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Seed Priming with Vegetal Protein Hydrolysate Enhances Germination and Early Seedling Growth in *Cucumis sativus* L.

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ABSTRACT

Cucumber is widely cultivated worldwide, occupying significant agricultural acreage. The present research aims to evaluate the seed priming potential of Vegetal Protein Hydrolysate (VPH) to improve early seedling growth and seed germination of cucumber. We assessed two concentrations of VPH (2 and 4 mL L^{-1}) under normal and salt stress conditions, with water as the control treatment. The experiment was conducted in a completely randomized factorial design. The cucumber seeds were primed for 4, 8, and 12 h, followed by observing seed germination traits, including germination rate index, mean germination time, germination percentage, vigorous index-2, and early seedling growth traits. Based on the results, VPH treatments with low concertation significantly affected morphological traits in cucumber seed germination. We found the apparent priming potential of VPH to improve early seedling growth and seed germination in cucumbers with different morphological features. Seed priming with VPH significantly improved germination traits and chlorophyll in salt stress conditions compared to the control treatment. The results showed that the seed germination was affected by priming durations of 4 and 8 h. However, the extended exposure to the same concentration (12 h) inhibited the seed germination process, thereby inducing a higher level of stress intensity in the seedlings. Thus, VPH seed priming can be regarded as a bio-stimulant for enhancing early seedling growth and seed germination in cucumbers. These results can assist in preparing vegetal protein-based compounds and improve vegetable production in greenhouses and plastic tunnels.

Introduction

Seed germination is a crucial process initiated by water absorption in dry seeds, leading to embryonic axis growth. This process is a prime factor in determining plant development in a specific environment. Environmental conditions, including temperature, water, light, and soil nutrients, significantly influence this vital stage of plant life (Steinbrecher and Leubner-Metzger, 2017). Environmental factors such as temperature, water availability, light, and soil nutrients can all affect seed germination. For instance, some seeds require specific temperature ranges to germinate. Some other seeds must be exposed to light to sprout. In addition, the availability of water and nutrients in the soil can be essential for seed germination success. According to the literature, different physiological processes, including antioxidant enzymes, hormones like Indole-3-acetic acid (IAA), Abscisic acid (ABA), Gibberellic acid (GA3), soluble sugars levels, starch metabolism, and

redox potential, have a collective impact on seed germination. These factors are crucial parameters that must be investigated comprehensively to understand the germination procedure in a specific environmental condition (Nambara et al., 2010; Liu X et al., 2013; Wojtyla et al., 2016; Liu Y et al., 2018). Techniques such as genetic modifications of seeds and the application of chemicals can aid germination and improve the ability of seeds to grow and overcome germination barriers (Wang et al., 2016). Seed priming involves treating seeds with water and drying them to activate specific biochemical processes. Although the seed has not started sprouting yet, priming has been proven to aid germination and overcome obstacles (Yan, 2015; Ibrahim, 2016). In addition, seed priming can potentially enhance plant growth by increasing resistance to pathogens and promoting early growth (Johnson and Puthur, 2021). This resistance enhancement can develop a developed root system and improve plant growth. However, the effectiveness of seed priming partially reflects the concentration and duration of the priming chemical used.

Plant bio-stimulants (PBs) are among the new and innovative methods in agricultural management for sustainability promotion. They benefit various aspects of productivity and plant growth, such as nutrient utilization efficiency (NUE), flowering, fruit set, and ability to withstand abiotic stress (Colla et al., 2017; Rouphael et al., 2017). Using vegetable transplants can enhance the stem diameter, dry weight, leaf area development, and ability to create new roots after transplantation. Moreover, they improve nutritional status and reduce transplant shock. Protein hydrolysates (PHs) have particularly beneficial effects on plant transplants' quality traits, including germination stimulation, higher root length and density, and better plant establishment after transplantation. Therefore, PHs can help attain high-quality standards for vegetable transplant traits and positively affect plant growth, development, and crop performance after transplantation. The effectiveness of PHs derived from plants is attributable to their composition. This composition combines several bioactive compounds like amino acids, vitamins, peptides, carbohydrates, and phytohormones. PHs can have multiple functions. These functions include but are not limited to stimulating carbon and nitrogen metabolism by activating enzymes in the N-assimilation pathway and TCA cycle, exhibiting hormone-like activities similar to auxin and gibberellin, and enhancing secondary metabolism by producing metabolites and antioxidant enzymes. Researchers have investigated these effects in multiple cases of study (Halpern et al., 2015; Colla et al., 2017; Rouphael et al., 2017; Carillo et al., 2019; Giordano et al., 2020).

Hydrolyzed plant proteins can be a good option for germinating plant seeds because of their importance in plant growth. Due to the lack of sufficient information in this area, we have investigated the effects of hydrolyzed plant proteins on cucumber seed germination at different concentrations and times and under normal and salt stress conditions. The impacts of hydrolyzed plant proteins on cucumber seed germination under both conditions were reportedly significant at low concentrations and short times. Thus, our results are vital to comprehending the role of vegetal protein hydrolase as a priming agent in improving cucumber seed germination. Furthermore, they can present a platform to lower autotoxicity and counteract cropping obstacles that considerably reduce cucumber production.

Materials and Methods

Treatments and experimental conditions

All the experiments were performed using cucumber seeds. Following surface sterilization, the seeds were treated with 2 and 4 mL L^{-1} for both normal (C2 and C4) and salt stress (C2S and C4S) conditions by placing 120 seeds in 50 mL in a rotary shaker maintained at 25 °C for 4, 8, and 12 h. The protein hydrolysates trainer derived by vegetal® (Hello Nature Italia SRL, Rivoli Veronese (VR), Italy), a commercially accessible product, was used for this trial. This product is obtained through enzymatic hydrolysis of legume biomass. Amino acids, i.e., Arg, Ala, Asp, Glu, Cys, His, Gly, Leu, Ile, Met, Phe, Lys, Ser, Pro, Trp, Thr, Val, Tyr, and soluble peptides were the components of the PHs containing 5% of the total nitrogen content, accompanied by soluble sugars and phenolic compounds.

The seeds were primed with distilled water for the mentioned periods for the control treatment. Of the air-dried treated seeds, 40 were selected from each treatment for germination bioassay and distributed evenly over a double-layered filter paper bounded by a petri dish (11 cm × 7 cm). The moisture content was provided using 4 mL of distilled water (normal condition), while a 4 mL salt solution (40 mL L^{-1}) was used as the source of salt stress. The seeds were kept in the dark in a growth chamber at temperatures below 25 °C. Three replicates were performed for each treatment. After seed germination, the seedlings were watered (2-3 mL of water) on alternative days. The seeds were considered to have

germinated when seedlings had ≥3 mm radicle length. The germinated seeds were counted every 7 days at 24 h intervals.

Measuring the germination efficiency and morphological parameters

Morphological indices were measured by the growth of the seedlings over ten days. Vernier callipers were used for measuring root and hypocotyl length. Also, an electronic balance was used for taking the roots and aerial parts that determined the fresh weight immediately. Then, the samples were placed in an oven for 20 h at 80 ℃, followed by recording the dry weights (Gokul et al., 2016). The data were recorded using 15 seedlings per sample and measuring their mean values. The germination efficiency was expressed according to the final germination percentage (FGP), mean germination time (MGT), germination rate index (GRI), vigor index II (VI-II), and germination index (GI), as described by Alsaeedi et al. (2018).

Chlorophyll and carotenoid analyses

Obtaining chlorophyll and carotenoids involved grinding fresh leaf tissues (0.5 g) in 80% acetone, followed by separating the resulting extracts through centrifugation at 3000 g for 5 min. The levels of chlorophyll a and chlorophyll b were measured using a Beckman DU-50 UV-visible spectrophotometer (manufactured by Beckman Instruments, Inc., based in Fullerton, CA, USA) by measuring the absorbance at 470, 647, and 667 nm (Lichtenthaler and Wellburn, 1983).

Statistical analysis

The seeds were set in a completely randomized factorial experimental design (3 × 5). The collected data were exposed to the analysis of variance (ANOVA). Also, the mean values were separated according to Tukey's HSD test ($p<0.05$) using the SAS (ver. 9.4) statistical program.

Results

Based on ANOVA (Table 1), the interactive effects of VPH and salt stress, except for root length, were significant in other measured traits.

Table 1. Two-way ANOVA table for the cucumber priming experimental test.

Treatments	Fresh Weight	Dry Weight	Final Root Length	Final Stem Length	FGP	MGT	GRI	GI	VI-2
Salt	0.015^{*}	0.0003^{ns}	12.12^{**}	0.31^{ns}	2229.36^{**}	1.13^{**}	68.26^{**}	276.1^{**}	14.7^{**}
VPH	0.099^{**}	0.0003^{ns}	6.631^{**}	1.06^{*}	1543.05^{**}	3.08^{**}	97.68^{**}	289.45^{**}	12.3^{**}
Salt*VPH	0.020^{**}	0.00039^{*}	2.99^{**}	0.20^{ns}	827.54^{*}	0.76^{**}	19.59^{**}	545.65^{**}	5.5^{**}
CV	**14.89**	**16.96**	**22**	**9.69**	**24.26**	**5.14**	**27**	20	18

(VPH= Vegetal protein hydrolysate, FGP= Final Germination Percentage, MGT= Mean Germination Time, GRI= Germination Rate Index, GI= germination index, VI-Ⅱ= Vigorous Index Ⅱ). * and ** significant different at 95% and 99% respectively. Ns= Not Significant.

Seed priming with vegetal protein hydrolysate in normal and stress conditions improved the germination (Fig. 1). In seeds treated with 2 mL L^{-1} for 4 and 8 h in both growth conditions, the germination percentage was significantly higher than the control treatment, and the maximum germination percentage was obtained for the seeds applied with 4 mL L^{-1} for 12 h. Interestingly, the germination percentage was reduced significantly by VPH at higher concentrations (4 mL L^{-1}) in a time-based manner. Furthermore, extended exposures (12 h) caused minimal germination percentage with a statistical difference that varied significantly from the control group (Fig. 1a).

The mean germination time increased by applying high-concentration VPH, and the time needed by the seed for germination was reduced. Germination was facilitated by priming the seeds with 2 and 4 (mL L^{-1}) for 12 h in a smaller time frame in both growth conditions. Moreover, stress was imposed on the seeds and extended by priming with 2 and 4 (mL L^{-1}) for 8 h (Fig. 1b).

Compared to the control, in seeds treated with 2 mL L^{-1} of VPH for 4 and 8 h, maximum germination was achieved. However, it was lowest in seeds treated with 4 mL L^{-1} for 12 h in both growth conditions. The effect of 2 mL L^{-1} VPH was non-significant when priming for 4, 8, and 12 h. Nevertheless, higher germination rates were obtained by extended durations (4 h) (Fig. 1c).

The VI-II was the highest using 2 mL L^{-1} of VPH for 4 and 8 h in normal growth conditions. Also, the 2 and 4 mL L^{-1} caused maximum values in both growth conditions in 4 h. The lowest VI-II occurred in response to using 4 mL L^{-1} VPH for 12

h (compared to the control). In this regard, 2 mL L^{-1} VPH for 4 h significantly increased VI-II. Likewise, a higher VI-II was found in seeds primed with 4 mL L^{-1} VPH for 4 h rather than 8 and 12 h, although with no significant difference being observed (Fig. 1d).

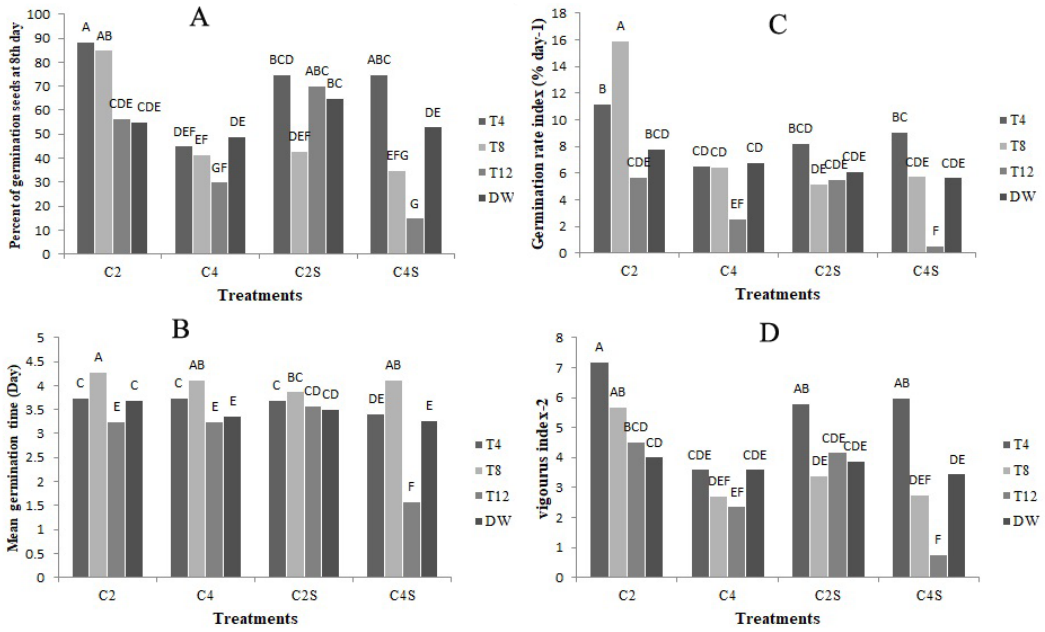

Fig. 1. Germination dynamics of vegetal protein hydrolysate and control seedling of cucumber. (A) Germinated seed percentage on the eighth day, (B) mean germination time, (C) germination rate index, (D) vigor index-2, DW (distillated water). C2 and C4 (VPH 2 and 4 mL L^{-1}) with 4, 8, and 12 h as priming durations. Each bar represents mean values and standard errors (n= 50). Similar letters on the bars indicate no significant difference ($p \leq 0.05$) (Tukey's).

Morphological enhancements in cucumber seedlings by seed priming

Different morphological parameters were improved significantly by seed priming in cucumber seedlings (Figs. 2 and 3). Data analysis indicated considerable effects of VPH on the morphological growth, concentrations, and the priming duration. Moreover, the priming duration of 4 h highly enhanced morphological parameters in the cucumber seedlings after 8 h with an intermediate effect and 12 h with the least positive effect. According to the results, the overall morphological characteristics (e.g., stem length, seedling root, and fresh or dry weights) improved significantly by 2 mL L^{-1} for growth conditions of VPH for 4 h compared to the control treatment. Furthermore, the stimulatory effect of 2 mL L^{-1} VPH in 8 h was less, and yet close to the effect of using 2 mL L^{-1} for 4 h (Fig. 4).

The VPH treatment for 4 and 8 h positively influenced seedling growth, thus significantly enhancing fresh weight and shoot length. However, high concentrations of VPH in salt stress growth conditions for 12 h had a negative effect on seedling growth and showed considerable inhibition effects compared to the other treatments.

According to data analysis, the priming duration interacted with treatment potential. When the cucumber seeds were treated with 2 mL L^{-1} for 4 and 8 h, the fresh weight positively increased in both growth conditions. On the other hand, treatment with 2 and 4 mL L^{-1} VPH for 4 and 8 h, compared to control, led to no significant differences in dry weight. However, the same treatment for 12 h showed significant differences with others. The highest shoot length was observed in the seedlings treated with 2 and 4 mL L^{-1} for 4 and 8 h, followed by the control treatment. Meanwhile, the lowest value was observed with the same treatment for 12 h. Moreover, significant root length improvements occurred. The application of 2 mL L^{-1} VPH for 4 and 8 h caused maximum value in normal growth conditions. Other treatments showed no significant effect on the root length. Meanwhile, the minimum level of root length was observed at 4 mL L^{-1} for 12 h in both growth conditions.

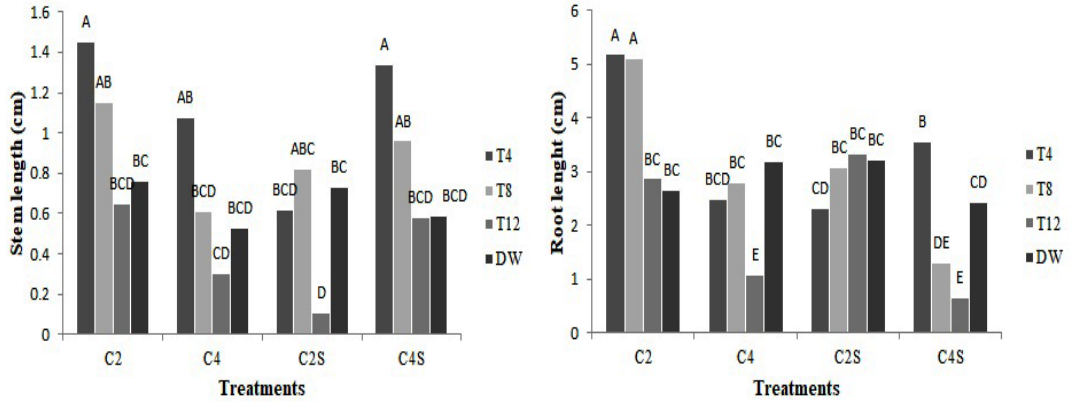

Fig. 2. Interaction effects of various priming condition on the morphological parameter, stem, and root length of cucumber seedlings. DW (distillated water). C2 and C4 (VPH 2 and 4 mL L^{-1}) with 4, 8, and 12 h as priming durations. Each bar represents mean values and standard errors (n= 50). Similar letters on the bars indicate no significant difference ($p \leq 0.05$) (Tukey's).

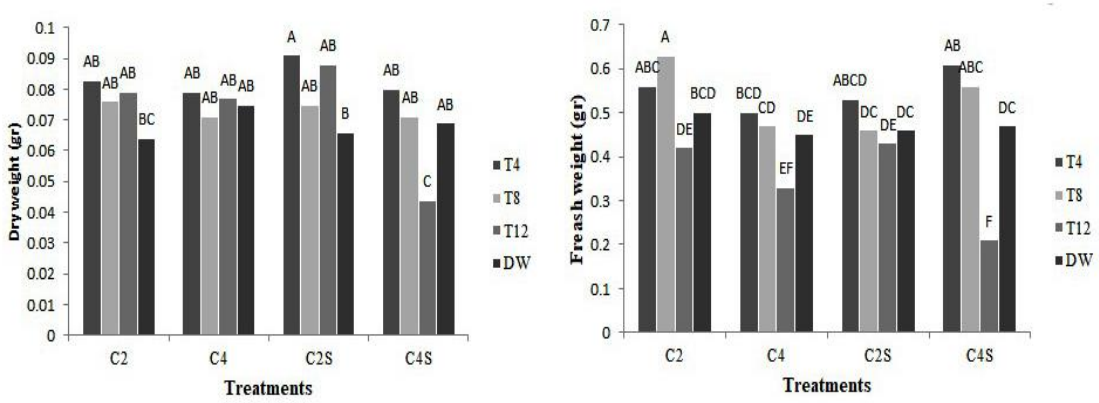

Fig. 3. Interaction effects of various priming condition on the dry and fresh weight of cucumber seedlings. DW (distillated water). C2 and C4 (VPH 2 and 4 mL L^{-1}) with 4, 8, and 12 h as priming durations. Each bar represents mean values and standard errors (n= 50). Similar letters on the bars indicate no significant difference ($p \leq 0.05$) (Tukey's).

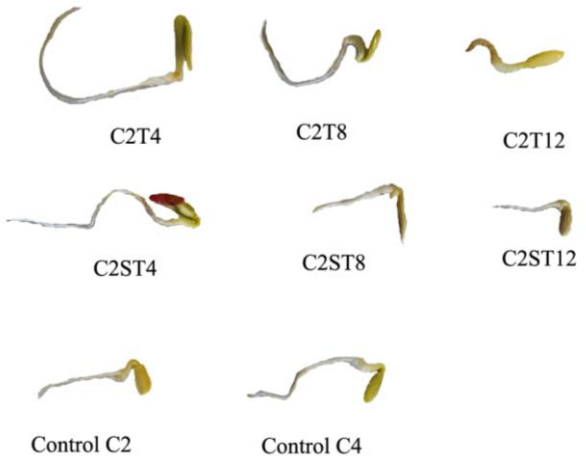

Fig. 4. Seedlings growth after germination of the seeds primed with 2 mL L^{-1} VPH and distilled water. Control (distilled water), C2T (normal condition treated with 2 mL L^{-1} VPH for 4, 8 and 12 h), C2ST (salt stress conditions treated with 2 mL L^{-1} VPH for 4, 8, and 12 h).

Total chlorophyll and a/b chlorophyll content

In normal conditions, total chlorophyll content increased significantly in cucumber leaves with 2 and 4 mL L^{-1} during 4 and 8 h. Also, there was a similar response regarding carotenoid content. On the other hand, in salt stress conditions, treatment with 2 and 4 mL L^{-1} in 4 and 8 h led to better results than the 12 h treatment. Chlorophyll a/b decreased during the 12 h treatment with 2 and 4 mL L^{-1} in both growth conditions. With increasing treatment time, it indicated a greater accumulation of chlorophyll b than chlorophyll a (Table 2).

Table 2. Interaction effects of various priming conditions on the chlorophyll content.

Treatments	Total chlorophyll (mg g^{-1} fresh weight)	Chlorophyll a/b	Carotenoids (mg g^{-1} fresh weight)
C2T4	2.1^{a}	2.4^{b}	0.67^{c}
C2T8	2.05^{a}	2.2^{bc}	0.57^{d}
C2T12	1.4^{e}	2.7^{a}	0.78^{b}
C2DW	1.8^{c}	2.6^{ab}	0.84^{b}
C4T4	1.9^{ab}	2.1^{b}	0.91^{a}
C4T8	1.8^{c}	2.6^{ab}	0.65^{c}
C4T12	1.3^{ef}	2.8^{a}	0.54^{d}
C2ST4	1.8^{c}	2.1^{c}	0.64^{cd}
C2ST8	1.8^{c}	2.4^{b}	0.56^{d}
C2ST12	1.04^{hi}	2.74^{bc}	0.43^{f}
C2SDW	1.7^{cd}	2.26^{bc}	0.73^{bc}
C4ST4	1.6^{d}	2.2^{bc}	0.86^{b}
C4ST8	1.5^{de}	2.21^{bc}	0.49^{ef}
C4ST12	1.1^{h}	2.78^{a}	0.34^{g}
C4SDW	1.1^{h}	2.8^{a}	0.62^{c}

Discussion

Based on the collected data, VPH had a biological effect on different physiological procedures during seed germination of cucumber, which was proportional to the VPH concentration and priming duration. Also, VPH affected early seedling growth and germination, representing a considerable effect of these growth regulators on seed priming. VPH enhanced seed germination and growth characteristics in stress growth conditions.

The results showed that the germination processes were affected by the VPH concentration in a dose-dependent manner. Seeds treated with 2 mL L^{-1} of VPH for 4 and 8 h had increased germination percentage, VI-II, germination rate, and MGT in both growth conditions. Furthermore, the increased germination traits were concentrated based on the priming duration. The maximum AGE concentration occurred by prolonged priming of the seeds (12 h).

According to Ali et al. (2019), osmotic stress declined by priming rice with biostimulants such as methyl jasmonate (MeJA) by changing the physiological procedures in the germinating seedlings. Our results strongly agree with the biological VPH effect as seed priming. However, exposing the seeds to higher concentrations of VPH for a prolonged time (4 mL L^{-1} for 12 h) had a negative effect on the seedlings and germination traits. These results are consistent with garlic-derived compounds with formerly established dose-based effects (Cheng et al., 2016). These results proved the role of seed priming with VPH in stimulated seed germination and a resultant seedling development and growth. As described previously, several processes create ROS during cellular metabolism, and these ROS can signal a massive array of cellular responses for sustaining normal plant growth (Mittler, 2017). These ROSs are essential in cellular elongation processes and cell division, particularly during seed germination (Kranner et al., 2010). Thus, these seedlings had more elaborate physiological features and stable states regarding the elevated antioxidant enzymes. The possible negative effect of the ROS

(e.g., DNA denaturation or cellular destruction) was controlled effectively under these conditions. Therefore, the seedlings represented considerable morphological growth (Gill and Tuteja, 2010).

Moreover, antioxidant enzyme activity declined by exposing the seeds to higher VPH concentrations for lengthy periods and the oxidative burst responses were caused by ROS elevation. Thus, the seedling growth was inhibited (Baxter et al., 2014; Jeevan Kumar et al., 2015). Seed priming may improve physiological activity and the germination process in cucumber seedlings, thus improving morphological growth. These features were followed by increased shoot length, root length, and seedling dry and fresh weights.

Our results showed significant responses in the morphological growth patterns and cucumber seedling germination traits. In this respect, it can be suggested that using VPH as a seed priming agent could possibly overcome the challenges during early cucumber growth. As a result, it can be an alternative to other priming methods.

This research revealed that the Chl a/b ratio decreased progressively by increasing the VPH dosage. This finding is consistent with previous studies that reported the same outcome when more nitrogen was available to the plants (Sonobe et al., 2020). The Chl a/b ratio indicates nitrogen availability and is positively linked to the ratio of photosystem II cores to the light harvesting chlorophyll-protein complex. When plants have more nitrogen available, the allocation to photosystem II decreases, allocation to rubisco becomes higher, and N allotment to the light harvesting chlorophyll-protein complex remains constant. Consequently, the ratio of photosystem II to the light harvesting chlorophyll-protein complex declines, thereby increasing the Chl a/b ratio with more nitrogen availability (Kitajima and Hogan, 2003). These results confirm that VPHs likely provide nitrogen benefits to the plants. In conclusion, the application of VPH did not impact the secondary metabolite content, such as total phenols and carotenoids. Overall, the biostimulant application mainly boosted primary metabolic processes that directly contributed to the growth and development of vegetable seedlings.

These substances may enhance plant growth and photosynthesis, leading to noticeable growth enhancements (Hayat et al., 2018). In this research, VPH was applied for seed priming for the first time in the literature. Future research can consider VPH treatment effects on the physiological traits of seedlings and clarify their unknown impacts.

Conclusions

This study showed the positive effects of seed priming on early growth in cucumber seedlings and their germination. Based on the results, VPH (2 mL L^{-1}) for 4 and 8 h was identified as the best priming treatment. Moreover, VPH enhanced early seedling growth and seed germination in salt-stress growth conditions. As a result, it is confirmed that VPH can induce priming effects on seed germination, chlorophyll, and carotenoid content in both normal and stress conditions. According to our findings, germination was stimulated by seed priming, and morphological traits were enhanced. Applying a high-concentration VPH decreased seed germination and chlorophyll content due to increasing osmosis conditions. VPH also successfully improved the cucumber germination properties by reducing the time taken for germinating and increasing the number of germinated seeds in a time frame. Our results provide a platform for future studies to comprehend the physiological functions of VPH during seed germination. These conditions include ROS regulation and scavenging through antioxidant enzymes or the accumulation of soluble proteins that interact with the germinating seeds.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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