PLANT SCIENCE TODAY, 2020
Vol 7(2): 257–263
https://doi.org/10.14719/pst.2020.7.2.743
ISSN 2348-1900 (online)

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RESEARCH ARTICLE

Assessment of exogenous application of plant growth regulators on Cress seed germination and β-Galactosidase activity

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ARTICLE HISTORY
Received: 01 February 2020
Accepted: 06 April 2020
Published: 03 May 2020

KEYWORDS
acclimation; development; enzyme production; *Lepidium sativum*; protein content

ABBREVIATIONS
β-Galactosidase (BGAL), Gibberellic acid (GA), Indole acetic acid (IAA), Kinetin (Kin), Ortho-Nitrophenyl-β-galactoside (ONPG), Percentage germination (GP), Plant Growth Regulators (PGRs), Salicylic Acid (SA)

ABSTRACT

Plant growth regulators (PGRs) were involved in several types of abiotic stress responses by means of improving seed germination and modifying the growth and development of medicinally important *Lepidium sativum* via alleviating the negative effects of abiotic stresses. Therefore, the present research was carried out to investigate the effects of exogenous application of PGRson seed germination, protein content and β-galactosidase activity of *L. sativum*. Germination of *L. sativum* seeds was monitored for a short interval after the start of incubation until growth became 100%. While cytokinin treatment showed a positive effect on seed germination more than Gibberellic acid (GA), salicylic acid (SA) produced a higher negative effect than auxins. Quantifying changes in total protein content during seed germination as influenced by PGRs revealed that all PGRs have to exert a positive effect arranged in the following order: SA > auxin > cytokinin > GA. Parallel to changes in germination percentage and total protein content of seed, a negative effect was attainedon β-galactosidase specific activity in response to PGRs with the following arrangement: SA > auxin > cytokinin > GA. In conclusion, the present study proposed the potential importance of the type and magnitude of exogenously applied PGRs during the germination of easily or even more difficult-to-germinate seeds.

Introduction

Seed science is one of the most active research fields in plant physiology investigating possible physiological processes involved in seed germination. Germination occurs through the growth of seed and essential structures needs to proceed under seemingly favorable conditions to produce a normal plant. For that, the seed, particularly the embryo, is structurally and physiologically equipped (1).

Lepidium sativum, commonly known as garden cress, belongs to the Brassicaceae family. It is indigenous to southwest Asia and was referred to over many centuries ago in Western Europe. Its seed, oil, and powder contain a significant amount of nutritional components (2). Cress seeds are small, oval-shaped, reddish-brown and are about 2–3 mm long and 1–1.5 mm wide (3). The powder of the seeds is yellow-colored and creamish (4). Seeds have a gel layer in its outer membrane which contains a mucilage (6.5–15%) that consists of cellulose (18.3%) and uronic acid-containing polysaccharides (5).

Plant growth regulators, widely used in agriculture, are actively regulating plant developmental processes and they are involved in plant responses to several types of abiotic stresses such as salinity and water stress (6, 7). Seed germination is a process, in which seed's morphological and physiological characters sequentially changes leading to the protraction of embryonic axis from the seed coat. With the emergence of the radicle from seed layers, the process of seed germination is completed (8). The plant hormones play a significant role to protect plants from fluctuating ecological factors, evolving numerous adaptive responses (9). Every hormones has different actions on regeneration in plants (10). Each

To cite this article: Alghonmeen OO, Alsharafa KY, Al-limoun MO, Khleifat KM, Al-Ramamneh EDM. Assessment of exogenous application of plant growth regulators on Cress seed germination and β-Galactosidase activity. Plant Science Today. 2020;7(2):257–263. https://doi.org/10.14719/pst.2020.7.2.743

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recognized group of hormones shares in some sides of seed evolution (11).

β-Galactosidase (BGAL) is a ubiquitous enzyme, whose activity has been indicated in microorganisms, plants and animals (12). The β-galactosidase activity could be detected and possibly amplified during seed germination and in various plant functions. These include fruit mellowing, seed germination, and the evolution of vegetative organs. The optimal pH of the plant enzyme is acidic (13).

This work aimed to study the influence of the exogenous application of different concentrations of plant growth regulators on the seed germination of Cress (*Lepidium sativum*). It is also worthy to develop new insights about the regulation of beta-galactosidase enzyme production in response to different plant growth regulator substances and its influence on seed germination. Finally, to understand the cross talk between plant growth regulators and the regulation of beta-galactosidase enzyme production to reach the typically recommend level for food technology.

Material and Methods

Seed sterilization

Seeds were surface sterilized using 1% sodium hypochloride followed by dipping the seeds in 70% ethanol for 3 min and then rinsed in distilled water. After drying, they were used through spreading in plates.

Plant growth regulator treatment

For each treatment, a triplicate of thirty seeds of *L. sativum* were placed in 9 cm Petri dishes, lined with two layers of filter paper, and moistened with 6 ml distilled water (control) or the tested PGR solution and left in darkness. Treatment with growth regulators included: gibberellic acid (GA) at (29, 144, 289 µM) (14), auxin, namely Indole acetic acid (IAA) at (1, 5, 10 µM) (15), cytokinin, namely Kinetin (Kin) at (0.05, 0.25, 2.5 µM) (16), and salicylic acid (SA) at (0.05, 0.25, 2.5 mM) (17). Seeds were considered as germinated when the radicle had emerged from the seed coat. The number of germinated seeds counted every two hours until growth becomes 100% after the start of seed incubation in each specific plant growth regulator.

Percentage germination (%)

Percentage germination was recorded for each treatment at 0, 12, 16, 18 and 24 h after the start of incubation at room temperature and was calculated as indicated previously (18).

Percentage germination (GP) of seeds (%) = (Number of germinated seeds/ Total number of seeds) × 100.

Protein extraction

After each treatment, germinated seeds were harvested in liquid nitrogen and stored at -80C° till the next analysis. Seeds ground into fine powder in mortar and pestle were dissolved in lysing buffer consisting of 0.22 M mannitol, 0.07 M sucrose, 1 mM EDTA, 0.3% (w/v) PVP and 0.05% (w/v) bovine serum albumin (BSA) maintaining a medium to tissue weight ratio of 2:1, then the homogenate was maintained at pH 7.2 by the addition of 1 N KOH. After mixing the homogenate, it was centrifuged for 20 min at 10.000 g. The supernatant was further centrifuged at 21.000 g for 75 min, and the resultant soluble fraction was used for enzyme assays. All operations were carried out at 4° C (19). Protein contents in the supernatant were determined by the Bradford method (20) with BSA as a standard.

β-Galactosidase activity

The β-galactosidase activity was determined according to Kishore and Kayastha (13). The reaction mixture consisted of Ortho-Nitrophenyl-β-Galactoside (ONPG) contained in a final volume of 500 µl, 50 mM glycine–HCl (pH 2.8), 20 mM ONPG substrate and 20 µl of the extracted enzyme. The reaction was carried out at 37 °C for 10 min. Liberated o-nitrophenolate (ONP) was measured spectrophotometrically at 405 nm after stopping the reaction with the addition of 1.5 ml Sodium tetraborate (20 mM). The specific activity of β-galactosidase is expressed as µmoles of ONP formed per minute per mg of protein.

Statistical analysis

For all experiments, samples of the selected seeds were analyzed. The results were expressed as mean± SD. Data were subjected to one-way analysis of variance (ANOVA) and comparison of sample means was done by Dunnett's multiple comparisons test. Results were considered significant at $P<0.0001$.

Results

The effect of plant growth regulators on GP of L. sativum seeds

The seeds of *L. sativum* were germinated under different concentrations of PGRs. GA promoted dark germination; for seeds exposed to 144 µM and 289 µM GA, the GP was significantly higher by 1.14-fold and 1.2-fold as compared with the response in control after 12 h of incubation, respectively. Continued exposure of seeds to 289 µM GA for 14 and 16 h resulted significantly in a further increase of GP by 1.1-fold. Seeds exposed to 289 µM GA reached 100% GP earlier than lower GA concentrations which exhibited 100% GP after 20 h (Fig. 1A).

On the other hand, seed exposed to increasing IAA concentrations resulted in reduced and delayed germination. Fig. 1(B) shows that whereas the reduction of GP was 0.9-fold after 16 and 20 h of 1 µM auxin exposure, 0.9-fold reduction of GP was evident at all interval records of incubation as a result of 5 µM auxin exposure. The higher early reduction of GP reached 0.6-fold, 0.6-fold, 0.9-fold, 0.9-fold and 0.9-fold after 12, 14, 16, 18 and 20 h of 10 µM auxin exposures respectively.

Application of 0.25 µM kin promoted dark germination early which reached 11 and 36% after 6 and 8 h of exposure as compared with the control. Besides, the GP was significantly increased by 1.3 and 1.1-fold compared with the control after 12 and 14 h of exposure, respectively and eventually reached

100% GP which was at least 4 h earlier than the control (Fig. 1C).

The examination of the effect of different SA concentrations on seed germination showed that SA inhibits seed germination in a dosage-dependent manner as shown in Fig. 1(D). Seed germination caused by 2.5 mM SA was significantly reduced by 0.08-fold, 0.2-fold, 0.2-fold, 0.4-fold, and 0.5-fold compared with the control after 12, 14, 16, 18 and 20 h of exposure, respectively. Furthermore, the inhibitory effect of SA raised the time required for 100% GP up to 24 h and for the high concentration of SA, 2.5 mM, it was extended to 30 hr.

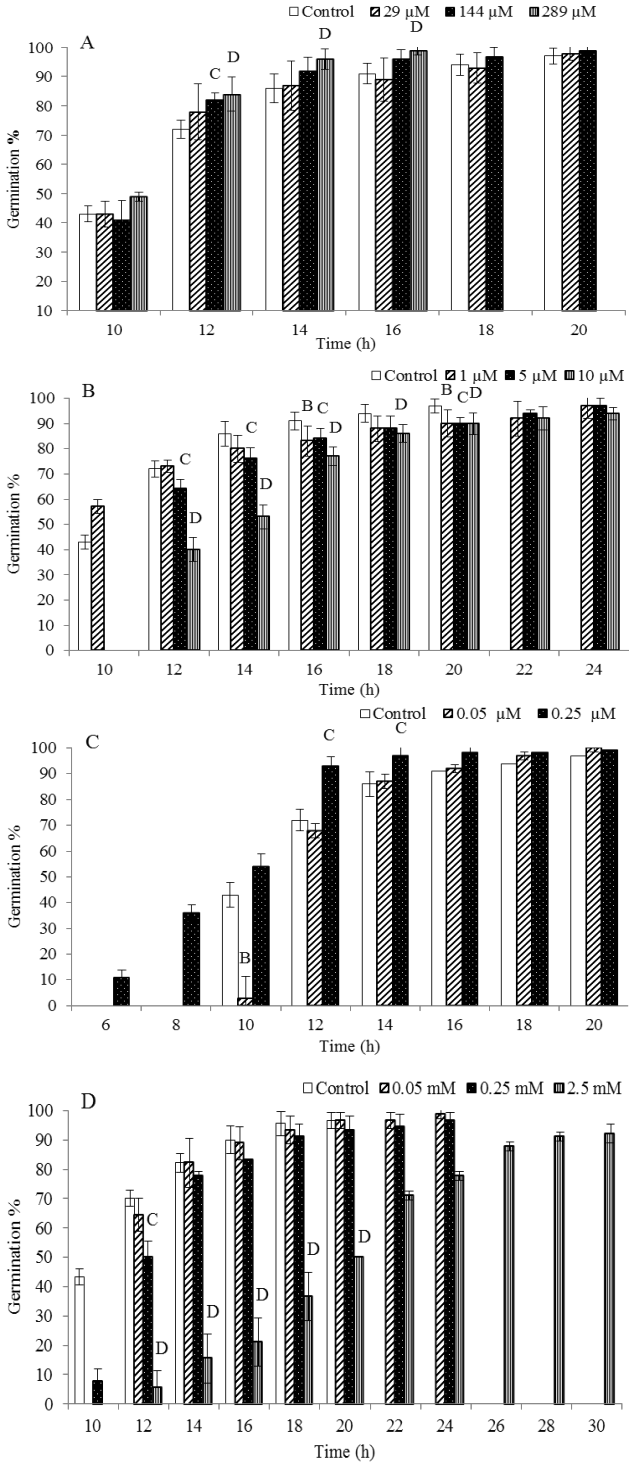

Fig. 1. *L. sativum* seed germination subjected to plant growth regulators (PGRs). A- Gibberellic acid. B- Auxin. C- Cytokinin. D- Salicylic acid. The effect of different PGRs on seed germination at different time points compared with the seed germination in controls. Data represent mean values ± SD, n=3 X 30 seeds are presented. The significance of differences was calculated using Dunnett's multiple comparisons test and the P value of <0.0001 indicating a significant difference.

Data comparing the influences of the different PGRs on *L. sativum* seed germination showed that cytokinin was more effective than GA treatment through its positive effects on seed germination which was earlier in the promotion and completion of germination. Auxin and SA treatments showed a negative effect on germination with SA exerting a higher effect than auxin.

The effect of plant growth regulators on L. sativum seed protein content

While investigating the influence of PGRs on germinating *L. sativum* seeds, we quantify changes in the total protein content during this process. Generally, protein content increased significantly to its highest level in response to all tested concentrations of PGRs compared to control as shown in Fig. 2. Comparing the positive effect on total protein content during seed germination showed that PGRs could be arranged as follows: SA > auxin > cytokinin > GA.

The effect of plant growth regulators on L. sativum seed β-galactosidase specific activity

To gain different perspectives about the influence of PGRs on germinating *L. sativum* seeds, we also have analyzed changes of β-galactosidase specific activity during this process. Generally, the β-galactosidase specific activity was reduced significantly to the lowest level in response to all tested PGRs and their concentrations compared to control as shown in Fig. 3.

The negative effects of PGRs on β-galactosidase specific activity during seed germination could be arranged in the following order: SA > auxin > cytokinin > GA. Notably, the influence of both SA and auxin was in a dosage-dependent manner.

Discussion

Seed germination begins with water uptake by the seed, followed by enzyme activation during which several biochemical events takes place, e.g. storage reserve mobilization that finally triggers the embryonic root to penetrate the surrounding structures of the seed coat (8, 21).

However, seed germination can also be influenced by environmental conditions whether stressful or and not, and by the presence of many elements as nitrogenous compounds and hormones for instance (14, 22, 23).

PGRs have been reported to enhance crop growth and yield (24–27). Their effects on modulating seed germination may be one of their most important functions in plant growth (28). The action of GA on seed germination is mediated through modulation of protein synthesis which requires cell wall modification by influencing cell wall- remodeling proteins (CWRPs). A hypothesis that may explain our results at the level of protein content and β-

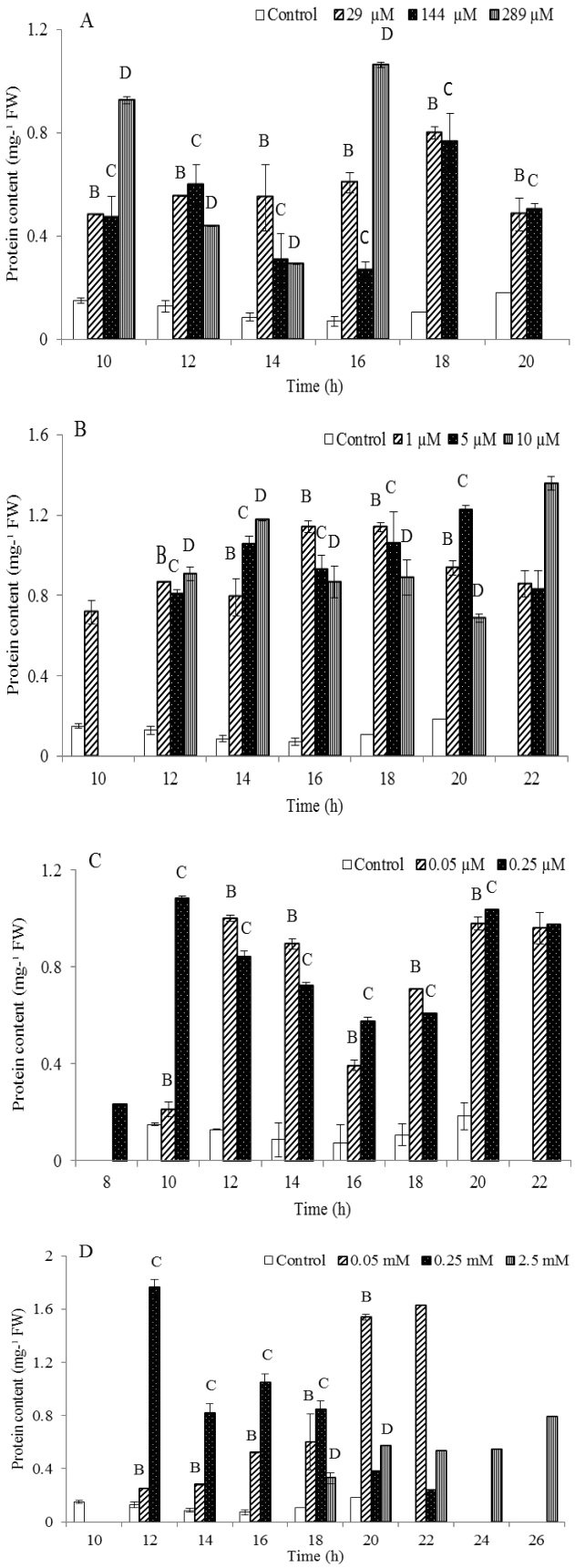

Fig. 2. *L. sativum* seed protein content subjected to various abiotic stresses. A- Gibberellic acid. B- Auxin. C- Cytokinin. D- Salicylic acid. The effect of different PGRs on protein content at different time points compared with the protein content in controls. Data represent mean values ± SD, n=3 X 30 seeds are presented. The significance of differences was calculated using Dunnett's multiple comparisons test and the P value of <0.0001 indicating a significant difference.

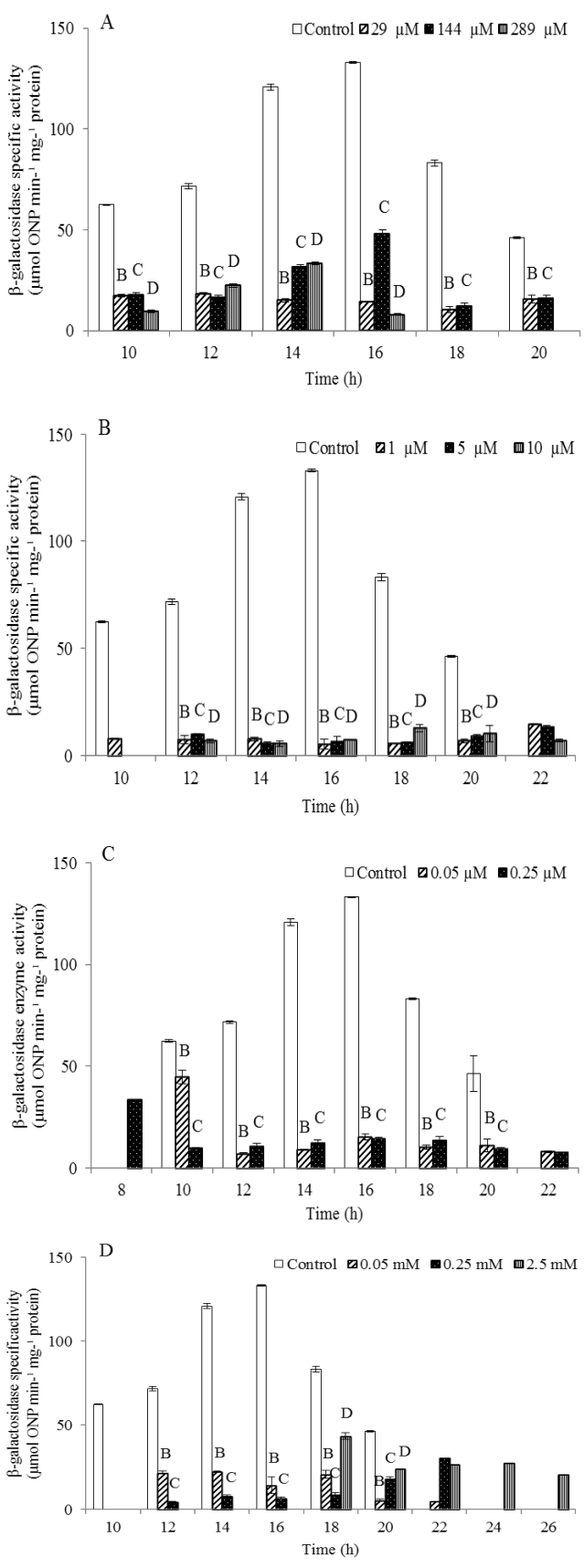

Fig. 3. *L. sativum* seed β-galactosidase specific activity subjected to various abiotic stresses. A- Gibberellic acid. B- Auxin. C- Cytokinin. D- Salicylic acid. The effect of different PGRs on β-galactosidase specific activity at different time points compared with the β-galactosidase specific activity in controls. Data represent mean values ± SD, n=3 X 30 seeds are presented. The significance of differences was calculated using Dunnett's multiple comparisons test and the P value of <0.0001 indicating a significant difference.

galactosidase specific activity during seed germination (29, 30). According to the present study, GA promoted the action of β-galactosidase more than the other PGRs indicating its role in seed coat relations. The activity of genes encoding β-galactosidase was suggested in modifying seed coat mucilage and its expansion upon hydration (31). The promotion of seed germination in response to GA treatment in the present study is also related to its role in food reserve mobilization during the germination process. It was reported that binding of GA with its receptor gibberellin insensitive dwarf1 (GID1) can provoke degradation of DELLA protein, which in turn induces the transcription of α-amylase important for its role in carbohydrate metabolism (32). Besides, Glycosyl hydrolases family targeted in the present study possess ubiquitous distribution and are involved, as a part of a complex physiological process, in the metabolism of complex carbohydrates during germination (19).

In agreement with our results, the exogenous application of IAA was found to have delayed wheat seed germination (33). Similarly, IAA can also result in such responses by a wild-type plant and under high salinity (8, 15, 34). These observations imply that auxin plays a role in regulating seed germination possibly due to its role in mediating ABA and GA biosynthesis (15, 35, 36).

Cytokinins promoted seed germination in the present study which could be, as for GA, through modulation of protein synthesis (26, 37, 38). This group of growth-promoting PGRs has been indicated in regulating seed germination and is active in all stages (16, 39). Their promotion effects on seed germination may be also attributed to the stimulation of hydrolyzing enzymes and antagonizing the inhibitory effect of ABA on the germination process (40).

Salicylic acid plays an important role in determining the sensitivity of plants to various abiotic stresses. It induced several effects ina dosage-dependent manner (41, 42). Whereas lower concentrations (0.05 mM and 0.25 mM) did not affect the germination speed, higher concentration (2.5 mM) caused the seed germination process to be dramatically impeded. Similar results on germination have been reported (17, 42, 43). SA precise role during germination and the molecular mechanisms involved have not been fully elucidated (17). However, processes affected by SA, e.g. the quality of protein translation, the synthesis of antioxidant enzymes, and the mobilization of seed storage proteins during germination may be associated with protein synthesis during early stages of germination (42, 44). It was suggested that SA plays as a bifunctional modulator. It acts under normal growth conditions by inhibiting the expression of GA-induced α-amylase genes which then inhibits germination (45). In contrast during stress conditions (salinity), SA promotes germination via another pathway that reduces oxidative damage (17). The reported results of the changes in total protein content upon SA treatment are consistent with highly active protein metabolism in response to SA exposure during seed germination as reported by (42). Reports indicated that SA under salt stress leads to catechol accumulation to a high level and may though acts as an antioxidant, removing synthesized H_2O_2 (17) but at high concentrations have toxic effects possibly by inducing ROS biosynthesis (46).

Conclusion

In conclusion, this study suggests that the effects of exogenous application of PGRs on seed germination vary depending on the type and concentration of applied PGRs. The results provided more insights on the importance of exogenous application of PGRs and the subsequent response in β-galactosidase enzyme production on germination of more difficult-to-germinate seeds having either hard coatings or physiologically dormant. For such plants, attempts can be made to explore conditions, for instance could enhance β-galactosidase specific activity during their germination. However, further investigation on *L. sativum* seed germination is required, including the application of other PGRs such as jasmonic acid and brassinosteroids, or combinations of different PGRs, in addition to extensive measurements of reactive oxygen species and antioxidants, which would clarify the molecular mechanisms underlying PGRs regulation of germination.

Acknowledgements

We acknowledge the support offered by the College of Graduate Studies- Mu'tah University- Mu'tah-Karak-Jordan.

Authors' Contributions

OA performed the research. KA designed the project, supervised and discussed the results and wrote the article. MA, KK and EA discussed the results and wrote the article. Percentage participation is equal for all co-authors.

Competing interest

The authors declared that they have no competing interests.

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