applied sciences

MDPI

Article

Priming of *Solanum melongena* L. Seeds Enhances Germination, Alters Antioxidant Enzymes, Modulates ROS, and Improves Early Seedling Growth: Indicating Aqueous Garlic Extract as Seed-Priming Bio-Stimulant for Eggplant Production

Muhammad Ali [1,†], Sikandar Hayat [1,2,†], Husain Ahmad [1,†], Muhammad Imran Ghani [1], Bakht Amin [1], Muhammad Jawaad Atif [1] and Zhihui Cheng [1,*]

[1] Department of Vegetable Science, College of Horticulture, Northwest A&F University, Yangling 712100, China; muhammadali@nwsuaf.edu.cn (M.A.); sikander@nwsuaf.edu.cn (S.H.); husain@nwsuaf.edu.cn (H.A.); imran_pak@nwsuaf.edu.cn (M.I.G.); bakhtamin96@nwafu.edu.cn (B.A.); jawaadatif@nwafu.edu.cn (M.J.A.)

[2] College of Natural Resource and Environment, Northwest A&F University, Yangling 712100, China

* Correspondence: chengzh@nwafu.edu.cn; Tel.: +86-151-2918-3300

† These authors equally contributed in the manuscript.

Received: 8 May 2019; Accepted: 23 May 2019; Published: 29 May 2019

Abstract: The current study was aimed to evaluate the seed priming potential of AGE (aqueous garlic extracts) to enhance seed germination and early seedling growth of eggplant. Different concentrations (100, 200, and 300 μg mL^{-1}) of AGE were evaluated along with methyl jasmonate (MeJA) and salicylic acid (SA), plant growth regulators with reported seed priming potential whereas, water was taken as a control treatment. Eggplant seeds were primed for 4-, 8-, and 12-h and seed germination traits such germination rate index, germination percentage, mean germination time, and early seedling growth traits such as fresh and dry weights, root, and shoot lengths were observed. Moreover, plant antioxidant enzymes activities and lipid peroxidation levels, soluble protein contents and reactive oxygen species were monitored to establish the stimulatory/inhibitory effects of the treatments. Our results indicate priming potential of AGE, SA, and MeJA to enhance seed germination and early seedling growth in eggplant and the effects were obvious in various morphological and physiological traits. Seed priming significantly altered the antioxidant enzymes activities such as superoxide dismutase (SOD), and peroxidase (POD) with alteration in the reactive oxygen species (ROS). Interestingly, priming duration also affected the bioactivity of these chemicals because seed priming with 300 μg mL^{-1} AGE for 4 h had a positive influence, however, prolonged exposure to the same concentration inhibited the seed germination process and induced oxidative stress on the seedlings with elevated levels of malondialdehyde (MDA) content. We propose AGE seed priming as a bio-stimulant to enhance seed germination and early seedling growth in eggplant, and the results hence lay the foundation for the preparation of garlic-based compounds to improve vegetables production under plastic tunnels and greenhouse production units.

Keywords: seed priming; antioxidants; aqueous garlic extract; ROS; eggplant; germination

1. Introduction

By definition, seed germination is the incorporation of events that commence with the uptake of water by a dry seed and terminate with the elongation of the embryonic axis, resulting into visible germination [1]. It is the primal process in the plants life and may be regarded as the characteristic

phenomenon to establish the proceeding plant growth and development in a particular environment. It is therefore necessary to understand that any alteration, imbalance, or obstacle during this stage may affect or define the lateral development or growth pattern of the plants [2,3]. By nature, each seed is equipped with the necessary food and biochemical drive forces to establish its existence into the provided environment [4]. However, the conditions of the very environment sometimes interact with the seed germination. Various reports show that numerous physiological activities such as antioxidative enzymes [5], hormones such as Abscisic acid (ABA) [6], Indole-3-acetic acid (IAA) [7] and Gibberellic acid (GA_3) [8], starch metabolism [9] and soluble sugars content [10], the redox potential [11] etc., overall influence the germination process and may be considered important parameters to study the germination process in a particular environmental situation. To overcome germination obstacles or to enhance germination potential, numerous methods can be employed such as the use of genetically enhanced/modified seeds bank and chemical priming [12–14]. Priming is seed treatments that result in hydrating the seeds followed by re-drying to activate certain biochemical processes but the radicle protrusion, however, has not occurred yet. Seed priming has been shown to successfully enhance the germination process and alleviate or overcome germination obstacles [15]. Nevertheless, seed priming is suggested to induce resistance against pathogenic attacks [16] or to stimulate the early growth of the plants [17]. Therefore, a well-established root system anticipates improved growth and development of the resulting plants. However, priming potential of a proposed chemical depends on the concentration of the chemical as well as, the priming duration.

Previously, the use of Salicylic Acid (SA) has been reported to enhance seed germination and seedling vigor in eggplants by altering various physiological functions [18]. SA is a hormone signaling molecule suggested to induce defense responses of the plants upon pathogen attack [16]. Moreover, methyl jasmonate (MeJA), which is an endogenous plant growth regulator, regulates plants defense responses and alleviates the stress situations on the plants [19]. Exogenously applied MeJA can enhance primary defense responses of the subject plants and may affect plant growth depending on the concentration applied as reported by Nawaz et al. (2017a,b). MeJA with increased concentrations could inhibit the germination and plant growth of rice as compared to low concentrations [20,21].

Recent advancements into the use of agricultural bio-stimulants have gradually paved the way for the horticulturist community to explore various botanical bio-stimulants to enhance growth, development, and/or the defense system of the subject plants [22]. As the name suggests, a bio-stimulant could be a single biochemical or a formulation of numerous compounds extracted or obtained from organic origin that bear the potential to bring about considerable changes in the physiology of the receiver plant. Based on their activity, they can be further categorized as promotors, inducers, or stimulators [23–25].

Plant derived secondary metabolites have been proposed by various researchers to induce the defense responses or improve the growth and development of the subject plants [26,27]. Furthermore, plant allelopathy has also been suggested to induce signals in the receiving plants which alter their physiological and molecular responses and, therefore, influence their growth and development [28,29]. Garlic among the plant species is been regarded as an allelopathic plant, which influences soil microclimate, enhances biological activities therein, and results in improved plant growth and metabolism. Previously, intercropping with garlic has been suggested to alleviate continuous cropping obstacles [30,31]. Intercropping with garlic has also been proposed to improve cucumber growth and soil properties [32,33]. However, intercropping different plant species creates competition for nutrients and space and may affect the producibility. Apart from intercropping, numerous studies reported the direct influence of garlic extracts and garlic derived allelochemicals on the growth and physiology of various vegetables [34–38]. The presence of organosulfur compounds such as allicin and Diallyl disulfide (DADS) or Diallyl trisulfide (DATS) allows speculation on the bioactivity of garlic extracts in the physiology of receiving plants [34,35,39]. The concentrations of garlic biochemicals may influence the resulting growth of the plants [36], additionally, the method of application also affect the bioactivity of these biochemicals [40–42]. Nevertheless, these studies mostly involve the application of garlic

compounds either as a foliar spray or root fertigation upon the seedlings. The question therefore exists that whether or not garlic allelochemicals bear the potential as a seed-priming bio-stimulant to enhance seed germination, which not only may improve the resulting plant growth but also, if possible, alleviate the environmental stresses ensuring the utility of space, water and nutrients by the treated plants.

The present study demonstrates garlic extracts as plant bio-stimulant to induce priming effects on the biological activities of the eggplant seeds during seed germination and early seedling development. Our study encompasses germination potential and indices, morphological observations (size, weight), and biochemical processes particularly the soluble protein abundance, lipid peroxidation levels (MDA content), antioxidant enzymes activity, and reactive oxygen species modulation, which are often regarded important during early growth establishment in plants. Findings of the current study are therefore of vital significance to establish an understanding of garlic extracts as priming agents to enhance and improve seed germination of eggplant seeds and may probably provide a platform to alleviate autotoxicity or continuous cropping obstacles that significantly impair eggplant production, particularly under the plastic production units in China [31].

2. Materials and Methods

2.1. Preparation of Aqueous Garlic Extract (AGE), Salicylic Acid (SA), and Methyl Jasmonate (MeJA) Solution

The method previously described in Reference [36], was used to prepare AGE (aqueous garlic extracts). Briefly, fresh garlic bulbs of CV G025 were obtained from the garlic germplasm unit, College of Horticulture, Northwest A & F University, China. Randomly selected, 10 g of the sample was completely homogenized in a sterile mortar pestle with 100 mL sterile distilled water and centrifuged at 10,000 rpm for 10 min. The pellet was discarded, and the supernatant was filtered through a 0.22 µm membrane filter. This filtrate was serially diluted to final concentrations of 100 µg mL^{-1}, 200 µg mL^{-1}, and 300 µg mL^{-1}, respectively. Salicylic acid (Bo Di, TianJin, China) was prepared to a final concentration of 0.5 mM. Methyl jasmonate was bought from and prepared to a final concentration of 0.05 mM [16,43].

2.2. Germination of Eggplant Seeds and Seedling Growth in Petri Dishes

Eggplant seeds (cultivar Taikong Qiewang) were used to perform all the experiments. After surface sterilization, these seeds were treated with the above-mentioned concentrations of AGE (100, 200, and 300 µg mL^{-1}), MeJA (0.05 mM), and SA (0.5 mM) by placing 200 seeds in 50 mL for 4, 8-, and 12-h duration, respectively, in a rotary shaker maintained at 25 + 2 °C. For the control treatment, seeds were primed for these mentioned durations with distilled water. The treated seeds were air dried and 50 seeds were selected from each treatment for germination bioassay. The selected seeds were evenly distributed onto a double-layered filter paper enclosed in a petri dish (11 × 7 cm). To each petri dish, 5 mL of distilled water was applied to provide moisture, and the seeds were maintained under dark in a growth chamber under 25 ± 1 °C. Each treatment contained 4 replicates. The seed germination was monitored and watered with 2–3 mL of water on alternative days. Seeds germination (defined as seeds with radicle 3 mm or more in length) was determined by counting the number of germinated seedlings at 24 h intervals over a 7-day period [35]. When the germination rates reached 80%, the obtained seedlings were exposed to light (white light with a luminance of 60 µmol·m^{-2}·s^{-1}).

2.3. Measurement of Morphological Parameters and Germination Efficiency

When the seedlings grew to 10 days, morphological indices were measured. Hypocotyl and root length were measured with the help of Vernier calipers. Fresh weight of the aerial part and the roots were immediately taken using electronical balance and the samples were later put in an oven at 80 °C for 20 h and later, the dry weights were recorded [44]. Data were recorded using 20 seedlings per sample and the mean was calculated.

The germination efficiency was expressed in terms of germination percentage, mean germination time, germination rate index, and coefficient of velocity of germination (CVG) as described by Alsaeedi [45].

2.4. Estimation of Antioxidant Activity (SOD, POD, and CAT) and MDA Content

From treated seedlings, 0.5 g of leaf samples were homogenized in 2 mL of 0.05 M phosphate buffer (pH 7.8) under ice cold conditions. The homogenate was centrifuged with 6 mL of 0.05 M phosphate buffer (pH 7.8) for 20 min at 10,000× g and at 4 °C [46]. The supernatant was further used to estimate the antioxidant enzymes and MDA content. The SOD activity of the treated seedlings was determined by photoreduction of nitroblue tetrazolium (NBT) [47]. To 0.05 mL of the supernatant, 1.5 mL of 0.05 M phosphate buffer (pH 7.8), 0.3 mL 0.1 mM EDTA-Na_2, 0.3 mL 0.13 M methionine, 0.3 mL of 0.75 mmol·L^{-1} NBT, 0.3 mL of 0.02 mmol·L^{-1} riboflavin and 0.25 mL distilled water were added to achieve a total volume of 3 mL. The reaction mix was exposed to fluorescent light (86.86 µmol·m^{-2}·s^{-1}) for 10–20 min and the absorbance was read at 560 nm wavelength on a spectrophotometer (UV-3802, UNICO, MDN, WI, USA). SOD activity was estimated as 50% inhibition of the NBT reduction by superoxide generated from the reaction of photo-reduced riboflavin and oxygen. The total SOD activity was expressed in units per gram of fresh leaves (U g^{-1} Fw). To determine the POD activity, the guaiacol method was used [48]. To 0.5 mL extract, 50 mL of 0.05 M phosphate buffer (pH 7.8), 28 mL guaiacol, and 19 mL 30% hydrogen peroxide (v/v) was added. Increase in absorbance at 470 nm for 3.5 mL of the prepared mix was used recorded for 3 min at an interval of 30 s. Total POD activity was represented as D470 per minute per gram of fresh leaves (U g^{-1} min^{-1}). For the CAT activity, 0.1 mL of extract was mixed with 1.9 mL of 200 mM phosphate buffer (pH 7.0), and 1 mL of 0.3% hydrogen peroxide. The enzyme activity was determined by reading absorbance at 240 nm for 3 min. The CAT activity was obtained as OD at 240 nm min^{-1} g^{-1} [49].

The MDA (Malondialdehyde) content was estimated by a Thiobarbituric acid (TBA) reaction method [50]. 2 mL of the supernatant was heated with 0.6% (w/v) TBA solution [prepared in 5% (v/v) trichloroacetic acid (TCA)] for 10 min in boiling water and cooled to sediment the flocculate thus formed. The absorbance of the resulting solution was read at 450 and 532 nm and subtracted from the absorbance read at 600 nm. Total MDA content was therefore expressed as the amount of substance per gram of fresh leaves (nmol g^{-1} Fw).

2.5. Total Soluble Proteins, Superoxides and Peroxides Determination

The concentration of the superoxide radicle O_2^- in the shoots was determined by incubating intact seedling shoots in a solution containing 10 mM KCN (to inhibit Cu/Zn SODs), 10 mM H_2O_2 (to inhibit Mn and Cu/Zn SODs), 2% (*m*/*v*) SDS (to inhibit Mn and Fe SODs), 80 µM nitro blue tetrazolium chloride (NBT), and 50 mM potassium phosphate (pH 7.0) for 20 min. The shoots were then homogenized and centrifuged at 10,000× g for 5 min. The supernatant was spectrophotometrically analyzed at 600 nm. The superoxide concentration was determined by reading absorbance of the supernatant at 600 nm [44]. Hydrogen peroxide was measured to indicate the level of stress in the seedlings after inoculation. The samples were homogenized in 1 mL of: 0.25 mL of 0.1% Trichloroacetic acid + 0.5 mL of 1 M KI + 0.25 mL of 10 mM potassium phosphate buffer; at 4 °C for 10 min in dark. A control was prepared by replacing KI with water. After incubation, the homogenate was centrifuged at 12,000× g for 15 min at 4 °C. The supernatant was dispensed into UV-microplate wells and incubated at room temperature for 20 min. A calibration curve was generated with the standard H_2O_2 solution and concentration of the unknown (supernatant) was obtained [40]. Protein concentration of the seedling was determined colorimetrically for the total crude extraction of the protein sample according to Reference [51] using bovine serum albumin as standard.

2.6. Statistical Analysis and Preparation of Illustrations

The experiment was performed in a randomized complete block design with 4 replicates. The collected data were subjected to analysis of variance (ANOVA) and differences obtained in the mean values were categorized through least significant difference (LSD) at the 0.05 level probability using SPSS statistical program. The pictures and figures were prepared using Origin-pro version 16, MS Excel 2016 and Photoshop CC, respectively.

3. Results

3.1. Effect of Seed Priming on the Germination Traits of Eggplant

Seed priming with AGE, SA, and MeJA resulted in enhanced germination and the results are presented in Figure 1. The germination percentage was significantly higher in seeds treated with 100–200 μg mL^{-1} of AGE, MeJA, and SA compared to the control treatment and seeds applied with 200 μg mL^{-1} of AGE for 12 h obtained maximum germination percentage. Interestingly, AGE at higher concentrations (300 μg mL^{-1}) greatly reduced germination percentage in a time dependent manner and prolonged exposure (12 h) resulted in the minimum germination percentage, which was at a statistical difference with the control treatment (Figure 1a).

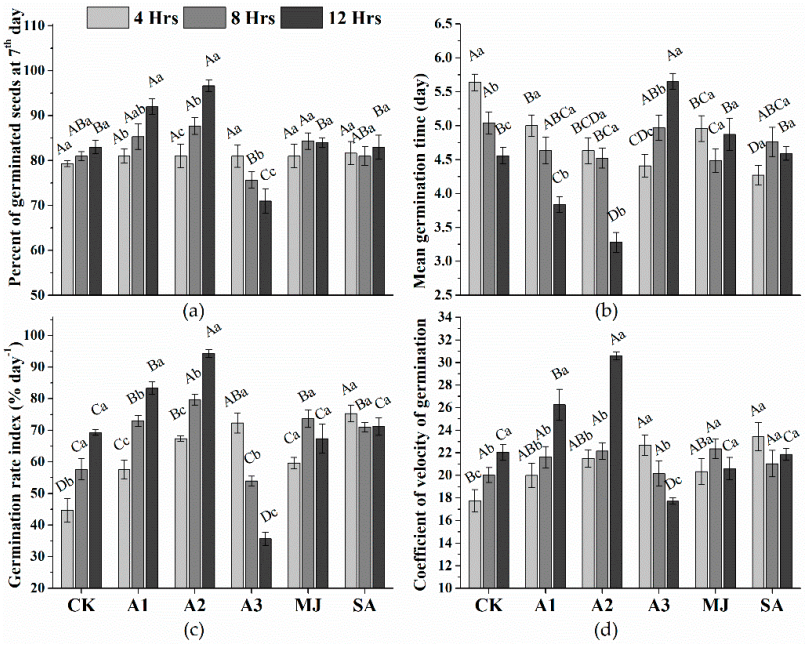

Figure 1. Germination dynamics of AGE (aqueous garlic extracts), methyl jasmonate (MeJA), salicylic acid (SA) and control seedlings of eggplant. (**a**) percent of germinated seeds at 7th day, (**b**) Mean germination time, (**c**) Germination rate index, (**d**) Coefficient of velocity of germination. Ck (Distilled water), A1, A2, A3 (AGE 100, 200, 300 μg mL^{-1}), MJ (MeJA 0.05 mM), SA (Salicylic acid 0.5 mM), whereas 4, 8-, and 12-h are priming durations. Bars represent mean and standard errors (n = 50). Data were subjected to analysis of variance ANOVA. Same letters on the bars bear no significant difference at p = 0.05 (LSD), whereas, capital letters represent a significant difference among chemical treatments and small letters represent a significant difference among the priming durations.

Not only did the germination percentage increase with AGE application, but also the time required by the seed to germinate was shortened. Priming the seeds with 200 μg mL^{-1} of AGE for 12 h facilitated germination in the least amount of time. On the other hand, priming with 300 μg mL^{-1} of AGE for 12 h, imposed stress upon the seeds and extended the germination time. Treatment with 100 μg mL^{-1} of AGE for 12 h and SA for 4 h also reduced the germination time appreciably (Figure 1b).

As compared to the control, the germination rate was highest in seeds treated with 200 μg mL^{-1} of AGE for 12 h and lowest in seeds treated with 300 μg mL^{-1} of AGE for 12 h compared to the control. The effect of 100 μg mL^{-1} AGE and MeJA were non-significant during the 4 h priming, however, the prolonged duration (8 h) resulted in higher germination rates. SA however, had promising effects on the germination rates in all the priming durations (Figure 1c).

The CVG was found to be highest with application of 200 μg mL^{-1} of AGE for 12 h and lowest with 300 μg mL^{-1} of AGE for 12 h (with respect to the control). 100 μg mL^{-1} of AGE for 12 h also showed a significant increase in CVG. Similarly, seeds primed with SA for 4 h resulted higher CVG than those of 8- and 12 h. Furthermore, MeJA priming for 8 h had more prominent effects on CVG than the rest of the priming durations (Figure 1d).

3.2. Seed Priming Promotes Morphological Indices of Eggplant

Seed priming significantly improved various morphological parameters in eggplant. The results for these morphological parameters can be observed in Figure 2, whereas the mean values which describe the overall effects of the treatments on these parameters, are shown in Table 1. Data analysis revealed significant effects of AGE, SA, and MeJA on the morphological growth and the effects correlated to concentrations or the priming duration. It can be observed from Table 1, that the priming duration of 12 h was the most effective in enhancing the morphological parameters of the eggplant seedlings. This was followed by 8 h having an intermediate effect and 4 h having the least positive influence. It can also be observed that 200 μg mL^{-1} of AGE significantly improved the overall morphological characters such as seedling length and fresh or dry weights in comparison to the control treatment. Moreover, 100 μg mL^{-1} of AGE exhibited an enhancing effect which was less, yet very close to the effect obtained by the application of 200 μg mL^{-1} of AGE. MeJA also positively influenced seedling growth, particularly improving shoot length. Compared to the control treatment, seed priming with SA improved the shoot length and fresh weight of the eggplant seedlings. AGE at high concentrations of 300 μg mL^{-1}, however, negatively impacted upon seedling growth and a significant inhibition was observed compared to the rest of the treatments.

The interactive effects of the treatments can be observed in Figure 2. Data analysis showed that priming duration may interact with the potential of the treatments. When the eggplant seeds were primed for a duration of 4 h, the fresh weights of the root and aerial parts of the plant showed a noticeable increment where 0.5 mM SA treatment delivered the highest fresh weight recorded for the aerial parts and roots (11.6 mg and 4.03 mg), followed by 300 μg mL^{-1} AGE (11.1 mg for aerial parts and 3.99 mg for roots). Treatment with MeJA produced an intermediate effect on an increase in fresh weights; 10.3 mg for aerial parts and 3.52 mg for roots. Control seedlings had the lowest levels of fresh weights. Highest dry weights were observed in the seedlings treated with 300 μg mL^{-1} AGE (0.49 mg and 0.26 mg, respectively) followed by SA (0.38 mg for aerial parts and 0.22 mg for root) treatment, whereas the lowest values were observed in the control seedlings. Additionally, considerable root and shoot length enhancement were observed and SA treated seedlings had maximum values (9.4 mm shoot length and 8.95 mm root length), control seedlings comparatively had lower root and shoot lengths (7.9 mm shoot length and 7.0 mm root length).

Similarly, when the seedlings were primed for 8 h with these biochemicals, noticeable increments were observed for fresh and dry weights as well as root and shoot lengths. Interestingly, 200 μg mL^{-1} AGE treated seedlings showed maximum fresh weights, which was 13.6 mg for aerial parts and 5.31 mg for roots. SA did not stimulate any significant increase in the fresh weights when compared to the control seedlings. Treatment with MeJA resulted in an increase in fresh weights (13.2 mg for aerial parts and 5.19 mg for roots). Nonetheless, 300 μg mL^{-1} AGE reduced the fresh weights of aerial parts in comparison to the other concentrations. Similar effects were observed on the dry weights, where 200 μg mL^{-1} AGE, 100 μg mL^{-1} AGE, and MeJA had almost similar results; SA had somewhat positive effects on the dry weight but were non-significant compared to the control. The AGE treatments (100 and 200 μg mL^{-1}) resulted in an increase in the root lengths, however, the highest concentration i.e., 300 μg

mL^{-1} inhibited the root lengths of the seedlings. Similar effects were observed in the seedlings treated with SA.

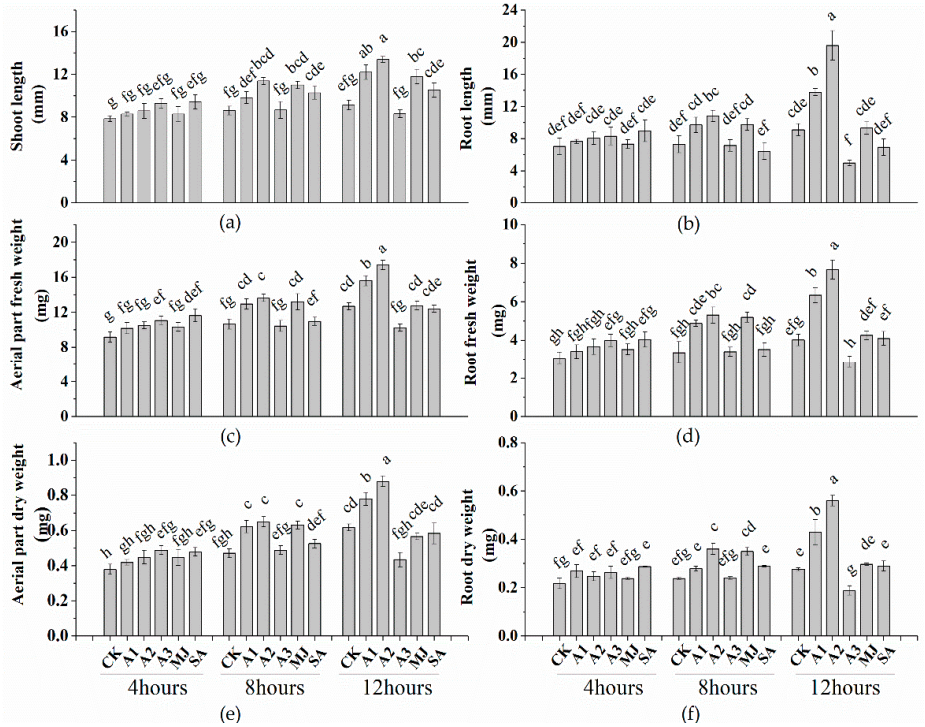

Figure 2. The interaction effects of various priming conditions on the morphological parameters of eggplant. (**a,b**) Shoot and root length, (**c,d**) Fresh weight of aerial part and roots, (**e,f**) Dry weight of aerial part and roots. CK (Distilled water), A1, A2, and A3 (AGE 100, 200, and 300 µg mL^{-1}), MJ (MeJA 0.05 mM) and SA (Salicylic acid 0.5 mM), whereas 4, 8-, and 12-h are priming durations. Bars represent mean and standard errors (n = 20). Data were subjected to analysis of variance (ANOVA) and LSD (p = 0.05). Means followed by same letters have no significant difference.

Table 1. Effect of treatments and priming durations on morphological parameters of eggplant seedlings.

Treatments	Shoot Length (mm)	Aerial Part Fresh Weight (mg $Plant^{-1}$)	Aerial Part Dry Weight (mg $Plant^{-1}$)	Root Length (mm)	Root Fresh Weight (mg $Plant^{-1}$)	Root Dry Weight (mg $Plant^{-1}$)
Treatments						
Control	8.53 ± 0.38c	10.83 ± 0.51de	0.488 ± 0.025cd	7.81 ± 0.94cd	3.47 ± 0.40d	0.243 ± 0.011d
AGE (100)	10.09 ± 0.47b	12.90 ± 0.60b	0.606 ± 0.029a	10.37 ± 0.55b	4.87 ± 0.30ab	0.325 ± 0.030b
AGE (200)	11.11 ± 0.43a	13.83 ± 0.45a	0.659 ± 0.033a	12.81 ± 1.34a	5.54 ± 0.43a	0.388 ± 0.021a
AGE (300)	8.76 ± 0.52c	10.57 ± 0.51e	0.469 ± 0.030d	6.80 ± 0.72d	3.41 ± 0.29d	0.230 ± 0.017d
MeJA	10.36 ± 0.57ab	12.09 ± 0.63bc	0.547 ± 0.028b	8.79 ± 0.67bc	4.32 ± 0.25bc	0.294 ± 0.009bc
SA	10.07 ± 0.64b	11.65 ± 0.55cd	0.528 ± 0.036bc	7.43 ± 1.14cd	3.88 ± 0.36cd	0.288 ± 0.009c
Priming Durations						
4 h	8.62 ± 0.49c	10.47 ± 0.56c	0.442 ± 0.029c	7.88 ± 0.84b	3.61 ± 0.34c	0.253 ± 0.017c
8 h	9.96 ± 0.51b	11.96 ± 0.59b	0.564 ± 0.027b	8.52 ± 0.87b	4.27 ± 0.33b	0.292 ± 0.010b
12 h	10.89 ± 0.52a	13.51 ± 0.48a	0.643 ± 0.034a	10.61 ± 0.98a	4.87 ± 0.34a	0.339 ± 0.021a
F-Test						
Concentrations (C)	***	***	***	***	***	***
Priming Durations (P)	***	***	***	***	***	***
C × P	**	***	***	***	***	***

Values are expressed as the mean ± SE (n = 20); different letters indicate significant differences between means within columns at $p < 0.05$ (LSD). Ck (distilled water), AGE (aqueous garlic extracts, 100, 200, and 300 µg mL^{-1}), MeJA (0.05 mM) and SA (0.5 mM), whereas 4, 8-, and 12-h are priming durations. Data in the treatment section represent the mean data for the particular treatment at the applied priming durations (4, 8, and 12 h), whereas, the data in the priming durations represent the mean data of the particular priming duration observed for the applied treatments to the seeds. *, **, ***, significant at 5, 1, and 0.1% level, respectively.

When the seeds were primed for 12 h, significant effects were observed on the fresh weights (17.4 mg for aerial parts and 7.66 mg for roots) in the seedlings treated with 200 μg mL^{-1}, which compared to the control (12.7 mg for aerial parts and 4.02 mg for root), was at the highest level of significance. On the other hand, a higher concentration of AGE (300 μg mL^{-1}) significantly inhibited the fresh weights compared to the control (from 12.7 mg in control to 10.2 mg) and roots (from 4.02 mg in control to 2.86 mg). The influence on fresh weights of the aerial parts and roots on treatment with MeJA (12.8 mg for aerial parts and 4.25 mg for root) and SA (12.4 mg for aerial parts and 4.09 mg for root) was almost similar to that observed in the control plants. Similar effects were observed in the root dry weights and the maximum concentration of AGE 300 μg mL^{-1} significantly inhibited the dry weights to 0.19 mg when compared to the control (0.28 mg). The highest values (0.56 mg) were recorded for the seeds primed with 200 μg mL^{-1} AGE. Furthermore, the same concentration resulted in the highest shoot and root length, whereas the maximum concentration (300 μg mL^{-1}) negatively influenced these growth parameters indicating the inhibitory effects of AGE at maximum concentration (Figure 3).

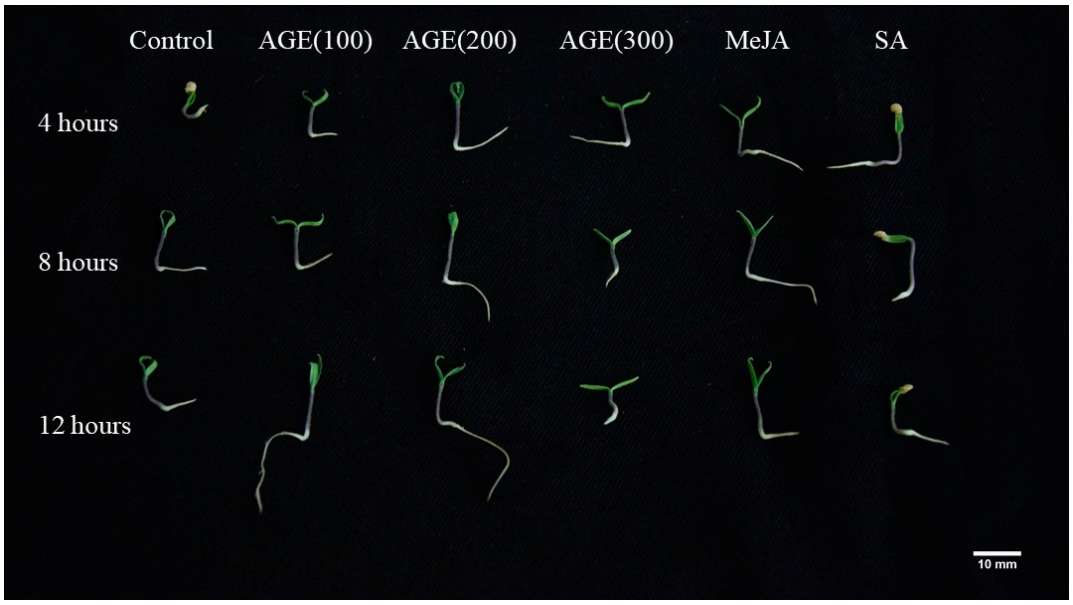

Figure 3. Seedlings growth after germination of the seeds primed with AGE, MeJA, SA, and distilled water. Control (distilled water), AGE (100, 200, and 300 μg mL^{-1}), MeJA (Methyl Jasmonate 0.05 mM), and SA (Salicylic acid 0.5 mM) whereas 4, 8-, and 12-h are the priming durations (Bar = 10 mm).

3.3. Seed Priming Alters the Antioxidant Enzymes and Indicates a Stress Like Response in the Resulted Eggplant Seedlings

Results for the antioxidant enzymes and the lipid peroxidation levels observed in the eggplant seedlings are presented in Figure 4. Data analysis revealed that seed priming had a significant influence on these enzymes activity and the effect relates to the concentration and duration of priming. Among the applied treatments, AGE with 200 μg mL^{-1} for 12 h had the maximum results for the activity of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). Similar effects were observed in the seeds primed with 100 μg mL^{-1} for the same duration. Seed priming with MeJA for 8 h yielded maximum results for SOD activity. Interestingly, SA priming for 4 h resulted in maximum SOD activity compared to the rest of the treatments. On the other hand, when the concentration of AGE increased to 300 μg mL^{-1}, the activity of CAT and POD significantly reduced in comparison to the control treatment, indicating inhibition of antioxidant enzymes activity due to prolonged exposure to higher concentrations of AGE. To indicate stress levels, we observed the lipid peroxidation by measuring the malondialdehyde (MDA) concentrations in the treated eggplant seedlings. As shown, seed priming

with 300 μg mL^{-1} of AGE for 12 h significantly increased the MDA content, followed by seeds primed with MeJA and SA for the same durations, indicating that prolonged priming with these chemicals imposed a stress situation on the seeds.

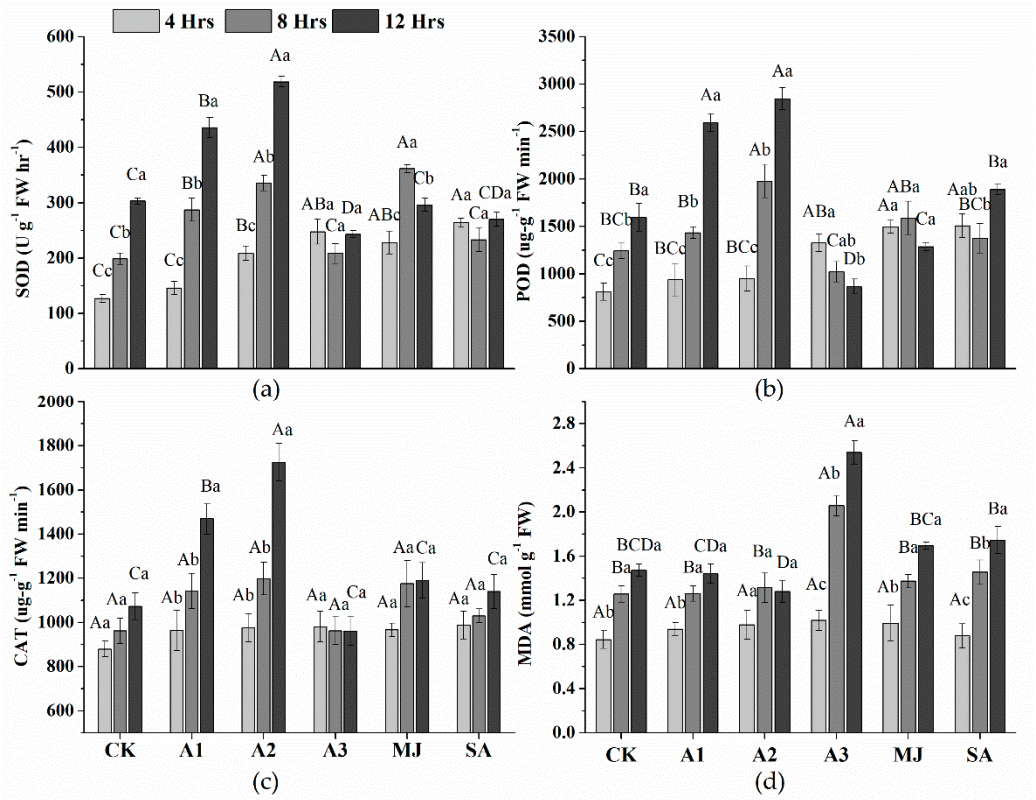

Figure 4. Priming effect of AGE, MeJA, and SA on the antioxidant enzymes and MDA content of eggplant seedlings. (**a**) Superoxide dismutase (SOD), (**b**) peroxidase (POD), (**c**) catalase (CAT) and (**d**) malondialdehyde (MDA). Ck (distilled water), A1, A2, A3 (AGE 100, 200, 300 μg mL^{-1}), MJ (MeJA 0.05 mM), SA (Salicylic acid 0.5 mM), whereas 4, 8-, and 12-h are priming durations. Bars represent mean and standard errors (n = 4). Data were subjected to analysis of variance ANOVA. Same letters on the bars bear no significant difference at p = 0.05 (LSD), whereas, capital letters represent significant difference among chemical treatments and small letters represent significant difference among the priming durations.

3.4. Effect of Seed Priming on the Reactive Oxygen Species Observed in Eggplant Seedlings after Germination

Figure 5 depicts the concentrations of reactive oxygen species observed in the eggplant seedlings after germination. Data analysis revealed that seed priming may influence the production of superoxide radicals and the effect may relate to the concentration of the chemicals and priming duration. As shown, a seed primed with 300 μg mL^{-1} AGE for 12 h had the highest superoxide radical's concentration followed by 8 h priming, indicating the negative or stress-like influence of AGE on the resulted seedlings. Evidently higher concentrations were also observed in the seedlings after priming with 100 and 200 μg mL^{-1} AGE for 8 and 12 h. Seeds primed with either MeJA or SA for 8 h also resulted in elevated levels of superoxide radicals compared to the control seedlings. It is important to notice that seed priming for 4 h did not influence the superoxide concentrations when compared with the control. Similar effects were observed on the hydrogen peroxide (H_2O_2) levels where the seeds priming with 300 μg mL^{-1} AGE for 8 and 12 h resulted in the highest concentrations, indicating severe stress situations in the eggplant seedlings. The prolonged exposure of seeds to AGE (100 and 200 μg mL^{-1}) or MeJA and SA also increased the H_2O_2 concentration in the resulting eggplant seedlings in comparison to the control treatments.

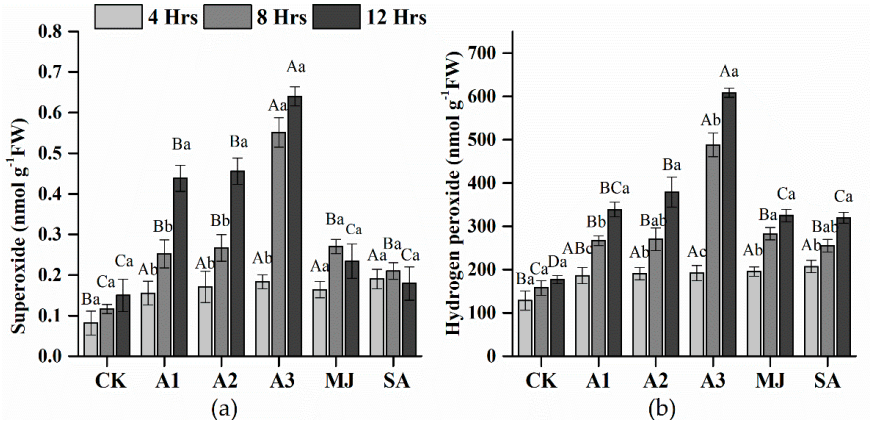

Figure 5. Effect of AGE, MeJA, and SA on the ROS concentration in eggplant seedlings. (**a**) Superoxide, (**b**) Hydrogen peroxide. Ck (distilled water), A1, A2, A3 (AGE 100, 200, 300 µg mL^{-1}), MJ (MeJA 0.05 mM), SA (Salicylic acid 0.5 mM), whereas 4, 8-, and 12-h are priming durations. Bars represent mean and standard errors (n = 4). Data were subjected to analysis of variance ANOVA. Same letters on the bars bear no significant difference at p = 0.05 (LSD), whereas, Capital letters represent significant difference among chemical treatments and small letters represent significant difference among the priming durations.

3.5. Seed Priming of Eggplant Indicates Alterations in the Total Soluble Protein Concentrations in the Obtained Seedlings

The total soluble protein concentrations were determined in the eggplant seedlings germinated after seed priming with distilled water, AGE, MeJA, and SA and the results showed that seed priming influenced the protein concentrations in the resulted seedlings (Figure 6). Data analysis revealed that seeds primed with 100 µg mL^{-1} and 200 µg mL^{-1} AGE for 12 h had maximum protein concentrations followed by MeJA priming for 8 h. The lowest values were observed in the seedlings treated with 300 µg mL^{-1} AGE for 12 h, indicating that the higher concentration of AGE may downregulate the protein abundance. Furthermore, our results indicated that priming duration also influenced the protein abundance and seeds primed with SA for 4 h had higher protein concentrations, whereas, MeJA resulted in higher protein concentrations when seeds were primed for 8 h.

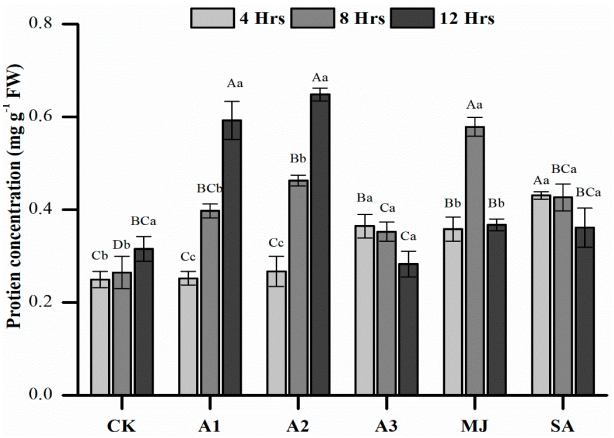

Figure 6. Total soluble protein concentration in eggplant seedlings after germination. Ck (distilled water), A1, A2, A3 (AGE 100, 200, 300 µg mL^{-1}), MJ (MeJA 0.05 mM), SA (Salicylic acid 0.5 mM), whereas 4, 8-, and 12-h are priming durations. Bars represent mean and standard errors (n = 4). Data were subjected to analysis of variance ANOVA. Same letters on the bars bear no significant difference at p = 0.05 (LSD), whereas, capital letters represent significant difference among chemical treatments and small letters represent significant difference among the priming durations.

4. Discussion

The observed data suggested biological influence of AGE on various physiological processes during seed germination of eggplant. The effect, however, was proportional to the concentration of AGE and priming duration. Additionally, SA and MeJA also influenced the seed germination and early seedlings growth, indicating significant influence of these growth regulators on seed priming.

Based on the results obtained, it was observed that the concentration of AGE influenced the germination processes in a dose dependent manner. Seeds applied with 100 μg mL^{-1} and 200 μg mL^{-1} AGE showed an increase in germination percentage, germination rate, and CVG. It is important to consider that the increase in the germination traits was concentration and priming duration dependent. The highest concentration of AGE inhibited these traits when the seeds were primed for prolonged (12 h) durations. Similar effects were observed for the enzymes such as SOD, and POD activities, where a concentration dependent effect was observed. These findings strongly suggest that the biological influence of AGE depends on its concentration and duration of the application. Previously, same concentrations of AGE have been reported to impose significant influence on the growth and physiology of subject plants [40–42], and therefore strongly agree to our results. Seed primed with MeJA and SA also exhibited alterations in the germination traits and the antioxidant enzymes activities, with MeJA being slightly more effective than SA. Previously, SA has been reported to induce defense responses and improve seed germination traits when the eggplant seeds were primed with various concentrations [16], which strongly agree with our findings that SA primed seeds have improved seed germination and seedling vigor. Furthermore, a study reported by Sheteiwey et al. (2018) suggested that priming with MeJA alleviates osmotic stress in rice by altering the physiological processes in the germinating seedlings and our findings are in strong agreement to the biological influence of MeJA as seed priming. The prolonged exposure of these seeds to higher concentration of AGE i.e., 300 μg mL^{-1}, however, negatively influenced the seedlings and the lipid peroxidation levels (MDA concentration) were significantly higher, indicating the onset of oxidative stress. These findings agree to the previously established dose dependent effects of garlic derived compounds [34,35]. Moreover, it has been reported that AGE application triggers antioxidant enzymes in cucumber, eggplant, tomato, and pepper seedlings [36,40–42], which are in line with current results. Antioxidant enzymes are the key players to counteract various stresses [52,53] and help sustain the growth and development of the plants. Elevated reactive oxygen species such as O_2^- and H_2O_2 were observed in the seedlings treated with different concentrations of AGE and the SA or MeJA treatments. These findings signify the role of seed priming in stimulated seeds germination and the resulting seedlings growth and development. As previously described, during cellular metabolism, numerous processes produce ROS, and these ROS signal a vast array of cellular responses to sustain normal plant growth [54]. Particularly during seed germination, these ROS play a pivotal role in the cell division and elongation processes [55,56]. The elevated amounts observed in current findings not only indicate a stress situation but also, it is likely associated with enhanced cellular metabolic processes. The elevation in the antioxidant enzymes, therefore, indicates that these seedlings possessed a more stable and elaborate physiological state where the possible negative influence of the ROS (e.g., cellular destruction or DNA denaturation) was effectively controlled and the seedlings therefore exhibited significant morphological growth [52]. On the other hand, when the seeds were exposed to higher concentrations of AGE for prolonged periods, the activity of antioxidant enzymes degraded and the ROS elevation caused oxidative burst responses, therefore inhibiting the seedling growth [53,57,58]. Additionally, priming also induced incrementing effects on the soluble protein content of the seeds where the highest amounts were observed in the seedlings treated with AGE. Seed priming with SA also induced an increased amount of protein production in the seedlings.

Seed priming enhanced germination process and physiological activity of the eggplant seedlings resulting in enhanced morphological growth of the seedlings. We observed increments in the hypocotyl length, root length, and fresh and dry weight of the seedlings. These findings clearly indicate that due to various treatments, the cocktail of physiological responses such as ROS and antioxidative responses,

as well as the soluble proteins, resulted in enhanced seedling growth and development as compared to those of the control seedlings. Nevertheless, the effect was concentration dependent and the higher concentrations of AGE impacted negatively upon the treated seeds, resulting in stunted or poor growth and development in the obtained seedlings.

Current findings not only signify the role of seed priming in the early growth of eggplant, but also, implicates the potential of garlic derived compounds to enhance seed germination and improve early seedling growth of the subjected seeds. To strengthen our hypothesis for the possibility of AGE as seed priming biomolecules, it is important to understand and consider the significant role of the studied physiological players (ROS and antioxidant enzymes) during seed germination and early seedlings growth of the plants. ROS such as $O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ are most often produced during cellular metabolism [59]. The production of these ROS, however, implicates a number of biological responses in the plants [58,60]. One aspect of these ROS is the perception of stress or stress like stimulus on the plants, which triggers the overproduction of these ROS to stress encounters, for example during a pathogenic attack. These ROS, therefore, induce cellular destruction to seize and control the spread of pathogenic growth in the affected areas [57,61,62]. Moreover, previous studies also suggest that abiological stresses such as salt and drought may induce higher concentrations of ROS in the cells, causing cellular destructions and impairing the growth of the plants [63,64]. However, a plethora of reports describes these ROS as signaling molecules inside the plants to govern various molecular responses such as gene translation or transcription and may therefore be considered pivotal for the normal life pace of these plants [58]. Additionally, ROS have been reported to play an active role during seed germination in cellular metabolism, cellular expansion, and division [55,56]. In addition, ROS production release dormancy as reported by Morad Shaban, 2014, sunflower seeds treated with ROS producing chemicals had significantly higher seed germination in comparison to the dormant, non-treated seeds [65]. Furthermore, ROS also involve in the carbonylation of biomolecules particularly proteins, resulting cellular damages, therefore, regulation of ROS is important to establish seed quality [66]. Certain scavengers that regulate and avoid the overproduction of ROS may be of potential to sustain seed quality and biological activities in the seeds, for example, methionine sulfoxide reductase (MSR), which repairs the oxidized methionine (Met) in methionine sulfoxide (MetO) and is a ROS scavenger involved in seed maturation and germination, which is suggested to be strongly related to seed longevity [67], strengthening our hypothesis that ROS regulation may be involved in the seed germination and early seedling growth after the seeds were primed with a particular concentration and appropriate duration. As discussed above, current findings revealed significant activities of certain ROS and their respective counterparts (antioxidant enzymes), indicating considerable influence of AGE on the cellular processes of the treated seeds during seed germination and early seedling growth. When the seeds are subjected to concentrations ranging up to 200 $\mu g\ mL^{-1}$, the redox balance of the seedlings is maintained, resulting in a boosted growth pattern of the obtained seedlings. However, at higher concentrations, the balance is perturbed, inhibiting the growth of the seedlings. Similarly, MeJA and SA result differently depending on the priming duration, which influence the ROS abundance and antioxidant enzymes activities. These findings further signify that priming induces biological influence on the eggplant seeds and confirms AGE as a bio-stimulant to alter the redox potential of the resulting seedlings.

Seed germination is a complex process and should be understood as the basic phenomenon to establish plant growth and development [2]. In horticultural produce, particularly in vegetable production, different crops encounter autotoxicity due to continuous cropping such as observed in the eggplant [68]. Thus, seed germination is sometimes negatively impacted by these hurdles, causing a significant reduction in the seedling development and plant production [69]. Intercropping with garlic has been reported to overcome these obstacles in eggplant production and garlic root exudates are reported to influence numerous physiological responses in these plants [31,70]. However, intercropping may sometimes compromise the space and nutrients from the cropping system and affect the producibility of the desired crops. As our results show significant responses in the eggplant seedlings

physiological and morphological growth patterns, it can be suggested that the application of AGE as a seed priming agent could possibly be a more effective way to overcome certain challenges during early growth of eggplant production and may therefore be proposed as alternative to intercropped garlic. Moreover, application of SA and MeJA has been reported to enhance seed germination and overcome various stress conditions, nevertheless, it can be noticed that the application of these substances is condition specific and, therefore, so is the resulted roles of these substances [71–73]. Current findings strongly suggest that the potential of AGE to stimulate the cellular metabolism is comparatively higher to those of the SA and MeJA and thus, indicate a promising role of AGE as a biological compound to stimulate and enhance the plant growth and development. Furthermore, garlic extracts contain carbohydrates, sugars, vitamins, minerals, and organosulfur compounds [39,74,75]. Application of these substances may improve the plant growth and enhance photosynthates abundance resulting in a pronounced growth [42].

The presence of allelochemicals such as DADS can trigger numerous physiological and molecular responses in the subject plants, enhancing cellular division and roots elongation [34,35], indicating that AGE is biologically active inside the plant cells. Nevertheless, the biological effects of AGE depend on concentration, hence exposure to high concentrations may negatively influence the miniature plants during seed germination. Usage of AGE as a priming agent to induce biological processes during seed germination, however, requires future advanced study approaches such as cell division or genetic approaches and our results therefore lay foundations not only to understand these processes, but also to prepare a garlic derived seed priming agent for enhanced vegetable production, in particular under plastic tunnel or greenhouse production units.

5. Conclusions

The outcome of the study is the positive effects of seed priming on eggplant seed germination and early seedlings growth. It is thoughtfully concluded that AGE at a concentration of 200 μg mL^{-1} for 12 h was the best priming treatment. Additionally, MeJA and SA also improved seed germination and early seedling growth, which confirm previously suggested functions of these plant growth regulators to induce priming effects on the seed germination of various plants. Our findings indicated that seed priming stimulated germination and enhanced numerous physiological responses such as antioxidant enzymes, ROS modulation, and soluble protein abundance in the randomly selected eggplant seeds. These physiological responses may, therefore, be suggested to have influenced the morphological improvements such as root/shoot length and fresh and dry weights. AGE could also successfully improve the germination properties of the eggplants by reducing the time taken to germinate and increase the number of seedlings germinated in a given time. Furthermore, stimulated antioxidant enzymes system may be important for the protection and maintenance during long-term survival of the resulting plants. Current findings also provide a platform for future research work to understand the biological functions of AGE, MeJA, and SA during seed germination involving ROS regulation and the scavenging through antioxidant enzymes or, the accumulation of soluble proteins interacting with the germinating seeds. Preparation of AGE is, nonetheless, handy and economical, and bears less or no potential hazards to the users and thus is proposed as a promising seed priming agent for enhanced seed germination and improved growth during early stages of plant development. Although the results are very convincing, additional studies involving molecular approaches and evaluating the anti-pathogenic effects of AGE treated plants should be performed.

Author Contributions: Conceptualization, M.A., S.H. and Z.C.; data curation, H.A., M.I.G., B.A. and M.J.A.; formal analysis, M.A. and H.A.; Funding acquisition, Z.C.; investigation, M.A., H.A. and M.I.G.; methodology, M.A. and S.H.; project administration, Z.C.; Resources, Z.C.; software, M.A.; supervision, Z.C.; Validation, M.A., S.H. and Z.C.; writing—original draft, M.A.; Writing—review and editing, M.A. and S.H.

Funding: This research received financial support from the Shaanxi Provincial Sci-Tech Innovation project (Grant Number 2016KTCL02-01) and National Natural Science Foundation of China (Grant Number 31772293).

Acknowledgments: The authors would like to extend their sincere appreciation to the China Scholarship Council and Shaanxi Provincial Science and Technology Innovation project of China for their financial support.

Conflicts of Interest: The authors have no conflict of interests to declare.

Abbreviations

SOD	superoxides dismutase
POD	peroxidase
CAT	catalase
MDA	malondialdehyde
ROS	reactive oxygen species
DADS	diallyl disulfide
DATS	diallyl trisulfide
ABA	Abscisic acid
IAA	Indole-3-acetic acid
GA3	Gibberellic acid
SA	Salicylic acid
MeJA	Methyl Jasmonate
DADS	Diallyl disulfide
DATS	Diallyl trisulfide
CVG	coefficient of velocity of germination

References

1. Bewley, J.D. Seed Germination and Dormancy. *Plant Cell Online* **1997**, *9*, 1055–1066. [CrossRef] [PubMed]
2. Bewley, J.D.; Bradford, K.J.; Hilhorst, H.W.M.; Nonogaki, H. Seeds physiology of development. In *Germination and Dormancy*; Springer: New York, NY, USA, 2013; ISBN 978-1-4614-4692-7.
3. Rosental, L.; Perelman, A.; Nevo, N.; Toubiana, D.; Samani, T.; Batushansky, A.; Sikron, N.; Saranga, Y.; Fait, A. Environmental and genetic effects on tomato seed metabolic balance and its association with germination vigor. *BMC Genom.* **2016**, *17*, 1047. [CrossRef] [PubMed]
4. Steinbrecher, T.; Leubner-Metzger, G. The biomechanics of seed germination. *J. Exp. Bot.* **2017**, *68*, 765–783. [CrossRef] [PubMed]
5. Guan, Y.; Hu, J.; Wang, X.; Shao, C. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *J. Zhejiang Univ. Sci. B* **2009**, *10*, 427–433. [CrossRef] [PubMed]
6. Seo, M.; Nambara, E.; Okamoto, M.; Kamiya, Y.; Tatematsu, K.; Yano, R.; Seo, M.; Kamiya, Y.; Nambara, E.; Okamoto, M.; et al. Abscisic acid and the control of seed dormancy and germination. *Seed Sci. Res.* **2010**, *20*, 55–67. [CrossRef]
7. Zhao, Y.; Liu, X.; Feng, Z.; Li, Q.; Luan, S.; Zhang, H.; Yang, H.-Q.; Li, J.; He, Z.-H. Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated ABI3 activation in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 15485–15490. [CrossRef]
8. Tran, L.-S.P.; Khan, M.A.; An, P.; Li, W.; Liu, X.; Yamaguchi, S. Roles of Gibberellins and Abscisic Acid in Regulating Germination of Suaeda salsa Dimorphic Seeds Under Salt Stress. *Front. Plant Sci.* **2016**, *6*, 1235. [CrossRef]
9. Liu, Y.; Han, C.; Deng, X.; Liu, D.; Liu, N.; Yan, Y. Integrated physiology and proteome analysis of embryo and endosperm highlights complex metabolic networks involved in seed germination in wheat (*Triticum aestivum* L.). *J. Plant Physiol.* **2018**, *229*, 63–76. [CrossRef] [PubMed]
10. Ferreira, C.D.S.; Piedade, M.T.F.; Tiné, M.A.S.; Rossatto, D.R.; Parolin, P.; Buckeridge, M.S. The role of carbohydrates in seed germination and seedling establishment of Himatanthus sucuuba, an Amazonian tree with populations adapted to flooded and non-flooded conditions. *Ann. Bot.* **2009**, *104*, 1111–1119. [CrossRef]
11. Wojtyla, Ł.; Lechowska, K.; Kubala, S.; Garnczarska, M. Different modes of hydrogen peroxide action during seed germination. *Front. Plant Sci.* **2016**, *7*, 66. [CrossRef]

12. Li, J.; Jian, H.; Wang, J.; Wei, L.; Li, C.; Wang, T.; Liu, L. Identification of microRNAs actively involved in fatty acid biosynthesis in developing *Brassica napus* seeds using high-throughput sequencing. *Front. Plant Sci.* **2016**, *7*, 1570. [CrossRef]
13. Savvides, A.; Ali, S.; Tester, M.; Fotopoulos, V. Chemical priming of plants against multiple abiotic stresses: Mission Possible? *Trends Plant Sci.* **2016**, *21*, 329–340. [CrossRef]
14. Saberi, M.; Shahriari, A.; Tarnian, F.; Jafari, M.; Safari, H. Influence of some chemical compounds on germination and early seedling growth of two range species under allelopathic conditions. *Front. Agric. China* **2011**, *5*, 310–321. [CrossRef]
15. Ibrahim, E.A. Seed priming to alleviate salinity stress in germinating seeds. *J. Plant Physiol.* **2016**, *192*, 38–46. [CrossRef]
16. Mahesh, H.M.; Murali, M.; Anup Chandra Pal, M.; Melvin, P.; Sharada, M.S. Salicylic acid seed priming instigates defense mechanism by inducing PR-Proteins in *Solanum melongena* L. upon infection with *Verticillium dahliae* Kleb. *Plant Physiol. Biochem.* **2017**, *117*, 12–23. [CrossRef]
17. 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]
18. Zhang, Y.; Liu, H.; Shen, S.; Zhang, X. Improvement of eggplant seed germination and seedling emergence at low temperature by seed priming with incorporation SA into KNO 3 solution. *Front. Agric. China* **2011**, *5*, 534–537. [CrossRef]
19. Król, P.; Igielski, R.; Pollmann, S.; Kepczyńska, E. Priming of seeds with methyl jasmonate induced resistance to hemi-biotroph *Fusarium oxysporum* f.sp. *lycopersici* in tomato via 12-oxo-phytodienoic acid, salicylic acid, and flavonol accumulation. *J. Plant Physiol.* **2015**, *179*, 122–132. [CrossRef]
20. Nawaz, A.; Sheteiwy, M.S.; Khan, S.M.; Hu, Q.; Guan, Y.; Bukhari, S.A.H.; Luo, Y.; Hu, J. Optimization of germination inhibitors for controlling pre-harvest sprouting in hybrid rice. *Pak. J. Agric. Sci.* **2017**, *54*, 261–270. [CrossRef]
21. Nawaz, A.; Sheteiwy, M.S.; Khan, S.M.; Bukhari, S.A.H.; Dawood, M.; Guan, Y.; Hu, J. Exploring the mechanism of exogenous applied Methyl jasmonate for germination inhibition in hybrid rice. *Pak. J. Life Soc. Sci.* **2017**, *15*, 60–71.
22. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41. [CrossRef]
23. Inderjit; Mukerji, K.G. Allelochemicals: Biological control of plant pathogens and diseases. In *Disease Management of Fruits and Vegetables*; Inderjit Mukerji, K.G., Ed.; Disease Management of Fruits and Vegetables; Springer Science & Business Media: Berlin, Germany, 2006; Volume 2, pp. 1–211. ISBN 9781402044458.
24. Andresen, M.; Wulff, E.G.; Mbega, E.R.; Stokholm, M.S.; Glazowska, S.E.; Zida, P.E.; Mabagala, R.B.; Lund, O.S.; Stokholm, M.S.; Mbega, E.R.; et al. Seed treatment with an aqueous extract of *Agave sisalana* improves seed health and seedling growth of sorghum. *Eur. J. Plant Pathol.* **2014**, *141*, 119–132. [CrossRef]
25. Inagaki, H.; Yamaguchi, A.; Kato, K.; Kageyama, C.; Iyozumi, H. Induction of systemic resistance to anthracnose in cucumber by natural components of *Allium* vegetables and shiitake mushrooms. *Sci. Against Microb. Pathog.* **2011**, 728–735.
26. Foyer, C.H.; Shigeoka, S. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiol.* **2010**, *155*, 93–100. [CrossRef]
27. Zhang, S.; Moyne, A.-L.L.; Reddy, M.S.; Kloepper, J.W. The role of salicylic acid in induced systemic resistance elicited by plant growth-promoting rhizobacteria against blue mold of tobacco. *Biol. Control* **2002**, *25*, 288–296. [CrossRef]
28. Zytynska, S.E.; Hanna, R.; Houmgny, R.; Weisser, W.W.; Singh, A. Reduce pests, enhance production: Benefits of intercropping at high densities for okra farmers in Cameroon. *Pest Manag. Sci.* **2017**, *73*, 2017–2027. [CrossRef]
29. Yang, F.; Chen, P.; Liu, X.; Shu, K.; Wang, X.; Yong, T.; Du, Q.; Yang, W.; Lei, L.; Hussain, S.; et al. Effects of reduced nitrogen inputs on crop yield and nitrogen use efficiency in a long-term maize-soybean relay strip intercropping system. *PLoS ONE* **2017**, *12*, e0184503. [CrossRef]
30. Liu, T.; Cheng, Z.; Meng, H.; Ahmad, I.; Zhao, H. Growth, yield and quality of spring tomato and physicochemical properties of medium in a tomato/garlic intercropping system under plastic tunnel organic medium cultivation. *Sci. Hortic.* **2014**, *170*, 159–168. [CrossRef]

31. Wang, M.; Wu, C.; Cheng, Z.; Meng, H. Growth and physiological changes in continuously cropped eggplant (*Solanum melongena* L.) upon relay intercropping with garlic (*Allium sativum* L.). *Front. Plant Sci.* **2015**, *6*, 262. [CrossRef]
32. Du, L.; Huang, B.; Du, N.; Guo, S.; Shu, S.; Sun, J. Effects of garlic/cucumber relay intercropping on soil enzyme activities and the microbial environment in continuous cropping. *HortScience* **2017**, *52*, 78–84. [CrossRef]
33. Xiao, X.; Cheng, Z.; Meng, H.; Khan, M.A.; Li, H. Intercropping with garlic alleviated continuous cropping obstacle of cucumber in plastic tunnel. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **2012**, *62*, 696–705. [CrossRef]
34. Ren, K.; Hayat, S.; Qi, X.; Liu, T.; Cheng, Z. The garlic allelochemical DADS influences cucumber root growth involved in regulating hormone levels and modulating cell cycling. *J. Plant Physiol.* **2018**, *230*, 51–60. [CrossRef]
35. Cheng, F.; Cheng, Z.; Meng, H.; Tang, X. The garlic allelochemical diallyl disulfide affects tomato root growth by influencing cell division, phytohormone balance and expansin gene expression. *Front. Plant Sci.* **2016**, *7*, 1199. [CrossRef]
36. Hayat, S.; Cheng, Z.; Ahmad, H.; Ali, M.; Chen, X.; Wang, M. Garlic, from remedy to stimulant: Evaluation of antifungal potential reveals diversity in phytoalexin allicin content among garlic cultivars; allicin containing aqueous garlic extracts trigger antioxidants in cucumber. *Front. Plant Sci.* **2016**, *7*, 1235. [CrossRef]
37. Perelló, A.; Gruhlke, M.; Slusarenko, A.J. Effect of garlic extract on seed germination, seedling health, and vigour of pathogen-infested wheat. *J. Plant Prot. Res.* **2013**, *53*. [CrossRef]
38. Rady, M.M.; Seif El-Yazal, M.A. Garlic extract as a novel strategy to hasten dormancy release in buds of "Anna" apple trees. *S. Afr. J. Bot.* **2014**, *92*, 105–111. [CrossRef]
39. Martins, N.; Petropoulos, S.; Ferreira, I.C.F.R. Chemical composition and bioactive compounds of garlic (*Allium sativum* L.) as affected by pre- and post-harvest conditions: A review. *Food Chem.* **2016**, *211*, 41–50. [CrossRef]
40. Hayat, S.; Ahmad, H.; Ali, M.; Hayat, K.; Khan, M.A.; Cheng, Z.; Hayat, S.; Ahmad, H.; Ali, M.; Hayat, K.; et al. Aqueous garlic extract as a plant biostimulant enhances physiology, Improves crop quality and metabolite abundance, and primes the defense responses of receiver plants. *Appl. Sci.* **2018**, *8*, 1505. [CrossRef]
41. Hayat, S.; Ahmad, H.; Ali, M.; Ren, K.; Cheng, Z. Aqueous garlic extract stimulates growth and antioxidant enzymes activity of tomato (*Solanum lycopersicum*). *Sci. Hortic.* **2018**, *240*, 139–146. [CrossRef]
42. Hayat, S.; Ahmad, H.; Ren, K.; Ali, M.; Cheng, Z. Response of tomato growth to foliar spray and root drenching of aqueous garlic extract: A cocktail of antioxidative defenses, chlorophyll, carotenoid and soluble sugar contents. *Int. J. Agric. Biol.* **2018**, *20*, 1251–1259. [CrossRef]
43. Kepczyńska, E.; Król, P. The phytohormone methyl jasmonate as an activator of induced resistance against the necrotroph Alternaria porri f. sp. solani in tomato plants. *J. Plant Interact.* **2012**, *7*, 307–315. [CrossRef]
44. Gokul, A.; Roode, E.; Klein, A.; Keyster, M. Exogenous 3,3'-diindolylmethane increases *Brassica napus* L. seedling shoot growth through modulation of superoxide and hydrogen peroxide content. *J. Plant Physiol.* **2016**, *196–197*, 93–98. [CrossRef]
45. Alsaeedi, A.; El-Ramady, H.; Alshaal, T.; El-Garawani, M.; Elhawat, N.; Al-Otaibi, A. Exogenous nanosilica improves germination and growth of cucumber by maintaining K+/Na+ ratio under elevated Na+ stress. *Plant Physiol. Biochem.* **2018**, *125*, 164–171. [CrossRef] [PubMed]
46. Gao, J. *Experimental Guidance for Plant Physiology*; Higher Education Press: Beijing, China, 2006.
47. Stewart, R.R.C.; Bewley, J.D. Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiol.* **1980**, *65*, 245–248. [CrossRef]
48. Polle, A.; Otter, T.; Seifert, F. Apoplastic Peroxidases and lignification in needles of Norway Spruce (*Picea abies* L.). *Plant Physiol.* **2016**, *106*, 53–60. [CrossRef]
49. Chance, B.; Maehly, A.C. Assay of catalases and peroxidases. *Methods Enzymol.* **1955**, *2*, 764–775. [CrossRef]
50. Dhindsa, R.S.; Plumb-Dhindsa, P.L.; Reid, D.M. Leaf senescence and lipid peroxidation: Effects of some phytohormones, and scavengers of free radicals and singlet oxygen. *Physiol. Plant.* **1982**, *56*, 453–457. [CrossRef]
51. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
52. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **2010**, *48*, 909–930. [CrossRef] [PubMed]

53. Das, K.; Roychoudhury, A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.* **2014**, *2*, 53. [CrossRef]
54. Mittler, R. ROS Are Good. *Trends Plant Sci.* **2017**, *22*, 11–19. [CrossRef] [PubMed]
55. Kranner, I.; Roach, T.; Beckett, R.P.; Whitaker, C.; Minibayeva, F.V. Extracellular production of reactive oxygen species during seed germination and early seedling growth in *Pisum sativum*. *J. Plant Physiol.* **2010**, *167*, 805–811. [CrossRef]
56. Muller, K.; Linkies, A.; Fry, S.C.; Leubner-Metzger, G.; Vreeburg, R.A.M.; Krieger-Liszkay, A. In vivo cell wall loosening by hydroxyl radicals during cress seed germination and elongation growth. *Plant Physiol.* **2009**, *150*, 1855–1865. [CrossRef]
57. Kumar, J.S.P.; Rajendra Prasad, S.; Banerjee, R.; Thammineni, C. Seed birth to death: Dual functions of reactive oxygen species in seed physiology. *Ann. Bot.* **2015**, *116*, 663–668. [CrossRef]
58. Baxter, A.; Mittler, R.; Suzuki, N. *ROS as Key Players in Plant Stress Signalling*; Oxford University Press: Oxford, UK, 2014; Volume 65, pp. 1229–1240.
59. Shafi, A.; Chauhan, R.; Gill, T.; Swarnkar, M.K.; Sreenivasulu, Y.; Kumar, S.; Kumar, N.; Shankar, R.; Ahuja, P.S.; Singh, A.K. Expression of SOD and APX genes positively regulates secondary cell wall biosynthesis and promotes plant growth and yield in *Arabidopsis* under salt stress. *Plant Mol. Biol.* **2015**, *87*, 615–631. [CrossRef]
60. Mittler, R.; Vanderauwera, S.; Suzuki, N.; Miller, G.; Tognetti, V.B.; Vandepoele, K.; Gollery, M.; Shulaev, V.; Van Breusegem, F. ROS Signaling: The New Wave? *Trends Plant Sci.* **2011**, *16*, 300–309. [CrossRef]
61. Torres, M.A.; Jones, J.D.G.; Dangl, J.L. Reactive oxygen species signaling in response to pathogens. *Plant Physiol.* **2006**, *141*, 373–378. [CrossRef]
62. Ali, M.; Cheng, Z.; Ahmad, H.; Hayat, S. Reactive oxygen species (ROS) as defenses against a broad range of plant fungal infections and case study on ROS employed by crops against *Verticillium dahliae* wilts. *J. Plant Interact.* **2018**, *13*, 353–363. [CrossRef]
63. Miller, G.; Suzuki, N.; Ciftci-Yilmaz, S.; Mittler, R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ.* **2010**, *33*, 453–467. [CrossRef] [PubMed]
64. Golldack, D.; Li, C.; Mohan, H.; Probst, N. Tolerance to drought and salt stress in plants: Unraveling the signaling networks. *Front. Plant Sci.* **2014**, *5*, 151. [CrossRef]
65. Shaban, M. Effect of reactive oxygen species on germination and lipid proxidation in sunflower seeds. *Int. J. Adv. Biol. Biomed. Res.* **2014**, *2*, 2086–2090.
66. Satour, P.; Youssef, C.; Châtelain, E.; Vu, B.L.; Teulat, B.; Job, C.; Job, D.; Montrichard, F. Patterns of protein carbonylation during Medicago truncatula seed maturation. *Plant Cell Environ.* **2018**, *41*, 2183–2194. [CrossRef]
67. Châtelain, E.; Satour, P.; Laugier, E.; Ly Vu, B.; Payet, N.; Rey, P.; Montrichard, F. Evidence for participation of the methionine sulfoxide reductase repair system in plant seed longevity. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 3633–3638. [CrossRef]
68. Shaoli, C.; Baoli, Z.; Shanshan, L.; Xia, L.; Xueling, Y. Accumulation of cinnamic acid and vanillin in eggplant root exudates and the relationship with continuous cropping obstacle. *Afr. J. Biotechnol.* **2011**, *10*, 2659–2665. [CrossRef]
69. Huang, X.; Bie, Z.; Huang, Y. Identification of autotoxins in rhizosphere soils under the continuous cropping of cowpea. *Allelopath. J.* **2010**, *25*, 383–392.
70. Wang, M.; Wu, C.; Cheng, Z.; Meng, H.; Zhang, M.; Zhang, H. Soil chemical property changes in eggplant/garlic relay intercropping systems under continuous cropping. *PLoS ONE* **2014**, *9*, e111040. [CrossRef]
71. Hussain, S.; Khan, F.; Hussain, H.A.; Nie, L. Physiological and biochemical mechanisms of seed priming-induced chilling tolerance in rice cultivars. *Front. Plant Sci.* **2016**, *7*. [CrossRef]
72. Anaya, F.; Fghire, R.; Wahbi, S.; Loutfi, K. Influence of salicylic acid on seed germination of Vicia faba L. under salt stress. *J. Saudi Soc. Agric. Sci.* **2018**, *17*. [CrossRef]
73. Sheteiwy, M.S.; Gong, D.; Gao, Y.; Pan, R.; Hu, J.; Guan, Y. Priming with methyl jasmonate alleviates polyethylene glycol-induced osmotic stress in rice seeds by regulating the seed metabolic profile. *Environ. Exp. Bot.* **2018**, *153*, 236–248. [CrossRef]

74. Bhandari, S.R.; Yoon, M.K.; Kwak, J.H. Contents of phytochemical constituents and antioxidant activity of 19 garlic (*Allium sativum* L.) parental lines and cultivars. *Hortic. Environ. Biotechnol.* **2014**, *55*, 138–147. [CrossRef]
75. El–Hamied, S.A.A.; El-Amary, E.-A. Improving growth and productivity of "Pear" trees using some natural plants extracts under north sinai conditions. *IOSR J. Agric. Vet. Sci.* **2015**, *8*. [CrossRef]