \text{Abs663} - 0.918 \times \text{Abs644}$$

$$\text{Chlb} = 9.7 \times \text{Abs644} - 3.87 \times \text{Abs663}$$

$$\text{Car} = 4.2 \times \text{Abs452.5} - (0.0264 \times \text{Chla} + 0.4260 \times \text{Chlb})$$

Whereas Chla = chlorophyll a, Chlb = chlorophyll b, Car = carotenoids, and Abs663, Abs644, and Abs452.5 are absorbed in 663, 644, and 452.5 wavelengths and expressed in  $\mu\text{g g}^{-1}$  FW [54].

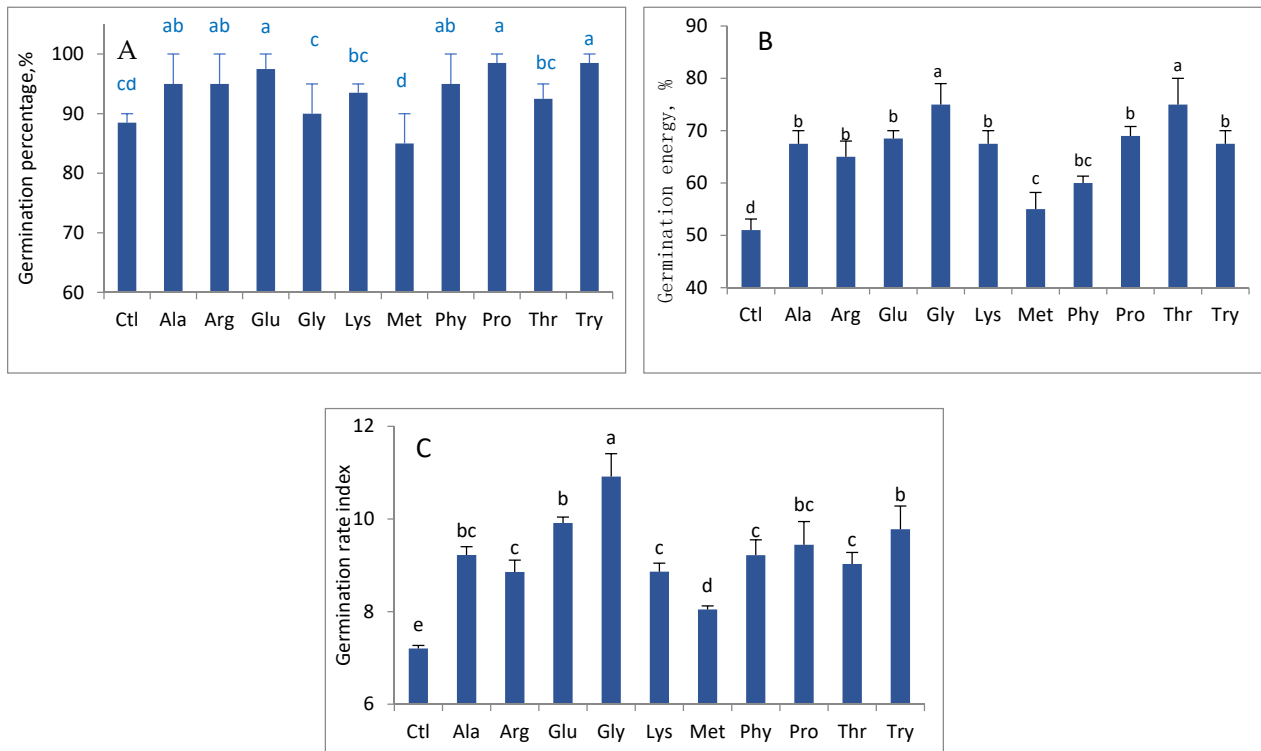
### 2.3. Statistical Analyses

The data were analyzed using one-way ANOVA with six replicates. The outputs expressed as mean  $\pm$  standard deviation followed Tukey's multiple range test at  $p \leq 0.01$  using MINITAB v.19. Principal component analysis (PCA) was also computed using XLSTAT v. 2022.1.

## 3. Results

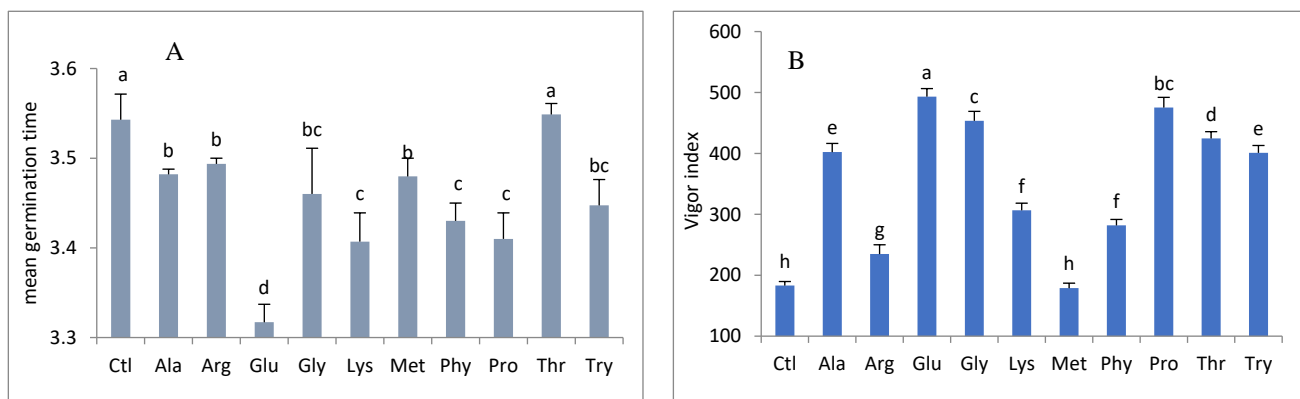
In general, the studied germination parameters were significantly ( $p \leq 0.01$ ) affected by amino acids (AAs) priming (Figures 1 and 2). Germination percentage ranged from 85% in methionine (Met) to 98.5% in proline (Pro) and tryptophan (Try), with 10% more than in the control (Ctl) treatment. The germination percentage (GP) data from all exogenous amino acids (EAAs) were statistically similar and improved germination percentage more than the control treatment except Met, which reduced GP by 3.5% compared with Ctl. Germination energy refers to the percentage of germinated seeds in a seed sample within four days in this experiment. Priming onion seeds with glycine (Gly) and threonine (Thr) gave the highest germination energy percentage (75%), while the least germination energy (GE) was obtained from Met (55%) and still more than non-primed seeds 4%. Figure 1C shows the effect of priming onion seeds with exogenous amino acids. The highest germination rate index (GRI) was observed in the Gly treatment (10.9%), with more than 3.7% compared with the non-primed Ctl. Priming seeds with Met gave the least GRI (8.0%), which statistically did not differ from the control treatment.

Mean germination time (MGT) accurately measures the time spent for seeds to germinate, focusing on the day when most germination occurred and not related to time spread or germination uniformity. The vigor index (VI) measures the percentage of viable seeds and expresses the ability to produce normal seedlings because, in some cases, seeds may not grow normally after germination. In this study, Glu treatment enhanced VI of onion seeds, giving the maximum ability (493) to produce normal seedlings, while non-primed and priming with Met shared the least VI that increased the risk of producing abnormal seedlings. The maximum MGT of onion seeds (3.5) was recorded from both non-primed and primed with Threonine, while the minimum MGT (3.2) was recorded when Glu was applied. The root length is considered the initial part (a growing plant embryo) arising from the seed through the germination process, and it is the embryonic root of the plant which grows downward firstly in the soil to fix the seedlings and absorb the nutrients and water.

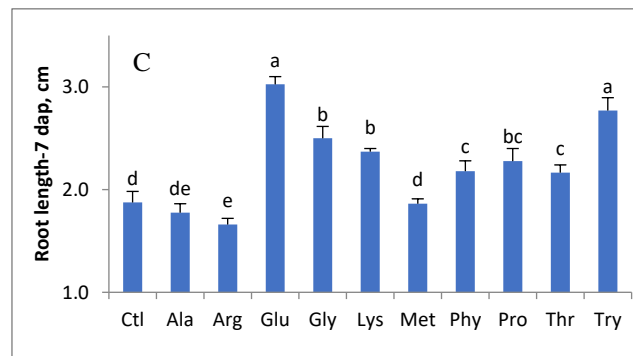


**Figure 1.** Effect of priming onion seeds with exogenous amino acids. (A)—Germination percentage, %; (B)—Germination index, %; (C)—Germination rate index, %. Ctl (Control); Ala (Alanine); Arg (Arginine); Glu (Glutamine); Gly (Glycine); Lys (Lysine); Met (Methionine); Phy (Phenylalanine); Pro (Proline); Thr (threonine); Try (tryptophan). Columns that share the same letter(s) are not statistically significant at  $p \leq 0.01$ .

According to the shown data of root length after seven days of priming (RL-7), pre-priming onion seeds with Glu and Try treatments produced a root length of more than 2.8 cm (Figure 2C). The longest radicle (3.0 cm) was obtained from seeds primed with Glutamine and increased by over 55% compared with non-primed (1.1 cm). At 14 days after priming, all AA increased the length of the root system compared with the control, except with the arginine (Arg) treatment (Figure 3B).

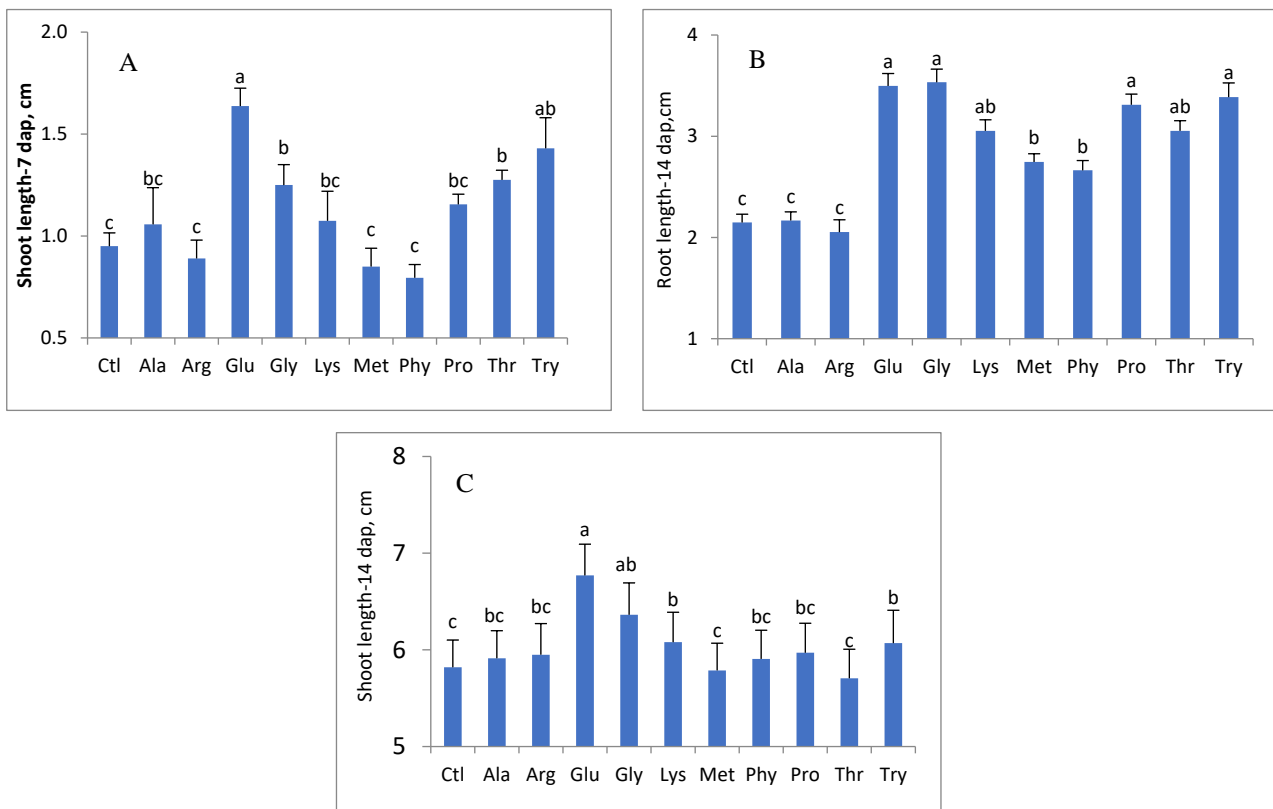


**Figure 2.** Cont.



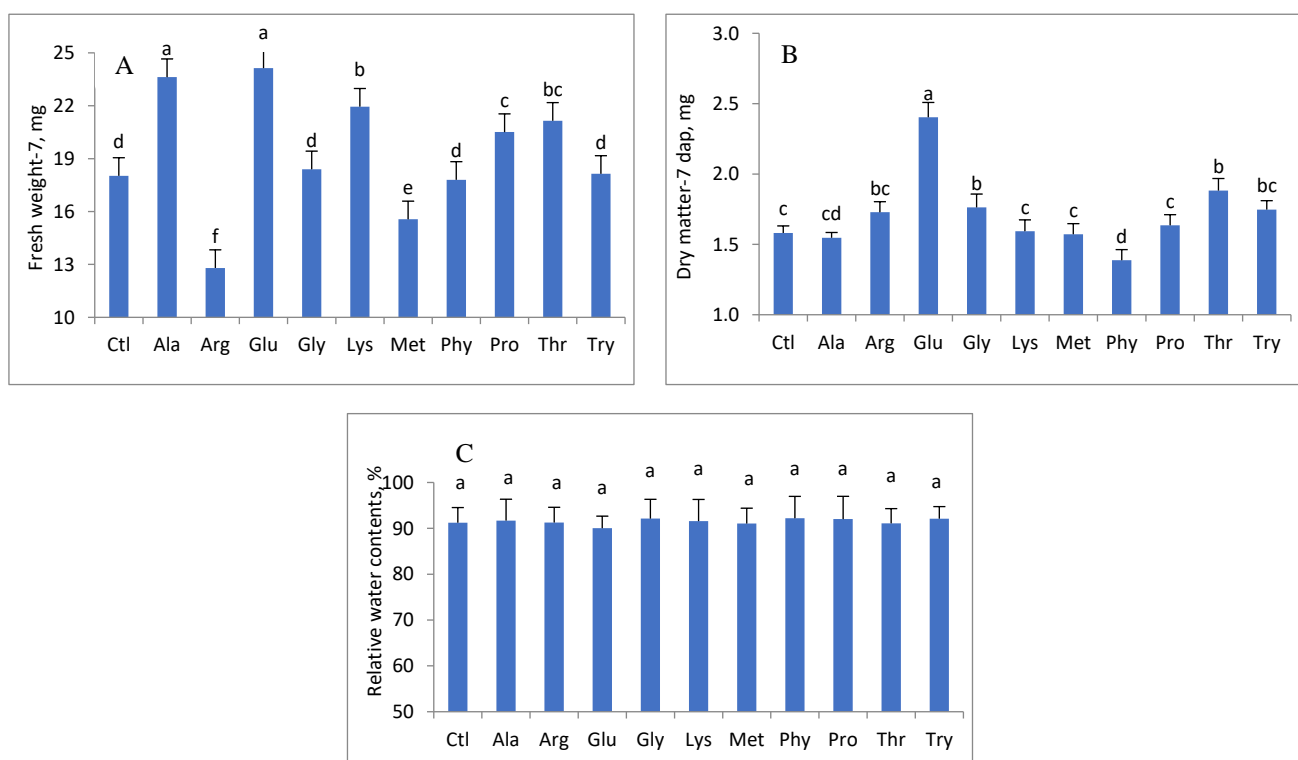
**Figure 2.** Effect of priming onion seeds with exogenous amino acids. (A)—Mean germination time, %; (B)—Vigor index, %; (C)—Radicle length, %. Ctl (Control); Ala (Alanine); Arg (Arginine); Glu (Glutamine); Gly (Glycine); Lys (Lysine); Met (Methionine); Phy (Phenylalanine); Pro (Proline); Thr (threonine); Try (tryptophan). Columns that share the same letter(s) are not statistically significant at  $p \leq 0.01$ .

The most extended root system ( $\geq 3.3$  cm) was obtained when the onion seed was primed with Glu, Gly, Pro, and Try, while the shortest was observed at Ctl, Ala, and Arg treatments ( $\leq 2.2$  cm).



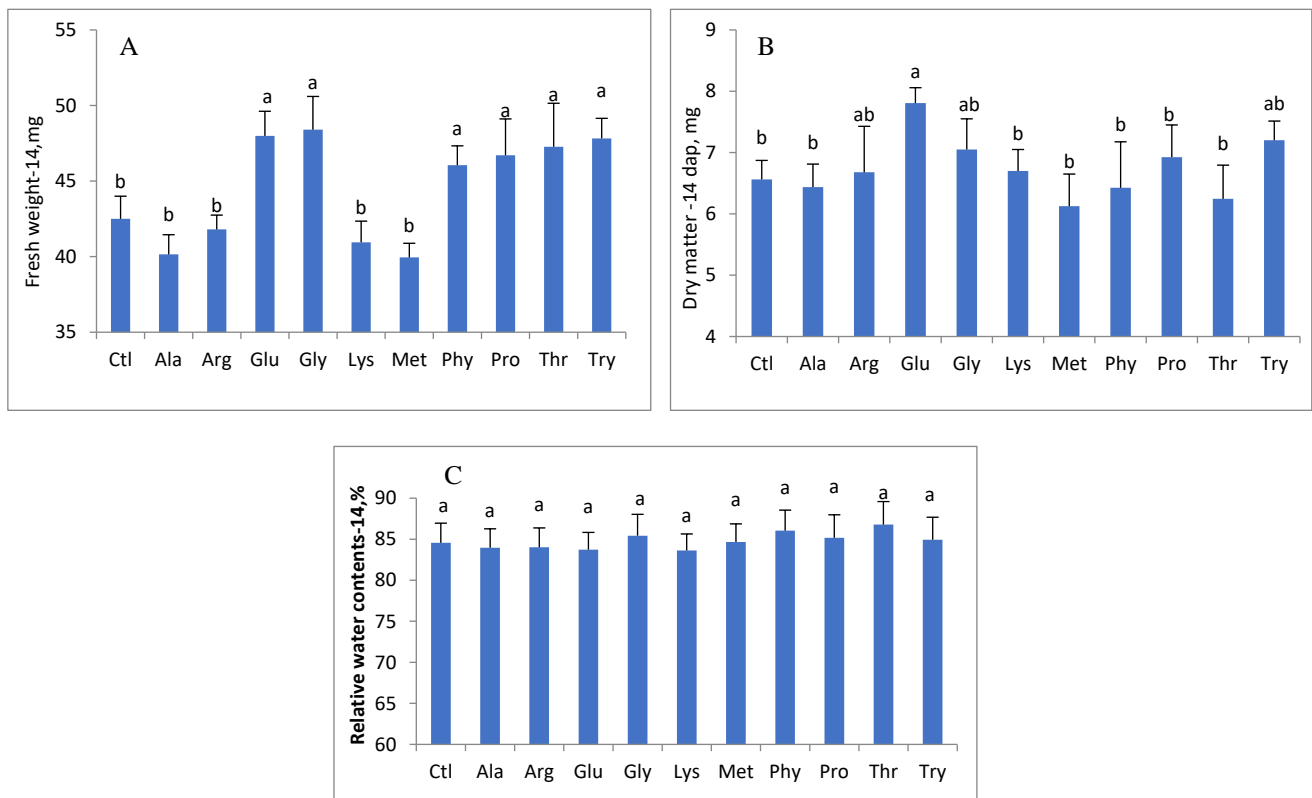
**Figure 3.** Effect of priming onion seeds with exogenous amino acids. (A)—Shoot length, cm (SL-7); (B)—Root length, cm (RL-14); (C)—Shoot length, cm (SL-14). Ctl (Control); Ala (Alanine); Arg (Arginine); Glu (Glutamine); Gly (Glycine); Lys (Lysine); Met (Methionine); Phy (Phenylalanine); Pro (Proline); Thr (threonine); Try (tryptophan). Columns that share the same letter(s) are not statistically significant at  $p \leq 0.01$ .

Shoots are the second growth part that emerges from seed germination that grows upward, where leaves will develop, and the photosynthesis process will start. Dry matter refers to all substances absorbed and formatted by a plant's tissues except water; it is a more reliable indicator to investigate the weight. The maximum shoot length at 7 days after planting was obtained from treatments (Glu and Try) with more than 1.4 cm representing more than 60% compared with non-primed (1.0 cm), while the minimum results were observed at Arg, Met, and Phe treatments ( $\leq 0.9$  cm). The same trend also was observed 14 days after planting, whereas Glu treatment produced the highest result (6.8 cm), while the minor result (5.7 cm) was obtained at Thr (Figure 3C). Illustrated data in Figures 4 and 5 show the impact of AAs on the fresh weight of the whole onion-primed seeds at 7 and 14 days after planting in addition to water contents percentages. At 7 days after planting, the Glu treatment registered the heaviest fresh and dry weight (24.1 and 2.4 mg, respectively) by more than 33% compared with the control treatment. The least fresh weight (FW) was registered from Met (17.6 mg), while the minimum dry matter (DM) content (1.4 mg) was observed at the Phe treatment. Figure 4C shows no statistically significant differences in relative water contents (RWC) among the treatments at 7 days after planting.



**Figure 4.** Effect of priming onion seeds with amino acids. (A)—Fresh weight-7, mg; (B)—Dry matter-7, mg; (C)—Relative water contents-7, %. Ctl (Control); Ala (Alanine); Arg (Arginine); Glu (Glutamine); Gly (Glycine); Lys (Lysine); Met (Methionine); Phy (Phenylalanine); Pro (Proline); Thr (threonine); Try (tryptophan). Columns that share the same letter(s) are not statistically significant at  $p \leq 0.01$ .

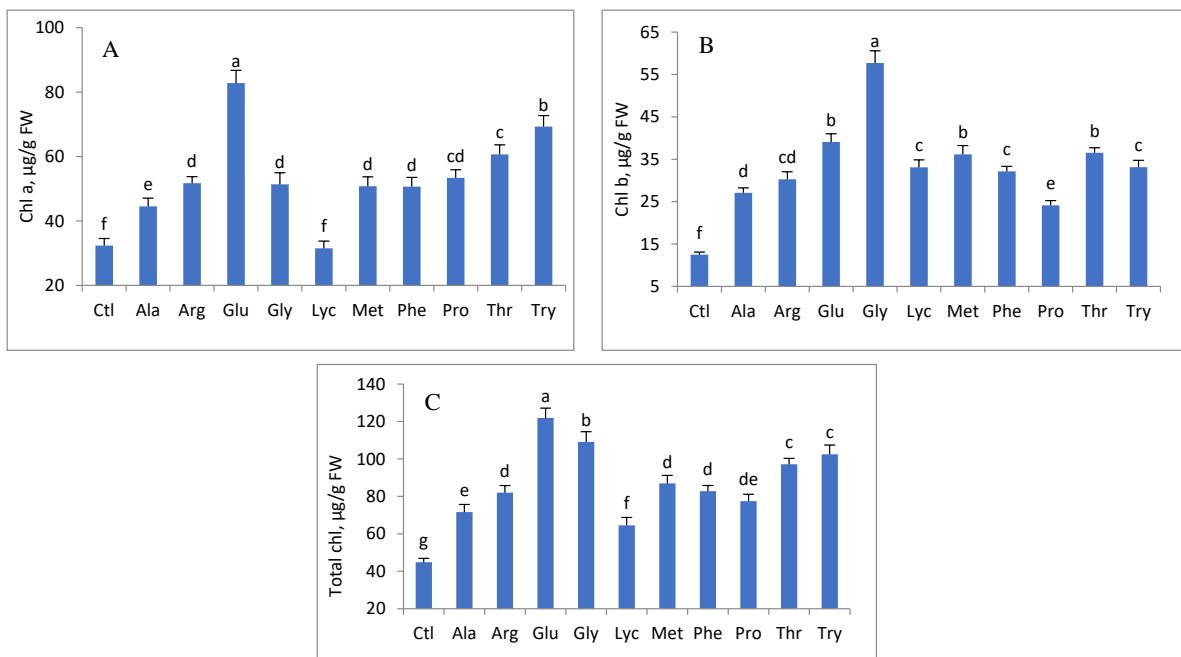
At 14 days after planting (Figure 5A), Glu and Gly treatments registered the heaviest fresh weight (48.0 and 48.5 mg, respectively), representing more than 14% compared with the control treatment. On the other hand, Ala, Arg, Lys, and Met treatments gave the minor FW compared with other treatments; their results statistically did not differ from the control treatment. The same trend also was observed in the accumulation of dry matter contents (DM) in the studied treatments (Figure 5B). The maximum DM was obtained from Glu and Gly with more than (7.5 mg) while the minimum DM was observed at Try treatment (6.0 mg), which contained the highest RWC (87.1%), as shown in Figure 5C.



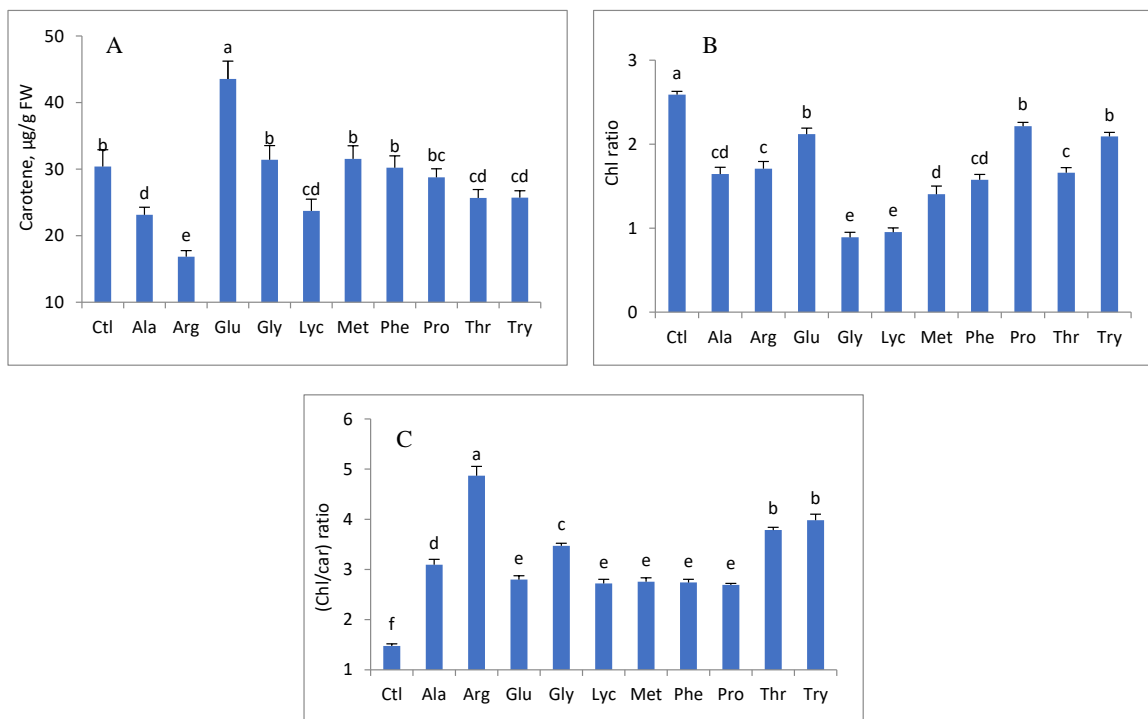
**Figure 5.** Effect of priming onion seeds with exogenous amino acids. (A)—Fresh weight-14, mg; (B)—Dry matter-14, mg; (C)—Relative water contents-14, %. Ctl (Control); Ala (Alanine); Arg (Arginine); Glu (Glutamine); Gly (Glycine); Lys (Lysine); Met (Methionine); Phy (Phenylalanine); Pro (Proline); Thr (threonine); Try (tryptophan). Columns that share the same letter(s) are not statistically significant at  $p \leq 0.01$ .

Photosynthesis is the primary process of recharging a plant for growth and development, and embryonic photosynthesis has a profound function in priming the next stage of the plant. Priming onion seed with Glu formatted the highest chlorophyll a (Chla) contents (82.8  $\mu\text{g/g}$  FW), and the results were significant over other treatments (Figure 6A) and increased by 160% over the control treatment (32.4  $\mu\text{g/g}$  FW), while Lys produced the minor Chla contents in onion shoots (31.5  $\mu\text{g/g}$  FW) compared with other AAs. A different trend was observed in chlorophyll b (Chlb) contents (Figure 6B), whereas Thr achieved the maximum contents (66.5  $\mu\text{g/g}$  FW), while Pro gave the minimum result (24.1  $\mu\text{g/g}$  FW) in comparison with other AAs treatments, while the least Chlb content (12.5  $\mu\text{g/g}$  FW) was observed from the Ctl treatment. When the total chlorophyll contents were calculated, the (Chla + Chlb) in shoot parts showed that all AAs increased the total chlorophyll contents compared with the Ctl. Glu and Thr had the highest amount (122 and 127  $\mu\text{g/g}$  FW, respectively) compared with other treatments (Figure 6C), and the most negligible contents came from Ctr (45  $\mu\text{g/g}$  FW), while the most negligible impact among AAs was observed in Lyc (64  $\mu\text{g/g}$  FW). Carotene contents in the onion seedling shoot were also determined (Figure 7A). Glu gave the highest carotene (Car) contents with 50% over control treatment (30.4  $\mu\text{g/g}$  FW), whereas the minimum content was registered in Arg (16.8  $\mu\text{g/g}$  FW) with a  $-50\%$  under control Chla/Chlb ratio (Figure 7B), which was very high in the Ctl treatment (2.6) but decreased in all AAs treatments. The minimum ratio was obtained at Glu (0.9). The ratio between total chlorophyll and carotene (Chl/Car) was also calculated in this work (Figure 7C).





**Figure 6.** Effect of priming onion seeds with exogenous amino acids. (A)—chlorophyll content, µg/g FW; (B)—chlorophyll b, µg/g FW; (C)—total chlorophyll, µg/g FW. Ctl (Control); Ala (Alanine); Arg (Arginine); Glu (Glutamine); Gly (Glycine); Lys (Lysine); Met (Methionine); Phy (Phenylalanine); Pro (Proline); Thr (threonine); Try (tryptophan). Columns that share the same letter(s) are not statistically significant at  $p \leq 0.01$ .

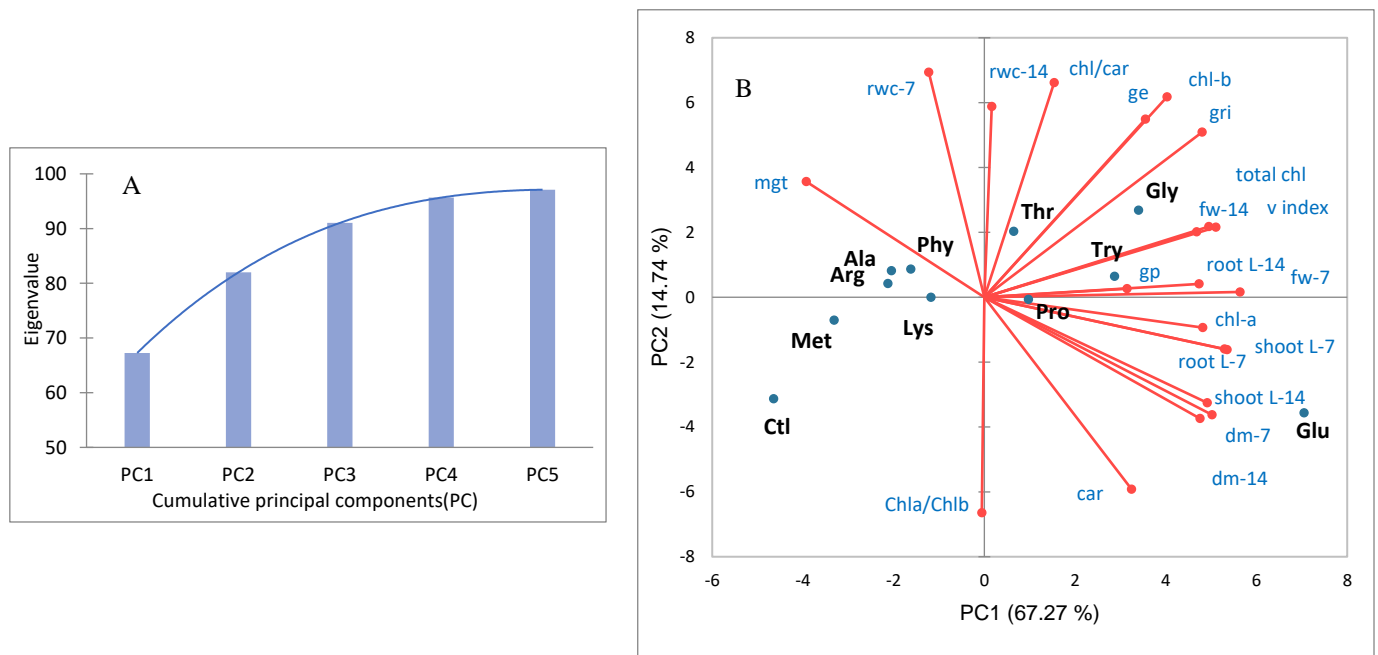


**Figure 7.** Effect of priming onion seeds with amino acids. (A)—Carotene contents, µg/g FW; (B)—Chl a/Chl b ratio; (C)—Chl/Car ratio. Ctl (Control); Ala (Alanine); Arg (Arginine); Glu (Glutamine); Gly (Glycine); Lys (Lysine); Met (Methionine); Phy (Phenylalanine); Pro (Proline); Thr (threonine); Try (tryptophan). Columns that share the same letter(s) are not statistically significant at  $p \leq 0.01$ .

The data showed that the maximum chl/car ratio was observed in Arg (6.0) and Thr (5.0) by 200% and 150% over Ctl (1.8). Most of the applied exogenous amino acids, such as (Glu, Gly, Lyc, Met, Phe, Pro, and Try) gave a pigments ratio ranging between 2.6–3.6.

#### Principal Component Analysis

Principal component analysis (PCA) was calculated to investigate the correlation of the studied parameters of onion seedlings with the different applied amino acids. Figure 8 illustrates that PC1 indicates 67.2% variability, and PC2 indicates 14.8% variability. This PCA biplot represents clear segregation into two clusters among the parameters. The first three components had eigenvalues of more than 90% of the variance (Figure 8A). The PCA biplot shows the loading of different variables on the first two PCA (PC1 and PC2), which explained more than 80% of the variance. All the variables are strongly indicated in the plot, as cleared from the long vectors. All studied variables were positively correlated with PC1 except germination time, relative water contents, and chlorophyll ratios. Half of the examined variables positively correlated with PC2, such as GRI, GE, VI, RWC, Chlb, chl/car ratio. The biplot provided sufficient details of the correlations of the variables. Strong positive correlations were observed between GP, RL, SL, DM, Chla, and Car (Figure 8B).

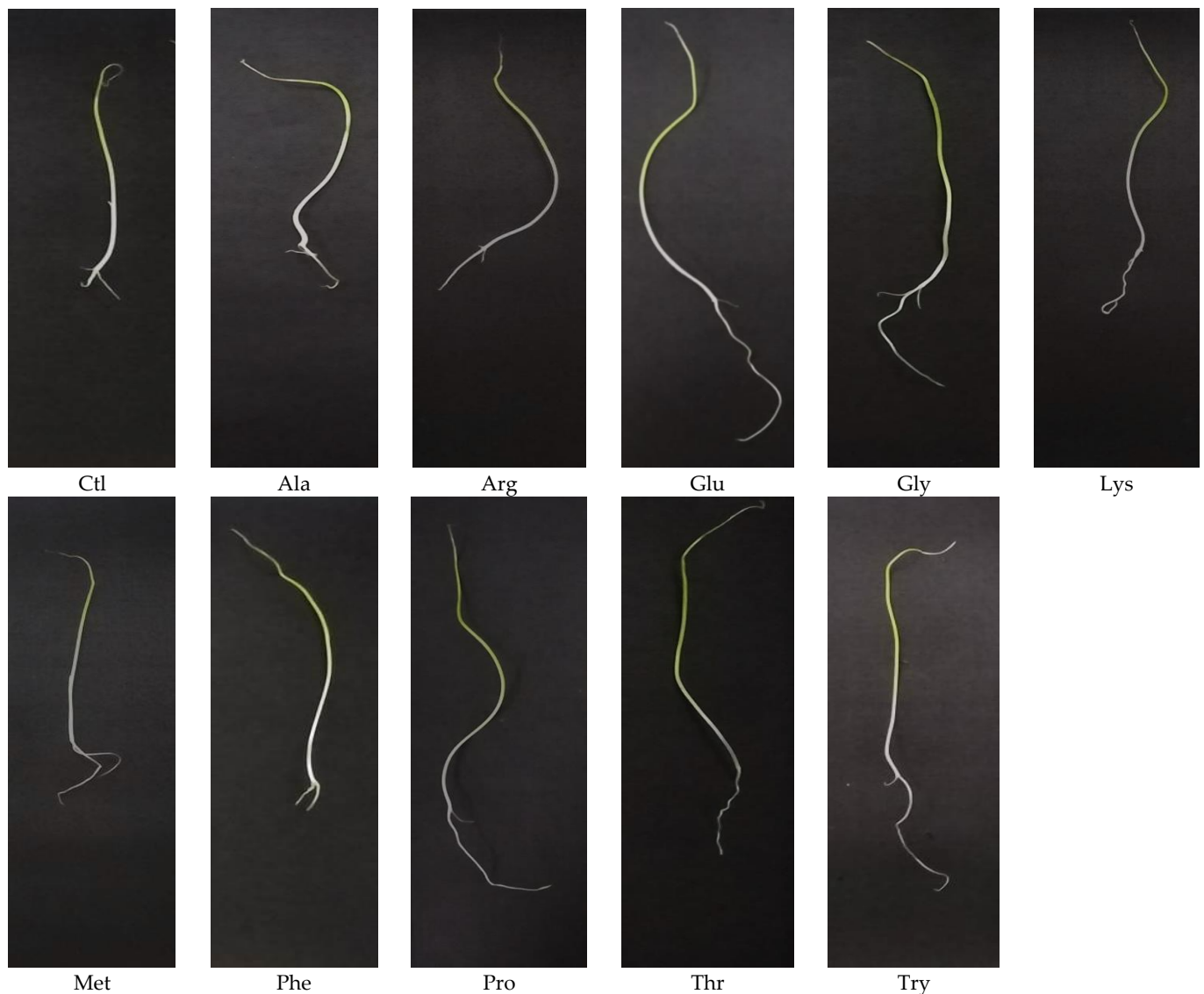


**Figure 8.** (A) Principal component analysis, PC1 and PC2 explained 82.01% of the total variation. (B) PCA components variability of growth, development, and pigment contents of onion seedlings primed with amino acids. Ctl (Control); Ala (Alanine); Arg (Arginine); Glu (Glutamine); Gly (Gly-cine); Lys (Lysine); Met (Methionine); Phy (Phenylalanine); Pro (Proline); Thr (threonine); Try (try-tophan).

#### 4. Discussion

The current study highlights the impact of priming onion seeds with amino acids on germination characteristics and pigment formations. Ten different AAs were applied at 500 ppm (0.5 g/L). The results showed that applying glutamine had the best germination parameters (GP, GRI, VI) and took the least germination time compared with other amino acid treatments. Furthermore, seedling growth and development parameters (shoot length, root length, fresh weight, dry matter, and relative water contents) are positively correlated with amino acid priming, and we can observe that glutamine and tryptophan registered the highest growth parameters. Glutamine enters the plant tissues through the amino acid transporters and serves as an essential nitrogen source for growth and development, and

this demonstrated that onion could effectively utilize glutamine as a nitrogen source. Based on the growth parameters of onion seedlings, such as root and shoot length and weight, we cleared that supplementation of 0.5 g glutamine significantly improved onion seedling growth parameters (Figure 9).



**Figure 9.** Primed onion seedlings with different exogenous amino acids. Ctl (Control); Ala (Alanine); Arg (Arginine); Glu (Glutamine); Gly (Glycine); Lys (Lysine); Met (Methionine); Phe (Phenylalanine); Pro (Proline); Thr (threonine); Try (tryptophan).

These conclusions proposed that glutamine is a primary nitrogen source in plants. The same trend was when the glutamine effect was studied in the growth and development of rice seedlings [55]. Priming improved germination rates in cauliflower and broccoli compared with non-primed under controlled conditions [56]. Primed seeds can be more profitable in farming than non-primed seeds for many reasons, such as reducing plant emergence time [57], which is considered an essential factor for crops to avoid environmental stresses and ensure high crop establishment [58,59].

The role of AAs on plants has been extensively investigated. Studies showed that AAs promote fertilizer assimilation, nutrients, water uptake, and photosynthesis of various vegetable crops [60], enhancing flowering, fruit set, and fruit yield [61,62]. AAs increased plant growth parameters of lettuce [63] and radish [64]. Furthermore, increased yield

quantity and quality in cucumber [65], garlic [66], potato and green bean [41], broccoli [67], and onion [68]. Amino acids have been reported to indicate growth regulator activity and activate plant metabolic processes [69,70].

The impact of seed priming in leaf biochemistry has been broadly studied, with an emphasis given to photosynthetic pigments. Despite leaves, chlorophyll content is an indicator of the plant's vigor [71]. It has been demonstrated that priming seeds can protect chlorophyll or enhance its concentrations under optimal conditions [15]. Priming techniques have also been shown to promote the concentration of carotenoids under normal and stressful conditions [18–20]. At the end of the experiment, photosynthetic pigments (Chla, Chlb, and Car), with contents as mg per g fresh weight of onion seedlings shoots, were performed then; from the data, pigments ratios were calculated. As a result, we observed that the maximum photosynthetic processes were obtained when Glutamine, Glycine, Proline, and Tryptophan were applied. The biostimulation impact of amino acids on plant growth and development has been repeatedly reported [47,72,73]. Due to their nature, low application doses, and effectiveness, amino acids are completely friendly to the ecosystem [74].

By far, glycine is one of the most widely applied AAs in plant nutrition. It is frequently used to produce various amino-chelate fertilizers (amino acid-chelated nutrients). The exogenous application of AAs is generally inverted into enhancing nitrogen status and concentration of nutrient elements in plant tissues [32]. Plants can directly uptake amino acids to promote their growth and development. Glutamine in 0.5 mM concentration enhances the length of shoots and roots and the chlorophyll content of rice seedlings. Glutamate is a highly active amino acid promptly converted to other nitrogen-containing compounds in plant tissues. For instance, glutamate is plants' primary nitrogen donor for most transamination reactions [75]. According to our study, proline application improved onion growth compared with non-treated plants due to enhancing cell membrane integrity, leaf water, and chlorophyll [76]. The growth of primed seeds with proline was improved due to enhancing mineral uptake and photosynthesis in canola [77]. Another study reported that a quarter of proline was found in seeds of Arabidopsis, whereas less than 5% of proline was registered in vegetative tissues, which indicates that proline has the primary function in seed metabolism [78]. Moreover, the additional proline supply via seed priming can be more beneficial during seed germination, seedling growth, and other growth stages [79]. Exogenous proline increased nitrogen metabolism [80], regulated the photosynthesis process [81], improved CO<sub>2</sub> exchange from stomata, and influenced the oxygen pentose phosphate pathway, which is considered a vital process for germination and growth [82,83].

Seed priming with tryptophan (1–100 µM) significantly promoted pepper seed germination and seedling emergence under salt stress conditions [84]. Tryptophan is an essential amino acid that has a vital role in protein biosynthesis and as a precursor of numerous biologically active compounds such as nicotinamide adenine dinucleotide, serotonin, quino-  
linic acid, and kynurenine [85].

## 5. Conclusions

To summarize everything identified so far, priming is a pre-sowing hydration technique that leads to a physiological condition that triggers seed germination and enhances uniformity. Furthermore, seed priming is implicated in promoting various ranges of morphological and biochemical responses. This technique is a cost-effective and less labour-intensive strategy, so the horticultural industry can easily apply this technique because improving the nutritional quality of horticultural produce by priming seeds before cultivation may reduce malnutrition issues, especially in underdeveloped countries. Priming onion seeds with exogenous amino acids is an attractive ecological approach for improving the germination rates of seeds. Priming exogenous amino acids (glutamine, proline, and tryptophan) at a concentration of 0.5 g/L of onion seeds can stimulate rapid and uniform germination and accelerate seedlings' growth.

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## References

- Abdelkader, M.M.; Gaplaev, M.S.; Terekbaev, A.A.; Puchkov, M.Y. The Influence of Biostimulants on Tomato Plants Cultivated under Hydroponic Systems. *J. Hortic. Res.* **2021**, *29*, 107–116. [\[CrossRef\]](#)
- Righini, H.; Roberti, R.; Cetrullo, S.; Flamigni, F.; Quintana, A.M.; Francioso, O.; Panichi, V.; Cianchetta, S.; Galletti, S. *Jania adhaerens* Primes Tomato Seed against Soil-Borne Pathogens. *Horticulturae* **2022**, *8*, 746. [\[CrossRef\]](#)
- Jardin, P.D. Plant biostimulants: Definition, concept, main categories and regulation. *Sci. Hortic.* **2015**, *196*, 3–14. [\[CrossRef\]](#)
- El-Nakhel, C.; Cozzolino, E.; Ottaiano, L.; Petropoulos, S.A.; Nocerino, S.; Pelosi, M.E.; Roupshael, Y.; Mori, M.; Di Mola, I. Effect of Biostimulant Application on Plant Growth, Chlorophylls and Hydrophilic Antioxidant Activity of Spinach (*Spinacia oleracea* L.) Grown under Saline Stress. *Horticulturae* **2022**, *8*, 971. [\[CrossRef\]](#)
- Yakhin, O.I.; Lubyantsev, A.A.; Yakhin, I.A.; Brown, P.H. Biostimulants in Plant Science: A Global Perspective. *Front. Plant Sci.* **2017**, *7*, 2049. [\[CrossRef\]](#) [\[PubMed\]](#)
- Alcazar, R.; Altabella, T.; Marco, F.; Bortolotti, C.; Reymond, M.; Koncz, C.; Carrasco, P.; Tiburcio, A.F. Polyamines: Molecules with regulatory functions in plant abiotic stress tolerance. *Planta* **2010**, *231*, 1237–1249. [\[CrossRef\]](#)
- Lönnerdal, B. Dietary Factors Influencing Zinc Absorption. *J. Nutr.* **2000**, *130*, 1378S–1383S. [\[CrossRef\]](#)
- Atilio, J.B.; Causin, H.F. The central role of amino acids on nitrogen utilization and plant growth. *J. Plant Physiol.* **1996**, *149*, 358–362. [\[CrossRef\]](#)
- Amin, A.; Gharib, F.A.; El-Awadi, M.; Rashad, E.-S.M. Physiological response of onion plants to foliar application of putrescine and glutamine. *Sci. Hortic.* **2011**, *129*, 353–360. [\[CrossRef\]](#)
- Meister, A. Selective Modification of Glutathione Metabolism. *Science* **1983**, *220*, 472–477. [\[CrossRef\]](#)
- Das, C.; Sengupta, T.; Chattopadhyay, S.; Setua, M.; Das, N.K.; Saratchandra, B. Involvement of kinetin and spermidine in controlling salinity stress in mulberry (*Morus alba* L. cv. S1). *Acta Physiol. Plant* **2002**, *24*, 53–57. [\[CrossRef\]](#)
- Zulfiqar, F. Effect of seed priming on horticultural crops. *Sci. Hortic.* **2021**, *286*, 110197. [\[CrossRef\]](#)
- Karim, M.N. Stimulatory effect of seed priming as pretreatment factors on germination and yield performance of yard long bean (*Vigna unguiculata*). *Horticulturae* **2020**, *6*, 104. [\[CrossRef\]](#)
- Sorrentino, M.; De Diego, N.; Ugena, L.; Spichal, L.; Lucini, L.; Miras-Moreno, B.; Zhang, L.; Roupshael, Y.; Colla, G.; Panzarová, K. Seed Priming With Protein Hydrolysates Improves Arabidopsis Growth and Stress Tolerance to Abiotic Stresses. *Front. Plant Sci.* **2021**, *12*, 626301. [\[CrossRef\]](#) [\[PubMed\]](#)
- Al-Amri, S.M. Improved growth, productivity and quality of tomato (*Solanum lycopersicum* L.) plants through application of shikimic acid. *Saudi J. Biol. Sci.* **2013**, *20*, 339–345. [\[CrossRef\]](#) [\[PubMed\]](#)
- Yan, M. Seed priming stimulate germination and early seedling growth of Chinese cabbage under drought stress. *S. Afr. J. Bot.* **2015**, *99*, 88–92. [\[CrossRef\]](#)
- Piri, R.; Moradi, A.; Balouchi, H.; Salehi, A. Improvement of cumin (*Cuminum cyminum*) seed performance under drought stress by seed coating and biopriming. *Sci. Hortic.* **2019**, *257*, 108667. [\[CrossRef\]](#)
- Ashraf, R.; Sultana, B.; Riaz, S.; Mushtaq, M.; Iqbal, M.; Nazir, A.; Atif, M.; Zafar, Z. Fortification of phenolics, antioxidant activities and biochemical attributes of radish root by plant leaf extract seed priming. *Biocatal. Agric. Biotechnol.* **2018**, *16*, 115–120. [\[CrossRef\]](#)
- Nessim, A.; Kasim, W. Physiological Impact of Seed Priming with CaCl<sub>2</sub> or Carrot Root Extract on Lupinus termis Plants Fully Grown under Salinity Stress. *Egypt. J. Bot.* **2019**, *59*, 763–777. [\[CrossRef\]](#)
- Valivand, M.; Amooaghaie, R.; Ahadi, A. Seed priming with H<sub>2</sub>S and Ca<sup>2+</sup> trigger signal memory that induces cross-adaptation against nickel stress in zucchini seedlings. *Plant Physiol. Biochem.* **2019**, *143*, 286–298. [\[CrossRef\]](#)
- Paul, S.; Dey, S.; Kundu, R. Seed priming: An emerging tool towards sustainable agriculture. *Plant Growth Regul.* **2021**, *97*, 215–234. [\[CrossRef\]](#)

22. Abdelkader, M.; Zargar, M.; Murtazova, K.M.-S.; Nakhaev, M.R. Life Cycle Assessment of the Cultivation Processes for the Main Vegetable Crops in Southern Egypt. *Agronomy* **2022**, *12*, 1527. [[CrossRef](#)]
23. Jisha, K.C.; Vijayakumari, K.; Puthur, J.T. Seed priming for abiotic stress tolerance: An overview. *Acta Physiol. Plant* **2012**, *35*, 1381–1396. [[CrossRef](#)]
24. Paparella, S.; Araujo, S.S.; Rossi, G.; Wijayasinghe, M.; Carbonera, D.; Balestrazzi, A. Seed priming: State of the art and new perspectives. *Plant Cell Rep.* **2015**, *34*, 1281–1293. [[CrossRef](#)] [[PubMed](#)]
25. Bryksová, M.; Hybenová, A.; Hernández, A.E.; Novák, O.; Pěňčík, A.; Spíchal, L.; De Diego, N.; Doležal, K. Hormoprimering to Mitigate Abiotic Stress Effects: A Case Study of N9-Substituted Cytokinin Derivatives With a Fluorinated Carbohydrate Moiety. *Front. Plant Sci.* **2020**, *11*, 599228. [[CrossRef](#)] [[PubMed](#)]
26. Carrillo-Reche, J.; Vallejo-Marín, M.; Quilliam, R.S. Quantifying the potential of ‘on-farm’ seed priming to increase crop performance in developing countries. A meta-analysis. *Agron. Sustain. Dev.* **2018**, *38*, 64. [[CrossRef](#)]
27. Gallardo, K.; Job, C.; Groot, S.P.; Puype, M.; Demol, H.; Vandekerckhove, J.; Job, D. Proteomic Analysis of Arabidopsis Seed Germination and Priming. *Plant Physiol.* **2001**, *126*, 835–848. [[CrossRef](#)]
28. Weitbrecht, K.; Müller, K.; Leubner-Metzger, G. First off the mark: Early seed germination. *J. Exp. Bot.* **2011**, *62*, 3289–3309. [[CrossRef](#)]
29. Steinbrecher, T.; Leubner-Metzger, G. The biomechanics of seed germination. *J. Exp. Bot.* **2016**, *68*, 765–783. [[CrossRef](#)]
30. Chen, K.; Arora, R. Priming memory invokes seed stress-tolerance. *Environ. Exp. Bot.* **2013**, *94*, 33–45. [[CrossRef](#)]
31. Teixeira, W.F.; Fagan, E.B.; Soares, L.H.; Umburanas, R.C.; Reichardt, K.; Neto, D.D. Foliar and Seed Application of Amino Acids Affects the Antioxidant Metabolism of the Soybean Crop. *Front. Plant Sci.* **2017**, *8*, 327. [[CrossRef](#)] [[PubMed](#)]
32. Khan, S.; Yu, H.; Li, Q.; Gao, Y.; Sallam, B.N.; Wang, H.; Liu, P.; Jiang, W. Exogenous Application of Amino Acids Improves the Growth and Yield of Lettuce by Enhancing Photosynthetic Assimilation and Nutrient Availability. *Agronomy* **2019**, *9*, 266. [[CrossRef](#)]
33. Selvi, D.T.; Saraswathy, S. Seed viability, seed deterioration and seed quality improvements in stored onion seeds: A review. *J. Hortic. Sci. Biotechnol.* **2017**, *93*, 1–7. [[CrossRef](#)]
34. El-Damarany, A.M.; El-Shaikh, K.A.A.; Obiadalla-Ali, H.A.; Abdel-Kader, M.M. Effect of Mother Bulb Size and Planting Space on Seed Production of Onion (*Allium cepa*, L.) Cultivar Giza 6 Mohassan. *J. Agril. Vet. Sci.* **2015**, *8*, 187–200. [[CrossRef](#)]
35. Khokhar, K. Part 1 Chapter 2 Onion: Seed Viability and Germination. In *Onion—An Ancient Crop and Modern Practices*; Noor Publishing: Pakistan, 2019.
36. El-Damarany, A.M.; El-Shaikh, K.A.A.; Obiadalla-Ali, H.A.; Abdel-Kader, M.M. Effect of Nitrogen and Potassium Fertilization on Seed Production of Onion (*Allium cepa* L.) Improved Giza 6 Cultivar. *Am.-Euras. J. Agric. Environ. Sci.* **2016**, *16*, 1296–1303.
37. Tan, J.W.; Kester, S.T.; Su, K.; Hildebrand, D.F.; Geneve, R.L. Seed Priming and Pericarp Removal Improve Germination in Low-Germinating Seed Lots of Industrial Hemp. *Crops* **2022**, *2*, 407–414. [[CrossRef](#)]
38. Malik, C.P. Seed deterioration: A review. *Int. J. Life Sci. Biotechnol. Pharma Res.* **2013**, *2*, 374–385.
39. Khan, W.; Rayirath, U.P.; Subramanian, S.; Jithesh, M.N.; Rayorath, P.; Hodges, D.M.; Critchley, A.T.; Craigie, J.S.; Norrie, J.; Prithiviraj, B. Seaweed Extracts as Biostimulants of Plant Growth and Development. *J. Plant Growth Regul.* **2009**, *28*, 386–399. [[CrossRef](#)]
40. Colla, G.; Roupael, Y.; Canaguier, R.; Svecova, E.; Cardarelli, M. Biostimulant action of a plant-derived protein hydrolysate produced through enzymatic hydrolysis. *Front. Plant Sci.* **2014**, *5*, 448. [[CrossRef](#)]
41. Abdel-Mawgoud, A.M.R.; El-Bassiouny, A.M.; Ghoname, A.; Abou-Hussein, S.D. Foliar application of amino acids and micronutrients enhance performance of green bean crop under newly reclaimed land conditions. *Aust. J. Basic Appl. Sci.* **2011**, *5*, 51–55.
42. Koukounaras, A.; Tsouvaltzis, P.; Siomos, A.S. Effect of root and foliar application of amino acids on the growth and yield of greenhouse tomato in different fertilization levels. *J. Food Agric. Environ.* **2013**, *11*, 644–648.
43. Bujalski, W.; Nienow, A. Large-scale osmotic priming of onion seeds: A comparison of different strategies for oxygenation. *Sci. Hortic.* **1991**, *46*, 13–24. [[CrossRef](#)]
44. Shooshtari, F.Z.; Souri, M.K.; Hasandokht, M.R.; Jari, S.K. Glycine mitigates fertilizer requirements of agricultural crops: Case study with cucumber as a high fertilizer demanding crop. *Chem. Biol. Technol. Agric.* **2020**, *7*, 19. [[CrossRef](#)]
45. Ambreen, S.; Athar, H.-U.; Khan, A.; Zafar, Z.U.; Ayyaz, A.; Kalaji, H.M. Seed priming with proline improved photosystem II efficiency and growth of wheat (*Triticum aestivum* L.). *BMC Plant Biol.* **2021**, *21*, 502. [[CrossRef](#)]
46. Alfosea-Simón, M.; Zavala-Gonzalez, E.A.; Camara-Zapata, J.M.; Martínez-Nicolás, J.J.; Simón, I.; Simón-Grao, S.; García-Sánchez, F. Effect of foliar application of amino acids on the salinity tolerance of tomato plants cultivated under hydroponic system. *Sci. Hortic.* **2020**, *272*, 109509. [[CrossRef](#)]
47. Noroozlo, Y.A.; Souri, M.K.; Delshad, M. Stimulation Effects of Foliar Applied Glycine and Glutamine Amino Acids on Lettuce Growth. *Open Agric.* **2019**, *4*, 164–172. [[CrossRef](#)]
48. Abdelkader, M.; Geioushy, R.A.; Fouad, O.A.; Khaled, A.G.; Voronina, L. Investigation the activities of photosynthetic pigments, antioxidant enzymes and inducing genotoxicity of cucumber seedling exposed to copper oxides nanoparticles stress. *Sci. Hortic.* **2022**, *305*, 111364. [[CrossRef](#)]
49. Kader, M.A. A comparison of seed germination calculation formulae and the associated interpretation of resulting data. *J. Proc. R. Soc. New South Wales* **2005**, *138*, 65–75.

50. Herrera, R.M.H.; Santacruz-Ruvalcaba, F.; Ruiz-López, M.A.; Norrie, J.; Hernández-Carmona, G. Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.). *J. Appl. Phycol.* **2013**, *26*, 619–628. [\[CrossRef\]](#)
51. Wang, J.; Lu, W.; Tong, Y.; Yang, Q. Leaf Morphology, Photosynthetic Performance, Chlorophyll Fluorescence, Stomatal Development of Lettuce (*Lactuca sativa* L.) Exposed to Different Ratios of Red Light to Blue Light. *Front. Plant Sci.* **2016**, *7*, 250. [\[CrossRef\]](#)
52. Wellburn, A.R.; Lichtenthaler, H. Formulae and Program to Determine Total Carotenoids and Chlorophylls A and B of Leaf Extracts in Different Solvents. In *Advances in Photosynthesis Research*; Springer: Berlin/Heidelberg, Germany, 1984; pp. 9–12.
53. Trineeva, O.V.; Slivkin, A.I. Comparative characteristics of pigment composition from raw materials and oil extract of nettle leaves. *Drug Dev. Regist.* **2016**, *1*, 142–148.
54. Abdelkader, M.M.; Elsayed, H.M.A. Biodiversity of Photosynthetic Pigments, Macronutrients Uptake and Fruit Quality of Tomato Genotypes. *Russ. J. Plant Physiol.* **2022**, *69*, 50. [\[CrossRef\]](#)
55. Kan, C.-C.; Chung, T.-Y.; Juo, Y.-A.; Hsieh, M.-H. Glutamine rapidly induces the expression of key transcription factor genes involved in nitrogen and stress responses in rice roots. *BMC Genom.* **2015**, *16*, 731. [\[CrossRef\]](#)
56. Wu, L.; Huo, W.; Yao, D.; Li, M. Effects of solid matrix priming (SMP) and salt stress on broccoli and cauliflower seed germination and early seedling growth. *Sci. Hortic.* **2019**, *255*, 161–168. [\[CrossRef\]](#)
57. Thakur, M.; Tiwari, S.; Kataria, S.; Anand, A. Recent advances in seed priming strategies for enhancing planting value of vegetable seeds. *Sci. Hortic.* **2022**, *305*, 111355. [\[CrossRef\]](#)
58. Gardarin, A.; Coste, F.; Wagner, M.-H.; Dürr, C. How do seed and seedling traits influence germination and emergence parameters in crop species? A comparative analysis. *Seed Sci. Res.* **2016**, *26*, 317–331. [\[CrossRef\]](#)
59. Wojtyła, Ł.; Lechowska, K.; Kubala, S.; Garnczarska, M. Molecular processes induced in primed seeds—increasing the potential to stabilize crop yields under drought conditions. *J. Plant Physiol.* **2016**, *203*, 116–126. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Sarojnee, D.Y.; Navindra, B.; Chandrabose, S. Effect of naturally occurring amino acid stimulants on the growth and yield of hot peppers. *J. Anim. Plant Sci.* **2009**, *5*, 414–424.
61. Neeraja, G.; Reddy, I.P. Effect of Growth Promoters on Growth and Yield of Tomato cv. Marutham. *J. Res. ANGRAU* **2005**, *33*, 68–70.
62. Fernández, V.; Eichert, T. Uptake of Hydrophilic Solutes Through Plant Leaves: Current State of Knowledge and Perspectives of Foliar Fertilization. *Crit. Rev. Plant Sci.* **2009**, *28*, 36–68.
63. Gunes, A.; Post, W.N.K.; Kirkby, E.A.; Aktas, M. Influence of partial replacement of nitrate by amino acid nitrogen or urea in the nutrient medium on nitrate accumulation in NFT grown winter lettuce. *J. Plant Nutr.* **1994**, *17*, 1929–1938. [\[CrossRef\]](#)
64. Gonzalez, C.; Zheng, Y.; Lovatt, C. Properly timed foliar fertilization can and should result in a yield benefit and net increase in grower income. *Acta Hortic.* **2010**, 273–286. [\[CrossRef\]](#)
65. Kamar, M.E.; Omar, A. Effect of nitrogen levels and spraying with aminal-forte (amino acids salvation) on yield of cucumber and potatoes. *J. Agric. Sci. Mansoura Univ.* **1987**, *12*, 900–907.
66. El-Shabasi, M.S.; Mohamed, S.M.; Mahfouz, S.A. Effect of foliar spray with amino acids on growth, yield and chemical composition of garlic plants. In Proceedings of the Sixth Arabian Conference for Horticulture, Ismailia, Egypt, 20–22 March 2005.
67. Shekari, G.; Javanmardi, J. Effects of foliar application pure amino acid and amino acid containing fertilizer on broccoli (*Brassica oleracea* L. var. *italica*) transplant. *Adv. Crop Sci. Technol.* **2017**, *5*, 280. [\[CrossRef\]](#)
68. Basha, D.; El-Aila, H.I. Response of foliar spraying with amino acids and integrated use of nitrogen fertilizer on radish (*Raphanus sativus* L.) plant. *Int. J. ChemTech Res.* **2015**, *8*, 135–140.
69. Kauffman, G.L.; Kneivel, D.P.; Watschke, T.L. Effects of a biostimulant on the heat tolerance associated with photosynthetic capacity, membrane thermostability, and polyphenol production of perennial ryegrass. *Crop Sci.* **2007**, *47*, 261–267. [\[CrossRef\]](#)
70. Kolomazník, K.; Pecha, J.; Friebrová, V.; Janáčková, D.; Vašek, V. Diffusion of biostimulators into plant tissues. *Heat Mass Transf.* **2012**, *48*, 1505–1512. [\[CrossRef\]](#)
71. Li, Y.; He, N.; Hou, J.; Xu, L.; Liu, C.; Zhang, J.; Wang, Q.; Zhang, X.; Wu, X. Factors Influencing Leaf Chlorophyll Content in Natural Forests at the Biome Scale. *Front. Ecol. Evol.* **2018**, *6*, 64. [\[CrossRef\]](#)
72. Garcia, A.L.; Madrid, R.; Gimeno, V.; Rodriguez-Ortega, W.M.; Nicolas, N.; Garcia-Sanchez, F. The effects of amino acids fertilization incorporated to the nutrient solution on mineral composition and growth in tomato seedlings. *Span. J. Agric. Res.* **2011**, *9*, 852. [\[CrossRef\]](#)
73. Zhou, Z.; Zhou, J.; Li, R.; Wang, H.; Wang, J. Effect of exogenous amino acids on Cu uptake and translocation in maize seedlings. *Plant Soil* **2007**, *292*, 105–117. [\[CrossRef\]](#)
74. Souri, M.K.; Hatamian, M. Aminochelates in plant nutrition: A review. *J. Plant Nutr.* **2019**, *42*, 67–78. [\[CrossRef\]](#)
75. Brian, G.; Lea, P.J. Glutamate in plants: Metabolism, regulation, and signaling. *J. Exp. Bot.* **2007**, *58*, 2339–2358.
76. Semida, W.M.; Abdelkhalik, A.; Rady, M.O.; Marey, R.A.; El-Mageed, T.A.A. Exogenously applied proline enhances growth and productivity of drought stressed onion by improving photosynthetic efficiency, water use efficiency and up-regulating osmoprotectants. *Sci. Hortic.* **2020**, *272*, 109580. [\[CrossRef\]](#)
77. Wahid, A.; Jamil, A. Inducing salt tolerance in canola (*Brassica napus* L.) by exogenous application of glycinebetaine and proline: Response at the initial growth stages. *Pak. J. Bot.* **2009**, *41*, 1311–1319.
78. Chiang, H.-H.; Dandekar, A.M. Regulation of proline accumulation in *Arabidopsis thaliana* (L.) Heynh during development and in response to desiccation. *Plant Cell Environ.* **1995**, *18*, 1280–1290. [\[CrossRef\]](#)

79. Szabados, L.; Saviouré, A. Proline: A multifunctional amino acid. *Trends Plant Sci.* **2010**, *15*, 89–97. [[CrossRef](#)] [[PubMed](#)]
80. Zhang, L.; Becker, D.F. Connecting proline metabolism and signaling pathways in plant senescence. *Front. Plant Sci.* **2015**, *6*, 552. [[CrossRef](#)]
81. Altuntaş, C.; Demiralay, M.; Muslu, A.S.; Terzi, R. Proline-stimulated signaling primarily targets the chlorophyll degradation pathway and photosynthesis associated processes to cope with short-term water deficit in maize. *Photosynth. Res.* **2020**, *144*, 35–48. [[CrossRef](#)]
82. Verslues, P.E.; Sharma, S. Proline Metabolism and Its Implications for Plant-Environment Interaction. *Arab. Book/Am. Soc. Plant Biol.* **2010**, *8*, e0140. [[CrossRef](#)]
83. Signorelli, S.; Dans, P.D.; Coitiño, E.L.; Borsani, O.; Monza, J. Connecting Proline and  $\gamma$ -Aminobutyric Acid in Stressed Plants through Non-Enzymatic Reactions. *PLoS ONE* **2015**, *10*, e0115349. [[CrossRef](#)]
84. Korkmaz, A.; Gerekli, A.; Yakupoğlu, G.; Karaca, A.; Köklü, Ş. Seed treatment with tryptophan improves germination and emergence of pepper under salinity stress. In Proceedings of the XXX International Horticultural Congress IHC2018: II International Symposium on Soilless Culture and VIII International 1273, Istanbul, Turkey, 12–16 August 2018; pp. 441–448.
85. Abbas, S.H.; Muhammad, S.; Saleem, M.; Mahmood, T.; Aziz, I.; Qamar, M.; Majeed, A.; Arif, M. Effect of L-tryptophan on plant weight and pod weight in chickpea under rainfed conditions. *Sci. Tech. Dev.* **2013**, *32*, 277–280.

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