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RESEARCH ARTICLE

Optimizing Germination and Seedling Vigour of True Shallot Seeds (TSS) Through Growth Regulators Priming

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Abstract Shallot cultivation with TSS is the right option for the challenges with shallot cultivation using bulbs. However, poor seed storage condition may diminish seed viability. This research aimed to optimize the TSS germination and seedling growth through a priming solution. The experiment was conducted in the Agro-Industry and Biomedical Laboratory greenhouse in the South of Tangerang. It was conducted in a randomized complete block design (RCBD) with 3 replications. The TSS were soaked with water as a control and plant growth regulator priming consisting of GA_3, NAA, BAP, B1, $Na_2S_2O_3.5H_2O$, $AgNO_3$, and eco enzyme. Then, TSS was planted in the growth medium, watered, and sprayed daily using priming solution. The research showed that the treatment significantly affected total germinated percentage (TGP), seedling height, root length, fresh weight, seedling dry weight, and root dry weight. The highest TGP was found in the treatment of soaking and watering with the eco enzyme (92%). Meanwhile, the treatment of soaking with GA_3 and NAA had the highest seedling (7.11 cm), the heaviest seedling fresh (310.40 mg), and the heaviest root fresh (72.17 mg).

Keywords: *Allium ascalonicum* L, eco enzyme, GA_3, NAA, priming solution.

Introduction

Shallot (*Allium cepa* L.) is an indispensable horticultural crop for food flavouring. Farmers employ shallots as a source of revenue because of their high economic value and ability to promote economic growth. Shallot prices, however, can vary due to unpredictable production. It rises during periods of low production and plummets during high production. Technology to increase shallots' productivity becomes essential to meet the demand for shallots in Indonesia [1,2].

The number of shallot bulbs required for seedlings is large and frequently transmits diseases. In addition, the large volume and seed dormancy are constraints in using shallot bulbs as seeds. In recent decades, there has been a growing interest in using true shallot seeds (TSS) as a source of seeds. The advantages of true shallot seeds are that they are disease-free, unbulky, cheaper, and portable [3]. Despite that, external factors in the post-harvest period caused seed quality deterioration, such as unstable temperature and humidity during storage, inadequate packaging, and gas composition in the storage room. The decline in seed quality will affect seed viability.

Seed preparation, such as priming application for shallot cultivation, is an important factor because it can increase the germination speed and percentage of germination and reduce the number of abnormal shallot tillers [4]. Several studies suggested that priming of true shallot seeds with various plant growth regulators can accelerate germination and improve seed viability and seedling growth [5]. Seed priming has been known to include hydro-priming, halopriming, osmo-priming, and hormonal priming. Seeds are treated with hydropriming, which is soaking the seeds in water and then drying before sowing, allowing the seeds to absorb water and begin metabolic activity. However, hydropriming can result in uneven hydration, non-uniform germination, and even seed damage because important nutrients are released from the seeds [6]. Halopriming is soaking seeds in an organic salt solution, such as NaCl, KNO_3, and

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$CaCl_2$. In addition, the process of soaking seeds in a solution of sugar, polyethylene glycol, glycerol, sorbitol, or mannitol, which have low osmotic potential, is called osmo-priming [7].

A plant growth regulator is an organic compound, either natural or synthetic, that plays an important role in modifying or controlling one or more specific processes within a plant [8]. Hormonal priming is the process of soaking seeds with a plant growth regulator. Gibberellin acid (GA_3) is the critical regulator that promotes germination, increases the number of tubers, and induces tillering [9]. Soaking the seeds in gibberellin 100 ppm can enhance the vigor index parameters, germination speed, and sprout power [10].

Benzyl amino purine (BAP) accelerates the number of tillers, boosts plant height [11,12]. Silver nitrate ($AgNO_3$) is used as a plant growth regulator, and it can increase shoot and root formation after germination and cell divisions in root and shoot tips [13]. Thiamin (B1) application increases the germination percentage, decreases the average germination time, and increases the emergence percentage and plant growth parameters such as seedling leaf area, fresh and dry weight of roots, and leaves of eggplant seedlings [14]. Sodium thiosulfate ($Na_2S_2O_3.5H_2O$) that is applied to seeds shows higher germination, faster germination, and growth of the seeds [15,16], and the application of an eco enzyme on red beans can increase the growth [17]. Therefore, this current research aimed to optimize the TSS germination and seedling growth by applying a priming solution. Further, we identified the effective pre-treatments for improving the quality and growth of seedlings.

Materials and Methods

Materials

True shallot seeds used in this research were the Lokananta variety. The growth media was made by mixing soil, rice husk, and manure in a 1:1:1 ratio and then sterilizing. The synthetic growth regulators used were gibberellic acid (GA_3), benzyl adenine (BAP) and 1-naphthaleneacetic acid (NAA). Other chemicals used were sodium thiosulphate ($Na_2S_2O_3.5H_2O$), silver nitrate ($AgNO_3$), and thiamine (vitamin B1), which were obtained from Merck® Chemicals. Eco enzyme was an organic liquid made from mixing fresh fruit waste (pineapple, papaya, orange, melon, watermelon), sugarcane and molasses. The growth media was put into a plastic pot, and the media depth was about 5 cm.

Methods

An experiment was conducted in a randomized complete block design (RCBD) with 3 replications. The seed soaking treatments tested were as follows: Table 1. Seeds were soaked for 12 hours before planting according to each treatment code, with 100 seeds in the experimental unit. After soaking, seeds were dried and coated with bactericide and fungicide, then planted for two weeks in growth media. Seeds with spray treatment with a plant growth regulator were sprayed according to each treatment code during the experiment. At the same time, seeds without spray treatment were sprayed with water to maintain humidity.

Table 1. Seed treatment

No	Treatment Code	Solution composition	Application technique
1	A (control)	Water	Soaking
2	B	GA_3 100 ppm; NAA 50 ppm	Soaking
3	C	BAP 50 ppm; NAA 50 ppm	Soaking
4	D	GA_3 100 ppm; NAA 50 ppm; B1 50 ppm	Soaking
5	E	BAP 50 ppm; NAA 50 ppm; B1 50 ppm	Soaking
6	F	B1 50 ppm	Soaking
7	G	$Na_2S_2O_3.5H_2O$ 18.94 ppm; $AgNO_3$ 1.6 ppm	Soaking
8	H	Eco enzyme	Soaking
9	B + Spraying	GA_3 100 ppm; NAA 50 ppm	Soaking, spraying
10	C + Spraying	BAP 50 ppm; NAA 50 ppm	Soaking, spraying
11	D + Spraying	GA_3 100 ppm; NAA 50 ppm; B1 25 ppm	Soaking, spraying
12	E + Spraying	BAP 50 ppm; NAA 50 ppm; B1 25 ppm	Soaking, spraying
13	F + Spraying	B1 50 ppm	Soaking, spraying
14	G + Spraying	$Na_2S_2O_3.5H_2O$ 18.94 ppm; $AgNO_3$ 1.6 ppm	Soaking, spraying
15	H + Spraying	Eco enzyme	Soaking, spraying

Total germinated percentage (TGP) was calculated by counting seedlings until 14 days after planting (DAP). Normal germinated percentage (GP) was calculated by counting only normal seedlings 14 DAP [18]. Germination rate (GR) was defined as:

$$GR = \frac{a + (a+b) + (a+b+c) + \cdots + (a+b+c+m)}{n(a+b+c+\cdots+m)}$$

Where a, b, and c were the number of seedlings in the first, second, and third counts; m was the number of seedlings in the final count; and n was the number of counts [19].

Seeds were measured for the seedling height, root length, fresh weight, and dry weight of the seedling and root at 14 DAP. Measurements of seedling height were taken from the base of the stem (soil surface) to the growing point using a ruler. Root length measurements were made from the stem's base to the longest root tip. Before measurement, the seedlings and the roots were washed with water and then air dried. The fresh weight of seedlings and roots was observed using an analytical balance. For the observations of dry weight, the seedlings and roots were dried using an oven at 50°C for 3 × 24 hours, then weighed again. The statistical analysis of treatments was tested using analysis of variance, and means were compared by Tukey's Honest Significant Difference (HSD) Test using the Statistical Tool for Agricultural Research (STAR).

Results and Discussion

The plant growth regulator treatments had a significant effect on TGP but no significant effect on GP (Table 2). The TSS soaked and sprayed with eco enzyme showed significantly higher TGP (92 %) than those of the other seed treatments and also showed the highest on GP, although not significant (79 %). Using eco enzyme in a 10-20% solution in a compost mixture produces a germination index of *Phaseolus vulgaris* seeds in the 87 – 100% [17]. Furthermore, eco enzymes can act as a seed surface sterilant agent because they inhibit the growth of gram-positive bacteria. Thus, they may suppress the population of antagonistic bacteria in seed germination [20]. Eco enzymes also contain organic C and NPK needed by plants, and then, in the fermentation process, they produce organic acid, which lowers pH [21]. This condition was optimum for producing phytohormones such as auxin, cytokinin, and gibberellin, which increase the vegetative growth of plants. Therefore, seeds soaking treatment with NAA and GA_3, which were external phytohormones, did not significantly increase TGP because seeds could germinate optimally with endogenous phytohormones stimulated by eco enzymes.

Table 2. Total germination percentage, normal germination percentage, and germination rate of TSS due to plant growth regulator treatments on 14 DAP

Treatments	Total germinated percentage (TGP) (%)	Normal germinated percentage (GP) (%)	Germination rate (GR)
A (Control)	85 ab	64	0.52
B	81 ab	65	0.51
C	51 cd	39	0.57
D	35 d	31	0.59
E	59 bcd	54	0.55
F	80 ab	69	0.51
G	68 abc	61	0.51
H	72 abc	67	0.51
B + Spraying	77 abc	55	0.54
C + Spraying	73 abc	56	0.58
D + Spraying	77 abc	66	0.53
E + Spraying	68 abc	59	0.54
F + Spraying	88 a	69	0.54
G + Spraying	87 ab	76	0.53
H + Spraying	92 a	79	0.52

Eco enzymes are categorised as a biological agent comprising a consortium of microbiomes that can produce essential phytohormones for plants [22,23]. Additonaly, eco enzymes are complex organic molecules believed to act indirectly to affect the structure and function of cell membrane permeability. However, it depends on the composition of the applied eco enzyme [24–28]. However, this needs to be proven by more in-depth studies.

In general, the treatment of spraying with growth stimulants during the experiment increased TGP and GP, although not significantly. Figure 1 showed the highest and lowest TGP. Repeated spraying allowed the seeds to receive more growth stimulants and be absorbed more than seeds not sprayed with growth stimulants. Therefore, the physiological and metabolic processes in seed germination were more stimulated.

Soaking in GA_3 100 ppm; NAA 50 ppm; B1 50 ppm (D)

Soaking in ecoenzyme (H)

Soaking and spraying with GA_3 100 ppm; NAA 50 ppm; B1 50 ppm (D + spraying)

Soaking and spraying with ecoenzyme (H + Spraying)

Figure 1. The highest and lowest total germinated percentage

Seed quality in this experiment was relatively low when referring to standards Certification of TSS by Degree of the Minister of Agriculture Number: 131/Kpts/SR.130/D/11/2015 about technical guidelines for seed certification of shallots. According to this, the minimum TSS GP requirement was 70%. However, the treatment of soaking and spraying with $Na_2S_2O_3.5H_2O$+$AgNO_3$ and the treatment with eco enzyme had GP values of 76% and 79%, respectively. The GR values of the seeds did not significantly differ due to treatment, and the average of the GR values ranged from 0.51 to 0.59.

The process of seed germination includes morphological, physiological, and biochemical changes. Seeds' germination began with water absorption by seeds, making the coats soft and hydrated from the protoplasm. The next stage began with the activities of cells and enzymes, as well as an increase in the level of seed respiration. After that, carbohydrate, fat, and protein materials were broken down into soluble forms and translocated to the growing point. The assimilation of the materials that have been decomposed in the meristematic area produces energy for the formation of components and the growth of new cells. The final stage was seedlings' growth through the division, enlargement, and division of cells at the growing points. Meanwhile, the leaves did not yet function as organs for photosynthesis. Therefore, the growth of seedlings was very dependent on the food supply in the seeds [29,30].

The plant growth regulator treatments significantly influenced leaf and root lengths (Table 3 and Figure 2). Seeds treated with GA_3 100 ppm + NAA 50 ppm (B treatment) showed higher seeds than other treatments (7.11 cm), followed by the eco enzyme treatment (H treatment) and $Na_2S_2O_3.5H_2O$+$AgNO_3$+spraying (G+spraying) treatment (6.78 cm, respectively). Meanwhile, the

longest seed roots were found in the B1+spraying treatment (F + Spraying), followed by $Na_2S_2O_3.5H_2O+AgNO_3$ treatment (G treatment), 2.88 cm and 2.85 cm, respectively. Ion Ag^+ affects the induction of cell division, root growth, hypocotyl, and shoots, also acts as an inhibitor of ethylene synthesis due to its solubility in water and low level of toxicity [13].

Table 3. Seedling height and root length at 14 DAP

Treatments	Leave length (cm)	Root length (cm)
A (Control)	5.27 abc	1.59 abc
B	7.11 a	2.36 ab
C	4.83 abc	1.20 bc
D	4.91 abc	1.31 bc
E	6.12 abc	1.22 bc
F	6.55 ab	2.58 ab
G	6.33 abc	2.85 a
H	6.78 ab	2.39 ab
B + Spraying	3.49 c	0.49 c
C + Spraying	4.04 bc	0.36 c
D + Spraying	4.73 abc	0.52 c
E + Spraying	4.03 bc	0.47 c
F + Spraying	6.14 abc	2.88 a
G + Spraying	6.78 ab	2.26 ab
H + Spraying	6.43 ab	2.12 ab

Note: Values with the same letters are not significantly different based on HSD Tukey, α=0.05

According to Pangestuti *et al.* [1], GA_3 and NAA increased leaf length and root length. Soaking seeds with NAA increased the content of the hormone auxin in seeds. Auxin plays a role in initiating the formation of lateral roots at advanced germination stages and early seedling growth stages. Combining soaking with gibberellin increased plant height, as in Table 3, by increasing cell division, extension, and replication [31].

Furthermore, auxin influenced cell elongation, especially roots, by making the cell wall flexible and softer. Auxin-activated proton pumps (H^+ ions) in the cell plasma membrane break some hydrogen cross-link chains of cellulose molecules that make up cell walls. It caused the cell walls to stretch easily and cell wall pressure to decrease, resulting in cell flexing. Apart from that, proton pumps also reduced the cell plasma membrane's pH value, activating certain enzymes in the cell wall. This enzyme degraded various proteins or polysaccharides spread across the soft and flexible cell walls, so cell elongation occurred, followed by cell division [31].

In contrast, applying soaking and spraying with GA_3 100 ppm and NAA 50 ppm on seeds inhibited leaf and root length, as described in Table 3. This result was in line with Evans *et al*. [32], where root elongation in seedlings of Arabidopsis was increased by exogenous auxin at low concentrations but inhibited at high concentrations. This inhibition occurred because excessive auxins produced ethylene. Based on the explanation from Wang *et al*. [33], the increasing concentration of auxins caused increasing ACC synthase, an enzyme that converted the precursor S-Adenosyl Methionine to Aminocycloheptane-1-carboxylic acid (ACC) which then became ethylene through the Yang Cycle. Ethylene inhibited the root length of seedlings because of the ethylene-triggered triple responses.

The analysis of variance showed that the application of plant growth regulator had a significant effect on leaf fresh weight, leaf dried weight, and root dried weight but had no effect on root fresh weight (Table 4). The highest fresh weight was 310.40 mg in treatment B (soaked in GA_3 100 ppm and NAA 50 ppm). Generally, the fresh weight treated by soaking and spraying the plant growth regulator tended to be lower than that of seeds treated with soaking without spraying. The highest dry weight was in treatment F, soaking with vitamin B1. In contrast to the fresh weight of the seeds, the plant growth regulator soaking and spraying treatments showed an increase in the leaf dried weight, although not significantly. The root fresh weight did not appear to be significantly different due to the plant growth regulator treatment, but was highest in treatment B. The root dried weight was highest in the G+spraying treatment (soaking and spraying with $Na_2S_2O_3.5H_2O$; $AgNO_3$), which was 5.20 mg. The soaking and spraying treatment produced a higher root dried weight than the soaking treatment without spraying, although it was not significantly different.

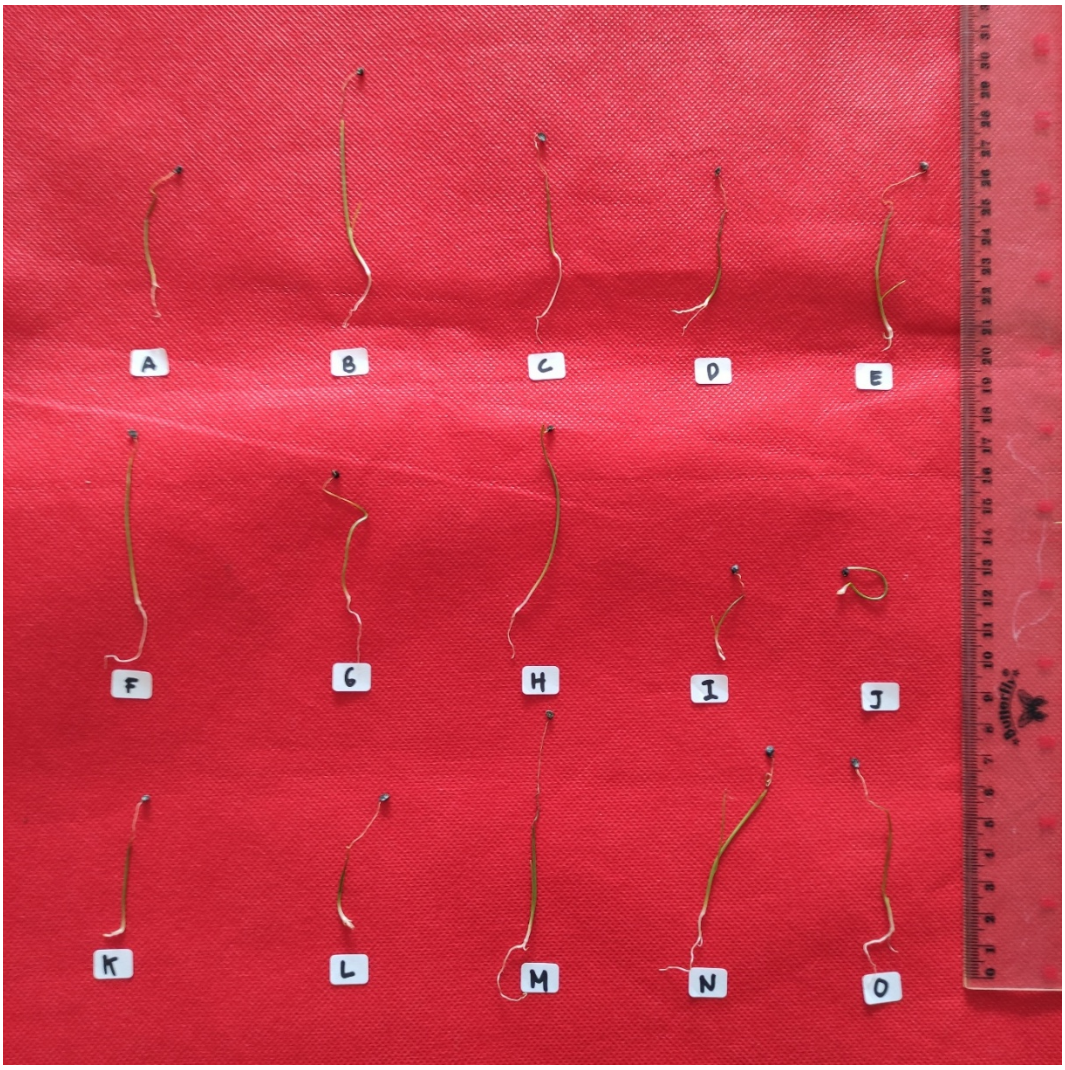

Figure 2. Seedling at 14 DAP : (A (control), B (soaking in GA_3 100 ppm; NAA 50 ppm), C (Soaking in BAP 50 ppm; NAA 50 ppm), D (Soaking in GA_3 100 ppm; NAA 50 ppm; B1 50 ppm), E (Soaking in BAP 50 ppm; NAA 50 ppm; B1 50 ppm), F (Soaking in B1 50 ppm), G (Soaking in $Na_2S_2O_3.5H_2O$ 18.94 ppm; $AgNO_3$ 1.6 ppm), H (Soaking in ecoenzyme), I (B treatment + spraying), J (C treatment + spraying), K (D treatment + spraying), L (E treatment + spraying), M (F treatment + spraying), N (G treatment + spraying), O (H treatment + spraying)

Similar results were also reported by Pangestuti *et al*. [1], and the seeds treated with 100 ppm GA_3 and 50 ppm NAA increased the TSS seeds' fresh and dried weight. Fresh weight was the weight of the seeds when they were still alive, and they were weighed directly after harvest, before losing water. Meanwhile, dry weight was the accumulation of organic and inorganic compounds from plant photosynthesis. Dry weight was a measure of plant growth and development because dry weight reflects the accumulated compounds that the plant has successfully synthesized. The increase in fresh and dry weight was due to more plant physiological activity with the addition of GA_3, which stimulated cell growth. If the physiological process that occurs in plants runs well, it increases the plant's dry weight. Applying GA_3 combined with NAA stimulated tissue growth and encouraged cell division, making growth more optimal.

Table 4. Fresh weight and dry weight of the seedling and root at 14 DAP

Treatments	Leave fresh weight (mg)	Leave dried weight (mg)	Root fresh weight (mg)	Root dried weight (mg)
A (Control)	180.10 ab	18.00 ab	50.57	3.77 ab
B	310.40 a	20.00 ab	72.17	4.37 ab
C	156.20 b	16.33 ab	36.30	2.53 b
D	147.33 b	9.33 b	42.30	2.70 ab
E	190.17 ab	14.00 ab	43.80	2.70 ab
F	239.00 ab	25.57 a	60.57	4.37 ab
G	231.30 ab	14.33 ab	67.00	4.60 ab
H	230.53 ab	14.17 ab	62.27	3.90 ab
B + Spraying	106.57 b	17.80 ab	35.90	3.53 ab
C + Spraying	118.97 b	17.30 ab	37.37	3.23 ab
D + Spraying	133.63 b	15.30 ab	59.53	5.07 ab
E + Spraying	126.57 b	15.33 ab	43.33	3.50 ab
F + Spraying	211.13 ab	20.30 ab	61.23	4.07 ab
G + Spraying	230.50 ab	20.00 ab	71.53	5.20 a
H + Spraying	213.73 ab	21.70 ab	62.40	4.47 ab

Note: Values with the same letters are not significantly different based on HSD Tukey, α=0.05

The correlation coefficients between several observation parameters are shown in Figure 3. Several parameters were positively correlated, except with the GR parameter. An increase followed the increase in TGP in GP, leave length, root length, fresh weight, and dried weight of leaves and roots. This showed that the seeds' quality affects the next growth stage. One indicator of good seeds was that they contained sufficient food reserves for the next stage of shallot growth, which will later develop into roots and leaves. The grown roots function to absorb nutrients, and the leaves function as a place for photosynthesis. The seeds then use the energy produced for the growth process.

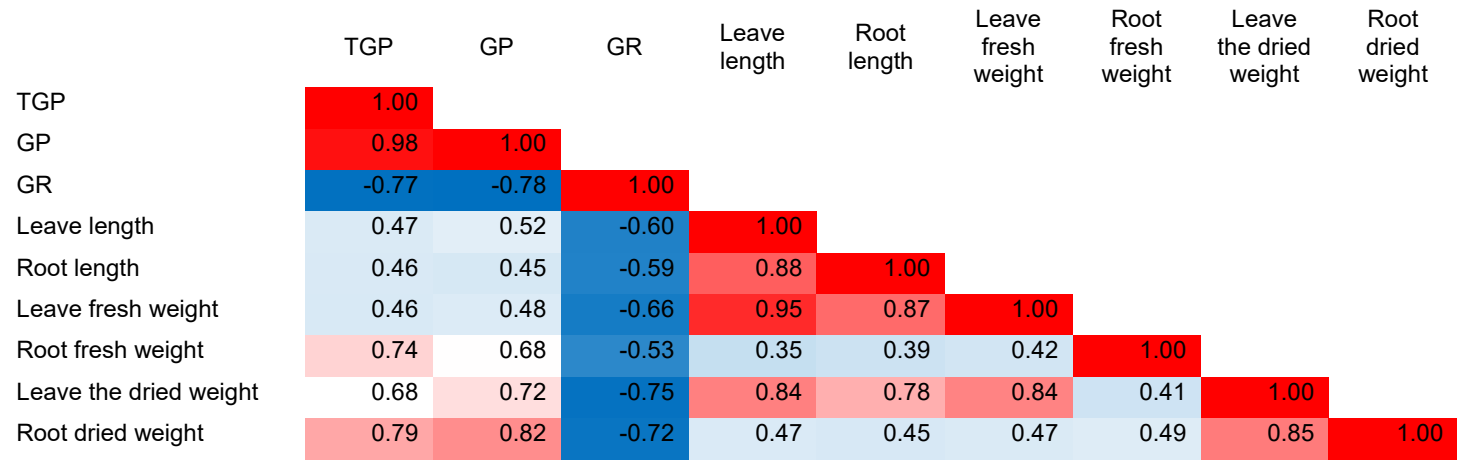

Figure 3. Correlation analysis between germination parameters of TSS

Conclusions

The treatment of seeds with plant growth regulators significantly affected total germinated percentage (TGP), seedling height, root length, fresh weight, seedling dry weight, and root dry weight. Soaking and spraying with eco enzyme produced the highest TGP, and the soaking treatment with GA_3 and NAA had the highest seedlings, the heaviest seedlings fresh, and the heaviest root fresh.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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