Improving of true shallot seeds germination by the application of plant growth regulators and osmoconditioning treatment

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Abstract. This study aims to determine the effect of the application of plant growth regulators and osmoconditioning treatment to improve the germination of true shallot seeds. This research was conducted in Asam Kumbang, Medan Selayang, Medan, Indonesia. The research method was a Randomize Block Design with 2 factors, the first factor is Plant Growth Regulators (Z) with 6 levels, namely Z0 (Without PGRs Application), Z1 (Gibberellin 500 ppm), Z2 (Putrescine 15 ppm), Z3 (Putrescine 20 ppm), Z4 (Putrescine 15 ppm + Gibberellin 500 ppm), Z5 (Putrescine 20 ppm + Gibberellin 500 ppm). The second factor was the osmoconditioning treatment with Polyethylene Glycol (PEG) 6000 (O) with 4 levels, namely O0 (Without Osmoconditioning Treatment), O1 (PEG 6000 3%), O2 (PEG 6000 4%), O3 (PEG 6000 5%). Parameters observed were germination rate, germination rate index, percentage of germination, germination ability, simultaneous growth of seeds, seedling length, root length, seedling dry weight, and catalase activity test. The results of this study were: application of plant growth regulators could improve true shallot seed germination, indicated by the observed values of all parameters which were significantly different from those of the control (without PGRs application). The plant growth regulator that produced the best increase in germination was Gibberellins 500 ppm, although the difference in effect with other PGRs was not significantly different. Meanwhile, the osmoconditioning treatment with PEG 6000 was also able to improve the germination of true shallot seeds as indicated by an increase in most of the observed parameters, but in the root length parameter it was seen that the tendency of PEG 6000 3% always gave the highest value but gave the lowest value for this parameter. The best concentration of PEG 6000 in the osmoconditioning treatment to improve true shallot seed germination was 3%.

1. Introduction
One of the leading horticultural crops and has been intensively cultivated by farmers in Indonesia is the shallot (Allium ascalonicum L.). This shallot plant functions as a food seasoning as well as a traditional medicinal ingredient and belongs to the group of spices that cannot be substituted. Shallots are a source of income for farmers and provide a high contribution to economic development in several areas [1].

The propagation of shallots usually uses bulbs, but actually shallots can also be propagated by using true shallot seed (TSS). Some of the advantages of using true shallot seed are the use of less seed because per hectare it only takes 3 - 7.5 kg of seed, while tubers require 1 - 1.5 tonnes / ha, the cost of
supply is cheap, seed storage is easier because it does not require space which is large for seed storage because the size of the seeds is much smaller than tubers, the shelf life of the seeds is long so that they are flexible and can be planted when needed, easy and cheap to distribute, low variety of seed quality, high productivity [2].

However, true shallot seeds are susceptible to quality degradation due to long storage. Decreased seed viability is one sign that TSS are experiencing a decline, because low viability will result in low seed vigor. Several things that can indicate a decrease in seed quality are the low germination ability and speed of seed germination. Actually, seeds that have deteriorate can still be improved by giving invigoration treatment. According to [3] invigoration is the treatment of seeds before sowing by balancing the water potential of the seeds to stimulate metabolic activities in the seeds so that the seeds are ready to germinate but the important structure of the embryo, namely the radicle, has not yet emerged.

One of the invigoration efforts that can be done is the priming method, where this method can help increase the speed of germination, increase the percentage of germination and reduce the number of abnormal shallot tillers [4]. In line with [5] which states that one method that can be used to accelerate germination is priming, where this method can accelerate and make germination simultaneously by controlling the absorption of water until finally seed germination can occur. One type of priming that can be used is osmoconditioning, namely the priming method by immersing the seeds in a solution with high osmotic pressure usually using PEG (Polyethylene Glycol) 6000.

Acceleration of germination and improvement of TSS viability can also be done with the application of plant growth regulators. One of the plant growth regulators are widely reported to help increase seed germination is gibberellins. In addition to increasing germination, gibberellins can also improve decreased seed viability and also play a role in breaking seed dormancy so that they can germinate. [6] reported that gibberellins play a role in breaking seed dormancy, assisting the mobilization of reserve endosperm during early embryonic growth, breaking bud dormancy, stem growth and elongation, flower and fruit development, cell expansion and division, even in rosette plants being able to extend internodes so that they grow. elongated. [7] stated that the exogenous gibberellin commonly used and available in the market is GA3 (gibberellin-3) which is also known as gibberelic acid.

Polyamines (Putrescine, Spermidine, and Spermine) are also plant growth regulators that can be used to increase germination and improve TSS viability. Polyamines are small polycationic molecules found in all organisms. Its function has been reported in various biological processes including transcription, RNA modification, protein synthesis and modulation of enzyme activity [8]. Polyamines are very important for growth, development, and cell differentiation in plants [9]. In addition, polyamines also accumulate in plant tissues and carry out their functions under normal and stress conditions. Exogenous polyamines can affect the germination of isolated embryos depending on the type of polyamine, its concentration, and the state of embryonic dormancy [10].

2. Materials and methods
This research was conducted in Asam Kumbang, Medan Selayang, Medan City, Indonesia. Held from May to August 2021, using a Randomized Block Design with 2 factors. The first factor is a plant growth regulator (Z) with 6 levels, namely Z0 (Without PGRs Application), Z1 (Gibberellin 500 ppm), Z2 (Putrescine 15 ppm), Z3 (Putrescine 20 ppm), Z4 (Putrescine 15 ppm + Gibberellin 500 ppm), and Z5 (Putrescine 20 ppm + Gibberellins 500 ppm). While the second factor is osmoconditioning treatment with Polyethylene Glycol (PEG) 6000 (O) with 4 levels, namely O0 (Without Osmoconditioning Treatment), O1 (PEG 6000 3%), O2 (PEG 6000 4%), and O3 (PEG 6000 5%). The number of replications was 3 with a total of 24 plots, so the total number of plots was 72 experimental plots.
2.1. Seed selection
The true shallot for this study came from captive farmers in Hinalang Village, Purba District, Simalungun, North Sumatra, Indonesia. The seed used are seeds that have been stored for one year. The selected seeds were put into water and those that sank to the bottom of the container were classified as pithy seeds and would be used in research, while the seeds that floated were removed. After soaking, the seeds are air-dried on paper towels so that the outer skin is not moist.

2.2. Chemical solution dilution
Preparation of PEG 6000 solutions with concentrations of 3%, 4%, and 5% by dissolving 3 grams, 4 grams, and 5 grams of PEG 6000 respectively into distilled water until the volume of each solution reaches 100 ml. Gibberellins (GA3) 500 ppm were obtained by dissolving 0.5 grams of GA3 into 1 liter of distilled water, 15 ppm putrescine solution (Put) was obtained by dissolving 0.015 grams of putrescine into 1 liter of distilled water, while 20 ppm putrescine solution (Put) was obtained by Dissolve 0.020 grams of putrescine into 1 liter of distilled water.

2.3. Osmoconditioning treatment with PEG 6000
TSS that have been selected as research materials are separated into 4 parts (according to the level of osmoconditioning treatment). The seeds to be given the osmoconditioning treatment were immersed in a solution of PEG 6000 with a concentration of 3%, 4%, and 5% for 12 hours.

2.4. Plant growth regulators application
Seeds were divided between those to be treated with GA3, Putrescine, and those not treated with PGR. The seeds that were given the gibberellin application were soaked in a GA3 solution, while the seeds to be given the putrescine application were soaked in a putrescine solution (15 ppm and 20 ppm) for 12 hours, respectively, then the seeds were dried. For seeds treated with putrescine + gibberellins, after being treated with GA3 immersion and after drying, they were soaked again with putrescine solution for 12 hours and then air-dried.

2.5. Seed nursery
Prior to the nursery, the seeds were soaked with a fungicide dose of 2 g/l. After that, the seeds were planted into poly bags that already contained planting media, where one poly bag for one TSS and one treatment plot consisted of 10 poly bags. Seeds are germinated with a depth of ± 1 cm. Seed germination is expected up to 6 weeks after germination.

2.6. Observation
Variables observed in this study were germination rate, germination rate index, percentage of germination, germination ability, simultaneous growth of seeds, seedling height, root length, seed dry weight, and catalase enzyme activity.

2.6.1. Germination rate (days after seedling). According to [11] the germination rate can be measured by counting the number of days required for the emergence of radicles or plumules.

\[
\text{Germination Rate} = \frac{N_{TI} + N_{T2} + \ldots + N_{Tx}}{\text{Total number of germinated seeds}}
\]

N = number of seeds that germinate in a certain time unit, T = shows the amount of time between the start of the test to the end of a certain interval of an observation.

2.6.2. Germination rate index. Germination rate index was calculated using the formula according to [12] as follows:
Dn
Gn....
D4
G4
D3
G3
D2
G2
D1
G1

\[ \text{GRI} = \frac{G1}{D1} + \frac{G2}{D2} + \frac{G3}{D3} + \frac{G4}{D4} + \ldots + \frac{Gn}{Dn} \]  

GRI: Germination Rate Index, \( G \): Number of seeds that germinate on a certain day, \( D \): The time corresponding to that amount, \( n \): Number of days on final assessment/calculation.

2.6.3. Germination percentage (%). The percentage of seed germination was observed by counting the germinated seeds in each experimental unit. Observations were made starting from the first day after the seeds were germinated with a span of 1 – 42 days after sowing. If more than 42 days after sowing the seeds are categorized as not germinating. According to [13] the calculation uses the following formula:

\[ \text{Germination Percentage} = \frac{\text{Total number of germinated seeds}}{\text{Total seed tested}} \times 100\% \]  

2.6.4. Germination ability test (%). Germination ability was determined by counting the number of seeds that germinated normally during a period of 14 days. By using the formula according to [13]:

\[ \text{Germination Ability} = \frac{\text{Total number of normal sprouts produced}}{\text{Total seed tested}} \times 100\% \]  

2.6.5. Simultaneous growth of seeds (%). Simultaneous growth of seeds is the percentage of strong normal germination in a certain germination period. The maximum value for germination synchronously is 100%. Simultaneous seed growth was calculated using the percentage of strong normal germination on the count between the first and last observations, according to [14] with the formula:

\[ \text{KST} = \frac{KK}{TB} \times 100\% \]  

KST = Simultaneous Growth of Seeds, KK = Number of strong sprouts, TB = Total seeds analysed

2.6.6. Seedling height (cm). Measurement of seedling height was carried out using a ruler by measuring the height from the base of the stem (soil surface) to the growing point. Seedling height parameters were measured at the end of the observation.

2.6.7. Root length(cm). Observation of root length was carried out by dismantling the seeds that were used as sample plants. The roots are washed thoroughly by spraying water until the remaining sand is gone and the roots are clean, then air-dried, then measurements are made from the base of the stem to the tip of the longest root. Root length observations were made at the end of the observation.

2.6.8. Seedling dry weight (g). Observation of the dry weight of sprouts was carried out by taking all parts of the sample seeds that had been cleaned and air-dried, then dried using an oven at 85°C for 2 x 24 hours. Then weigh the dry weight of the sprouts using an analytical balance.

2.6.9. Catalase enzyme activity test (μmol enzyme/ mg protein). The activity of the catalase enzyme was measured according to the modified in [15] method. Enzyme activity was measured by mixing 1.99 ml of 50 mM phosphate buffer pH 7.0 with 10 µl of crude extract of the enzyme, then adding 1 ml of 30 mM H2O2 solution at the end when the absorbance value was measured (total volume of reaction 3 ml). Determination of the activity of the catalase enzyme was carried out based on the decrease in the absorbance value as measured by a UV-VIS spectrophotometer (Shimadzu UV-1280, Japan) at a wavelength of 240 nm for 1 minute. The decrease in the absorbance value indicates the
decomposition of H2O2 in the reaction and the value of the H2O2 exclusion coefficient is 40/mM/cm. Enzyme activity is expressed in mol enzyme/mg protein [16].

The research data were analysed by means of variance or ANOVA (Analysis of variance), if there was a significant difference, then proceed with the mean difference test based on Duncan's Multiple-Distance Test (DMRT) at the 5% level [17].

3. Results and discussion

3.1. Germination rate, germination rate index, germination percentage, germination ability

From the results of analysis of variance, it can be seen that immersion of TSS with plant growth regulators and osmoconditioning treatment with PEG 6000 gave a significant effect on germination rate, germination rate index, percentage of germination, and germination ability. The results of the average difference test with DMRT (Duncan Multiple Range Test) on the effect of the application of plant growth regulators and osmoconditioning treatment are presented in figure 1, figure 2, figure 3, figure 4.

![Figure 1](image1.png)

**Figure 1.** Effect of the Application of Plant Growth Regulators on Germination Rate and Germination Rate Index of True Shallot Seed. Note: Numbers on bars followed by the same letter are not significantly different at the 5% level according to the DMRT average difference test (Duncan's Multiple Distance Test).

![Figure 2](image2.png)

**Figure 2.** Effect of the Application of Plant Growth Regulators on Germination Percentage and Germination Ability of True Shallot Seed. Note: Numbers on bars followed by the same letter are not significantly different at the 5% level according to the DMRT average difference test (Duncan's Multiple Distance Test).
Figure 3. Effect of the osmoconditioning treatment on Germination Rate and Germination Rate Index of True Shallot Seed. Note: Numbers on bars followed by the same letter are not significantly different at the 5% level according to the DMRT average difference test (Duncan's Multiple Distance Test).

Figure 4. Effect of the osmoconditioning treatment on Germination Percentage and Germination Ability of True Shallot Seed. Note: Numbers on bars followed by the same letter are not significantly different at the 5% level according to the DMRT average difference test (Duncan's Multiple Distance Test).

The application of Plant Growth Regulators was proven to increase the germination of True Shallot Seeds, indicated by the increase in the values of viability and vigor parameters observed compared to the control. The TSS germination rate was faster up to 41.52%, in line with the increasing value of the germination rate index up to 155.66% compared to the control. Germination percentage increased by 27.59% and was followed by germination ability which increased by 89.11% compared to control germination ability. Gibberellin gave the best results compared to other PGR treatments, although the difference was not significant.

The application of PGR can increase the germination of True Shallot Seed because PGR is a non-nutritive organic compound which in low concentrations can encourage, inhibit or qualitatively change plant growth and development [18]. Plant growth regulators play an important role in controlling biological processes in plant tissues [18] [19]. Its role includes regulating the speed of tissue growth and integrating these parts to produce the form we know as a plant. The activity of growth regulators...
in growth depends on the type, chemical structure, concentration, plant genotype and plant physiological phase [20].

[6] reported that gibberellins have a role in cell expansion and division, breaking seed dormancy, breaking bud dormancy, helping the movement of reserve endosperm during early embryonic growth. Gibberellins also play a role in stem growth and elongation, flower and fruit development, and in rosette plants they are able to lengthen internodes so that they grow lengthwise. According to [21], gibberellins are able to stimulate seed germination by activating hydrolytic enzymes that can remodel food reserves so that these food reserves are more easily utilized by the embryo during germination.

The results of the research by [22] regarding the application of PGR polyamines in increasing the germination of chili seeds are also in line with the results of this study, where the application of polyamines (putrescine, spermidine, and spermine) significantly increased germination and early growth of chili seed germination compared to seeds that did not given treatment. Polyamines have tremendous potential to increase the vigor of chili plants.

According to [8] polyamines play a role in various biological processes including transcription, RNA modification, protein synthesis and modulation of enzyme activity. The role of polyamines is very important in the growth, development, and differentiation of plant cells [9]. In addition, polyamines (including putrescine) also occur naturally in plants and carry out their functions in plants under normal and stressed conditions. Exogenous polyamines can affect the germination of isolated embryos, but depend on the type of polyamine, its concentration, and the state of embryonic dormancy [10].

Single factor osmoconditioning treatment with PEG 6000 was able to increase the viability and vigor of TSS as indicated by the faster germination rate of up to 24.28% compared to the control, the index of germination rate increased up to 57.58%, the percentage of germination increased by 19.56%, while the germination ability of seeds was also seen to increase up to 31.86%. The best concentration of PEG 6000 in increasing TSS seed germination was a concentration of 3%.

The apparent difference between TSS seeds given the osmoconditioning treatment and the control is thought to occur because the TSS given the osmoconditioning treatment occur slowly, and the incoming water is able to organize existing cell membranes, activate enzymes and organelles, especially mitochondria. [23] stated that with active mitochondria, the respiration process can take place immediately and be accelerated by enzymes that will remodel the food reserves in the seeds into simple molecular compounds that will be translocated to the embryonic axis, so that seeds that have decreased membrane permeability are able to germinate well.

The results of the germination rate index appear to be related to the germination rate value, namely that the lower the germination rate index value, the higher the number of days required for a germination process. According to [24] the longer the number of days required for germination indicates that the germination rate index is small. A low germination rate index value indicates that the seed requires a longer number of days required by a seed for the germination process.

The best concentration of PEG 6000 from the results of this study was a concentration of 3% followed by a concentration of 4%, meanwhile the concentration of 5% actually gave poor results even in some parameters showing values that were not significantly different from the control treatment (PEG 6000 0%). From these data it can be seen that to increase the germination of TSS seeds does not require a high concentration of PEG 6000, because a concentration of PEG 6000 that is too high will make the enzymes and reacting substrates dilute so that metabolism becomes slow [25].

Meanwhile, according to [26] that too high an osmotic solution concentration causes the seeds to stop absorbing water in phase II. According to [27] the process of water absorption by seeds follows a triphasic pattern (3 phases). Phase I is initiated by rapid absorption of water, this is due to the potential difference between water and seed. Water has a potential value of 0 Mpa, while the potential value for seeds (especially orthodox seeds) is between -50 and -350 Mpa [28]. Furthermore, in phase II, water absorption takes place slowly, because the water potential of the seed and its environment is in balance, but seed metabolism is actively taking place. In phase III, water absorption increases again, in which the germination process is complete, marked by the appearance of a radicle [26].
3.2. Simultaneous growth of seeds, seedling height, root length, and seed dry weight
From the results of analysis of variance, it can be seen that immersion of TSS with growth regulators and osmoconditioning treatment with PEG 6000 gave a significant effect on simultaneous growth of seeds, seedling height, root length, and seed dry weight. The results of the average difference test with DMRT (Duncan Multiple Range Test) on the effect of the application of plant growth regulators and osmoconditioning treatment are presented in Table 1.

| Treatment                          | Simultaneous Growth of Seeds (%) | Seedling Height (cm) | Root Length (cm) | Seedling Dry Weight (g) |
|------------------------------------|----------------------------------|----------------------|------------------|-------------------------|
| **Plant Growth Regulators**        |                                  |                      |                  |                         |
| Z0 (Without PGRs)                  | 58.33 a                          | 09.24 a              | 7.90 a           | 0.106 a                 |
| Z1 (Gibberellins)                  | 87.50 b                          | 11.88 b              | 9.30 b           | 0.142 b                 |
| Z2 (Putrescine 15 ppm)             | 79.17 b                          | 10.53 b              | 8.90 ab          | 0.133 ab                |
| Z3 (Putrescine 20 ppm)             | 80.00 b                          | 10.95 b              | 9.20 b           | 0.138 ab                |
| Z4 (Putrescine 15 ppm + gibberellins) | 75.00 ab                         | 10.51 b              | 8.70 ab          | 0.134 ab                |
| Z5 (Putrescine 20 ppm + gibberellins) | 75.83 ab                         | 10.66 b              | 9.20 b           | 0.134 ab                |
| **Osmoconditioning**               |                                  |                      |                  |                         |
| O0 (PEG 6000 0%)                   | 64.44 a                          | 07.42 a              | 8.80 ab          | 0.103 a                 |
| O1 (PEG 6000 3%)                   | 82.78 b                          | 12.71 b              | 8.10 a           | 0.151 b                 |
| O2 (PEG 6000 4%)                   | 81.11 ab                         | 11.35 b              | 9.10 ab          | 0.139 b                 |
| O3 (PEG 6000 5%)                   | 75.56 ab                         | 11.02 b              | 9.50 b           | 0.131 ab                |

Note: The numbers in the column followed by the same letter are not significantly different at the 5% level according to the DMRT average difference test (Duncan Multiple Range Test).

From the table and chart above, we can see that the application of plant growth regulators has been proven to increase the value of simultaneous growth of true shallot seeds, where the value of simultaneous growth of seeds without PGR application which is only 58.33% can be increased with PGR application up to 87.50% or occurs an increase of 50%. For seedling height, there was an increase with PGR application up to 28.49% compared to control, while root length increased up to 17.31%. Meanwhile, the dry weight of the seeds increased up to 34.65%.

We can see from the data that the PGR application gives significantly different results from the control (without PGR) in almost all the observed parameters. Gibberellin PGR gave the best results compared to other PGR treatments, although the difference was not significant. In other words, the application of PGRs given in this study was proven to increase the germination of true shallot seed.

Simultaneous growth of seeds which is a parameter of seed vigor can also be increased by the application of PGR. In line with the research results of [29] which proved that gibberellins were able to increase the simultaneous growth of sweet corn seeds both at high and low temperatures, similar thing was stated by [30] that the simultaneous growth of expired corn could be increased by giving gibberellins. The value of seed synchronously was also seen with the application of putrescine PGR, where the increase in the value of seed germination with the application of putrescine was up to 37.14% compared to the control.

It has also been previously reported that prime shallot seeds have higher levels of polyamine content than controls and that there is a positive correlation between seedling vigor and polyamine content in seeds [31]. This is thought to lead to the hypothesis that seed immersion treatment increases the level of polyamine content in the tissue which further increases seed vigor and seedling growth.
From the research results of [22], all seed treatments with polyamines increased shoot length, root length, and root to shoot ratio of chili seeds. Controls showed very low values for shoot and root length and root to shoot ratio compared to all other treatments.

The growth of seedlings from TSS in this study can also be increased by the application of putrescine, where these results can be clearly observed through higher root length and seedling height, dry weight of seedlings, and better simultaneous growth. This is thought to occur due to an increase in cell division in the apical meristem which causes increased seedling growth [32].

In root length parameters, osmoconditioning treatment with a concentration of 5% gave the highest root length results after the control treatment (PEG 6000 0%) where the two were not significantly different. While the concentration of 3% gave the lowest root length value which was significantly different from the control treatment. It can be seen that the higher the concentration of PEG 6000, the longer the roots.

The tendency that a concentration of 3% always gives the best results on other parameters, in fact the root length parameter actually gives the lowest value. This was presumably because the higher the concentration of PEG 6000 given, the slower the imbibition of TSS seeds. According to [33] a solution containing PEG 6000 with large molecules causes the process of entering water into the seeds to be slow. At the beginning of growth, seeds have not photosynthesized and are metabolized from energy obtained from food reserves (endosperm) which will be active when imbibition takes place optimally. PEG 6000 with high concentrations can indeed create drought conditions, in several studies PEG is often used to simulate drought stress environments because it can reduce the water potential of the media [33][34] and is used for screening drought tolerant genotypes [35]. Root length is thought to be related to the morphological and physiological adaptation of sprouts in meeting water requirements for normal metabolic processes. With drought conditions, the sprouts will adapt to the elongation of roots in order to get water. It is suspected that this is the reason why the concentration of PEG 6000 5% gives the longest root length results.

The dry weight parameter of the seeds in this study showed an increase of 31.84% compared to the control. This was thought to occur because the osmoconditioning treatment with PEG 6000 was able to produce normal sprouts so that the growth of sprouts to become seedlings also ran optimally. It was suspected that normal sprouts had good root development and hypocotyl system so that they had perfect growth which could be seen from their dry weight. Sprout dry weight is the accumulation of growth results during the germination process so that it becomes an indicator of seed viability [11]. Seeds with high germination will tend to have a high dry weight [36].

In line with the research of [37] which stated that osmoconditioning at a concentration of 15% showed a higher vigor index, hypocotyl length, and dry weight of normal sprouts than treatment without osmoconditioning. The results of research conducted by [38] also explained that at a concentration of 100-300 g liter-1 water, PEG compounds were able to increase plant dry weight and the relative speed of soybean plant growth.

For the simultaneous growth of seeds, the osmoconditioning treatment with PEG 6000 with a concentration of 3% was significantly different from the control treatment. Giving osmoconditioning treatment was able to increase the simultaneous growth of TSS seeds by 28.45%.

According to [14], the value of synchronous growth ranged from 40 – 70 percent, where if the value of synchronous growth was greater than 70%, it indicated the vigor of growing strength was very high and the simultaneous growth of less than 40% indicated a group of seeds that lacked vigor. Simultaneous growth of high seeds indicates high vigor of absolute growth strength because a group of seeds that show simultaneous and strong growth will have high growth strength.

3.3. Catalase activity test

The results of the average difference test with DMRT (Duncan Multiple Range Test) on the effect of the application of plant growth regulators and osmoconditioning treatment on Catalase Activity are presented in Figure 5.
Figure 5. Effect of (a) plant growth regulator application and (b) osmoconditioning treatment on Catalase Activity. Note: The numbers in the bars followed by the same letter are not significantly different at the 5% level according to the DMRT average difference test (Duncan Multiple Range Test).

The osmoconditioning treatment was able to increase the activity of the catalase enzyme in true shallot seeds. The activity of catalase enzyme without osmoconditioning treatment was only 1,961 µmoles enzymes/ mg protein, after osmoconditioning treatment it could reach 2,421 µmoles enzymes/ mg protein where the increase was about 23.46%. This is in line with the results of research by [39] who showed a positive effect of osmoconditioning on germination characteristics of spinach cultivars such as germination speed and root length and increased antioxidant enzyme activity due to seed priming which could result in increased tolerance of primed seeds to environmental stresses such as salinity.

This is presumably because soaking the seeds with PEG 6000 is able to make water enter slowly into the TSS seeds and is able to activate enzymes and organelles, one of which is the catalase enzyme. It is suspected that this is what causes the observed viability and vigor parameters to also show an increase compared to the control, because according to [40] that germination success is positively correlated with the activity of catalase and peroxidase enzymes as well as the degree of membrane stability in drought tolerant populations, priming treatment increased peroxidase activity, and catalase, and can reduce the content of malonyldialdehyde (MDA) and electrolyte leakage under water deficit.

The application of Plant Growth Regulators can also increase the activity of the catalase enzyme in true Shallot Seed, where the increase in catalase enzyme activity is up to 37.73% compared to the control, allegedly due to the increasing activity of enzymes (including catalase) due to the osmoconditioning treatment and the application of PGR. This is in line with [41] who stated that the seed germination process begins with the activity of enzymes that work to decompose food reserves such as carbohydrates, proteins and fats. After the absorption of water, the metabolism of embryonic cells begins, which consists of the breakdown reactions and the synthesis of cell components for growth. Among the reactions that occur are breaking down food reserves such as fat, starch and protein contained in the cotyledons into dissolved materials. The process of decomposition of food reserves is influenced by the activity of enzymes as catalysts. The enzymes that play a role in the metabolic process become more active by overhauling the food reserves in the seeds, resulting in changes in the biochemistry, physiology and morphology of the seeds. This process will take place continuously and is a supporter of sprout growth.
According to [42] seed deterioration can be identified biochemically which is characterized by decreased enzyme activity, decreased food reserves, decreased respiration rate and increased conductivity values. Therefore, the osmoconditioning treatment and the application of PGR in this study were able to improve the TSS seeds which had declined, allegedly indicated by the increasing activity of the catalase enzyme.

4. Conclusions
The application of plant growth regulators could improve the germination of true shallot seed which was indicated by the increase in the viability and vigor parameters of the observed seeds compared to the control, the PGR that gave the best results was gibberellins, although the difference was not significant with other PGR applications. Moreover, osmoconditioning treatment with PEG 6000 was able to improve TSS germination marked by an increase in most of the observed parameters, such as germination rate, germination rate index, percentage of germination, germination ability, growth simultaneously, seedling height, seedling dry weight, and catalase enzyme activity. The root length parameter also looks significantly different but the tendency of PEG 6000 3% always gives the highest value, it gives the lowest value for this parameter and 5% concentration gives the longest root length value. This is related to the adaptation of root morphology to water shortages where a high concentration of PEG 6000 can condition drought. Overall, the best concentration of PEG 6000 in the osmoconditioning treatment to improve the germination of TSS was a concentration of 3%.

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