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EVALUATION OF SEED PRIMING ON GERMINATION AND GROWTH OF BASIL (*Ocimum basilicum* L. cv. 'Genovese')

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ABSTRACT. The priming method is a technique that can greatly improve seed performance and provide high-quality seeds for successful production. In this study, the effect of hormopriming (GA_3 and IAA), halopriming ($MgSO_4$ and KNO_3), osmopriming (AA, H_2O_2) and hydropriming (H_2O) on the germination, as well as initial stages of growth and development of basil (*Ocimum basilicum* L. cv. 'Genovese') were investigated. The application of different priming methods not only improved the germination performances of basil, but also significantly influenced the growth of seedlings (root length, shoot length, fresh mass, and vigor index) with the best results achieved by priming with GA_3 and H_2O_2. In addition, it has been found that the concentration of photosynthetic pigments and soluble protein content can be improved by the appropriate priming treatment. The most favorable effect on the examined parameters was achieved during treatment with H_2O_2.

Keywords: basil, germination, photosynthetic pigments, proteins, RWC.

INTRODUCTION

Seed priming technology, a popular practice in recent years, has emerged as a promising and profitable strategy for managing abiotic and biotic stress without imposing genetic/transgenic modification (NOURI and HADDIOUI, 2021). Previous studies have shown that seed priming can improve germination rate and uniformity leading to reduced germination time of many agricultural and horticultural crops (BASRA *et al*., 2002; MASONDO *et al*., 2018; MORENO *et al*., 2018). This method involves soaking seeds in water and/or a solution containing other substances (natural/synthetic). In this way, a specific physiological state in the plant system is stimulated (MOULICK *et al*., 2018). Several different priming techniques have been identified for increasing germination and improving the uniformity of germination of various seeds. Each priming technique involves restricted hydration to allow imbibition and pre-germinative metabolic changes (SHARMA *et al*., 2014). A common technique in hydropriming implies the soaking of seeds in water for a specific time and drying of seeds to a certain moisture content before germination. It represents an effortless, economical, and environment-friendly technique. However, pure water enters seeds very fast, which may not be congenial for metabolic activation and cell elongation. To overcome this barrier, the seeds may be soaked in an osmotic solution followed by air drying. Osmosis controls the entry of excess water into the seed during imbibition, which reduces the accumulation of ROS and thus protects the cells from oxidative damages (SINGH *et al*., 2015). In addition to osmotic solutions, plant growth regulators applied in small quantities can also demonstrate direct effects on seed metabolism. Therefore, they are also used in the pre-sowing treatment of seeds as hormonal priming. These compounds not only stimulate growth and development but also mitigate the effects of some environmental stresses (NAWAZ *et al*., 2013).

Basil (*Ocimum basilicum* L.) is one of the most widespread and commonly cultivated plants around the world because it has various useful properties for humans. The application of basil in the food, perfume and medical industries, and thus the economic value of basil, is related to the chemical composition. It is known that basil plants are rich in essential oils (SHAHRAJABIAN *et al*., 2020; KULAK *et al*., 2021), but significant content of phenolic compounds including flavonoids, rosmarinic and caffeic acid with strong antioxidant activities can be found in various parts of *O. basilicum* L. cv. 'Genovese' (JAKOVLJEVIĆ *et al*., 2019).

In this paper, the influence of different priming treatments on certain physiological characteristics including germination, growth and development of basil were investigated. Given the global economic importance of *O. basilicum* L. cv. 'Genovese' and the priming efficiency, this study aimed to determine the most efficient priming agent to improve early growth characteristics and to provide a low-cost method that can be easily applied in basil cultivation.

MATERIALS AND METHODS

Seeds of basil (*Ocimum basilicum* L. cv. 'Genovese') were obtained from commercial sources ("Semesemena d.o.o." Belgrade). The application of priming methods on seeds was done according to KANJEVAC *et al*. (2021) with minor modifications. After surface-sterilization with sodium hypochlorite seeds were washed several times with distilled water. Sterilized seeds were primed with plant hormones (gibberellic acid – GA_3 and indole-3-acetic acid – IAA, in a concentration of 1 mM), salt solutions (2.5% KNO_3 and 1% $MgSO_4$), hydrogen peroxide (1% H_2O_2), ascorbic acid (0.01% AA) and distilled water (H_2O). Seeds were primed in the appropriate solution for 24 h and then exposed to the desiccation process on Petri dishes for 48 h, at room temperature. Unprimed seeds were used as a control. Basil seeds from all treatments (primed and unprimed seeds) were placed in Petri dishes with filter paper (Whatman No 1), soaked with 7 ml of distilled water and after that, they were incubated in a growth chamber (60% humidity, temperature 23 ± 2°C, photoperiod 16/8 h). Seeds were regarded as germinated after visible radicle protrusion. The growth characteristics of seedlings were evaluated two weeks after seed germination.

Germination characteristics (germination percentage – GP, mean time to germinate – MTG, rate of germination – RG, and uniformity – U) were calculated according to BEWLEY and BLACK (1994) and JAKOVLJEVIĆ *et al*. (2020), based on the following equations:

$$GP = \frac{Total\ seeds\ germination}{Total\ number\ of\ planted\ seeds} \times 100;$$

$$\mathrm{MTG} = \frac{\sum n_i \times t_i}{\sum n_i};$$

n_i = number of newly germinated seeds.
t_i = number of days from the start of the experiment to the observation.

$$RG = MGR \times 100;$$

MGR = mean germination rate, the reciprocal of the mean germination time.

$$U = \frac{GP}{MTG}.$$

The root and shoot length were measured using a digital caliper. Seedling length vigor index (SLVI) and seedling weight vigor index (SWVI) assessment was performed according to BOJOVIĆ *et al*. (2018), based on the following equations:

$$\text{Seedling Length Vigor Index (SLVI)} = (\text{Mean shoot length} + \text{Mean root length}) \text{x GP}$$

$$\text{Seedling Weight Vigor Index (SWVI)} = \text{Mean seedling weight x GP}$$

where GP = germination percentage.

The influence of the applied treatments on the relative water content in the leaf (RWC) (%) was measured and calculated according to DASTBORHAN *et al*. (2015) based on the following equation:

$$\text{RWC} = \frac{\text{fresh weitgh} - \text{dry weitgh}}{\text{turgid weitgh} - \text{dry weitgh}} \times 100$$

Determination of the concentration of chlorophyll a (*Chl a*), chlorophyll b (*Chl b*), total chlorophyll (*Chl a + b*) and carotenoids (*C x + c*) was performed by the spectrophotometric method according to BOJOVIĆ and STOJANOVIĆ (2005). The calculation was done according to WELLBURN (1994) and expressed in relation to fresh weight (mg g^{-1} FW):

$$Chl\ a + b = 8.02 \times A_{663} + 20.20 \times A_{646}$$
$$Chl\ a = 12.21 \times A_{663} - 2.81 \times A_{646}$$
$$Chl\ b = 20.13 \times A_{646} - 5.03 \times A_{663}$$
$$C\ x + c = (1000 \times A_{470} - 3.27 \times Chl\ a - 104 \times Chl\ b) \div 198$$

Determination of the concentration of total soluble proteins was performed according to LOWRY *et al*. (1951) with bovine serum albumin (BSA) as a standard and expressed in relation to the fresh sample weight (mg g^{-1} FW).

Each of the individual experiments was done in three replicates. The results were obtained by using the SPSS software package for statistical data processing (SPSS for Windows, version 21), and expressed as the mean of three replicates ± standard error (SE). The collected data were analyzed by ANOVA and LSD test with a significance threshold of $p \leq 0.05$.

RESULTS AND DISCUSSION

Seed germination characteristics

The obtained results for the percentage of germination, mean germination time, germination rate and uniformity of basil seeds are shown in Table 1. The most significant effect on GP and U was achieved by treatment with H_2O_2 (GP = 76.33%, U = 30.67), and the obtained values were higher compared to the values obtained for unprimed seeds. Most favorable treatment for MTG and RG was treatment with GA_3 (MTG = 2.21, RG = 45.73). Overall, except for KNO_3 treatment, which showed negative effects on basil seed germination, all treatments generally improved the germination characteristics of basil.

According to the obtained results, there was an improvement in germination performance after the application of priming agents compared to non-primed seeds, where a dominant effect had the priming with H_2O_2 and GA_3. This can be explained by the fact that the seeds primed with H_2O_2 quickly completed the first phases of germination which enables fast and uniform germination, leading to an increase in the total percentage of germination (GAMMOUDI *et al.*, 2021). Similar observations were noted by LI *et al.* (2017) and HEMALATHA *et al.*, (2017). According to ELLOUZI *et al.* (2021), priming with H_2O_2 during the imbibition phase stimulates mechanisms that are strongly associated with cellular and energy metabolism, gene expression, and early mobilization of starch reserves. The importance of gibberellic acid for seed germination processes is well known (KHAN *et al.*, 2002; IQBAL and ASHRAF, 2013). During seed germination, gibberellins induce amylase synthesis in the aleurone layer, which leads to starch hydrolysis and thus provides substrates and energy to stimulate embryonic development (WANG *et al.*, 2018).

Table 1. Effect of different priming treatments on germination characteristics of basil (*O. basilicum* cv. 'Genovese').

Treatment	GP	MTG	RG	U
GA_3	65.67 ± 8.69	2.21 ± 0.17	45.73 ± 3.63	30.42 ± 6.02
IAA	71.00 ± 7.58	2.38 ± 0.28	43.38 ± 5.79	30.00 ± 1.38*
$MgSO_4$	65.33 ± 7.88	3.05 ± 0.17	32.98 ± 1.74	21.79 ± 3.64
KNO_3	56.67 ± 5.24	3.27 ± 0.13	30.72 ± 1.31	17.36 ± 1.45
AA	73.33 ± 10.84	2.58 ± 0.20	39.28 ± 2.99	28.95 ± 5.53
H_2O_2	76.33 ± 6.67*	2.54 ± 0.22	40.02 ± 3.71	30.67 ± 4.46
H_2O	68.67 ± 5.67*	2.89 ± 0.41	36.23 ± 5.93	25.55 ± 6.46
Control	59.00 ±1.00	2.78 ± 0.30	36.94 ± 4.47	21.70 ± 2.23

An asterisk (*) indicates a statistically significant difference ($p \leq 0.05$) of the applied treatments compared to the control based on the LSD test.

Growth characteristics, vigor indices and relative water content

The monitored characteristics of basil growth under the influence of applied priming treatments are shown in Table 2. Priming treatment with GA_3 achieved the most significant effect on shoot length (2.36 cm) as well as root length (2.94 cm). All treatments improved basil seedling fresh weight with the highest value recorded in treatment with H_2O_2 and GA_3 (27.7 and 27.1 mg, respectively).

The results obtained for SLVI and SWVI (Table 2) showed that halopriming with $MgSO_4$ and KNO_3 only in the case of SLVI had a negative impact on the vigor index, while all other treatments manifested positive effects. In the case of SLVI, the best results were achieved with GA_3 and AA treatment (284.99 and 271.33, respectively), while for SWVI the highest value was recorded in treatment with H_2O_2 (SWVI = 2.13).

Furthermore, an increase in the RWC value of basil under the influence of priming agents was observed. The highest values were recorded during treatment with H_2O_2 (RWC = 94.68%) and AA (RWC = 91.68%). In addition, it is important to note that treatments with GA_3 and $MgSO_4$ induced relatively low RWC values compared to those in the control.

The emergence of seedlings, one of the most important and sensitive phases in the life cycle of a plant, directly affects the future growth of plants (SUN *et al*., 2021). Treatments with GA_3 and H_2O_2 showed the most significant effects on the monitored growth characteristics of basil seedlings. Gibberellins are known as growth stimulators that mediate many physiological and biochemical processes in plants, starting with seed germination (MIRHEIDARI *et al*., 2021). The beneficial effect of GA_3 on crop germination, growth and yield was previously confirmed (IQBAL and ASHRAF, 2013). Accordingly, the positive influence of GA_3 in this paper can be attributed to its vital role in the first processes of synthesis and the activity of hydrolytic enzymes that play a key role in promoting the germination and growth of plants. Similarly, BAIG *et al*. (2021) recently pointed out the importance of the application and positive effect of ascorbic acid in the form of priming on the growth and physiological processes of plants. Hydrogen peroxide in low concentrations acts as a signaling molecule with a significant role in plant growth and development by controlling morpho-physiological and biochemical processes (NAZIR *et al*., 2020). The improved growth performance of basil after the application of priming with H_2O_2 can be attributed to its role in the regulation of endogenous growth regulators such as GA_3, IAA and ABA (ORABI *et al*., 2015).

Table 2. Effect of different priming treatments
on growth characteristics of basil (*O. basilicum* cv. ‘Genovese’).

Treatment	Shoot length (cm)	Root length (cm)	Fresh weight (mg)	SLVI	SWVI	RWC (%)
GA_3	2.36 ± 0.01*	2.94 ± 0.19*	27.1 ± 0.00*	284.99 ± 37.70*	1.77 ± 0.23*	53.04 ± 5.45*
IAA	1.74 ± 0.05*	2.04 ± 0.14	16.8 ± 0.00*	237.14 ± 25.29	1.21 ± 0.13*	88.86 ± 2.05
$MgSO_4$	1.36 ± 0.05	1.49 ± 0.14*	12.6 ± 0.00	175.75 ± 21.20	0.85 ± 0.10	75.53 ± 3.48*
KNO_3	1.38 ± 0.20	1.58 ± 0.14	25.3 ± 0.00*	167.73 ± 15.51	1.42 ± 0.13*	89.90 ± 4.88
AA	1.51 ± 0.07*	2.30 ± 0.19	26.4 ± 0.00*	271.33 ± 40.10*	1.91 ± 0.28*	91.68 ± 2.31
H_2O_2	1.68 ± 0.07*	1.81 ± 0.17	27.7 ± 0.00*	230.53 ± 20.13	2.13 ± 0.19*	94.68 ± 1.08
H_2O	1.56 ± 0.06*	1.85 ± 0.12	14.3 ± 0.00	226.60 ± 18.70*	0.96 ± 0.08*	86.93 ± 4.92
Control	1.30 ± 0.05	2.01 ± 0.13	12.4 ± 0.00	191.75 ± 3.25	0.71 ± 0.01	89.88 ± 4.01

An asterisk (*) indicates a statistically significant difference ($p \leq 0.05$) of the applied treatments compared to the control based on the LSD test.

Concentration of photosynthetic pigments and soluble proteins

In leaves of basil seedlings concentration of total chlorophyll, chlorophyll *a* and chlorophyll *b* were enchased by applying AA and H_2O_2 treatments, while KNO_3 treatment caused a decrease in the concentration of investigated photosynthetic pigments (Figure1). The highest concentration of total carotenoids was measured after the treatment with H_2O_2.

The values of the concentrations of total soluble proteins of the examined plants varied greatly depending on the applied treatment (Figure 2). Measured protein concentrations ranged from 11.15 to 22.41 mg/g FW in basil, with statistically significant differences compared to the values obtained in the control. In basil seedlings, the highest value was measured in the control samples and was followed by the values in the treatment with H_2O_2 and H_2O. The lowest protein concentration was measured after priming treatment with KNO_3.

By analyzing the obtained results for the concentration of photosynthetic pigments and soluble proteins it can be noticed that priming treatments with AA and H_2O_2 had a significant impact. A priming treatment with AA leads to a series of physicochemical changes that modify the protoplasmic characteristics of plant cells, improving the physiological activity of embryos and future seedlings (SINGH *et al.*, 2018). Since it is a molecule with multiple roles, H_2O_2 in lower concentrations is involved in the regulation of growth, development, and physiological processes of plants (NAZIR *et al.*, 2019). The application of priming with H_2O_2 results in the maintenance of protein structure and regulation of enzymes responsible for protein synthesis (GUZEL *et al.*, 2013). In addition, H_2O_2 has been shown to stimulate photosynthetic processes by improving gas exchange, chlorophyll content and protection, as well as ROS removal (ASHFAQUE *et al*. 2014). These facts were also confirmed by FARIDUDDIN *et al*. (2014) and SUN *et al* (2016).

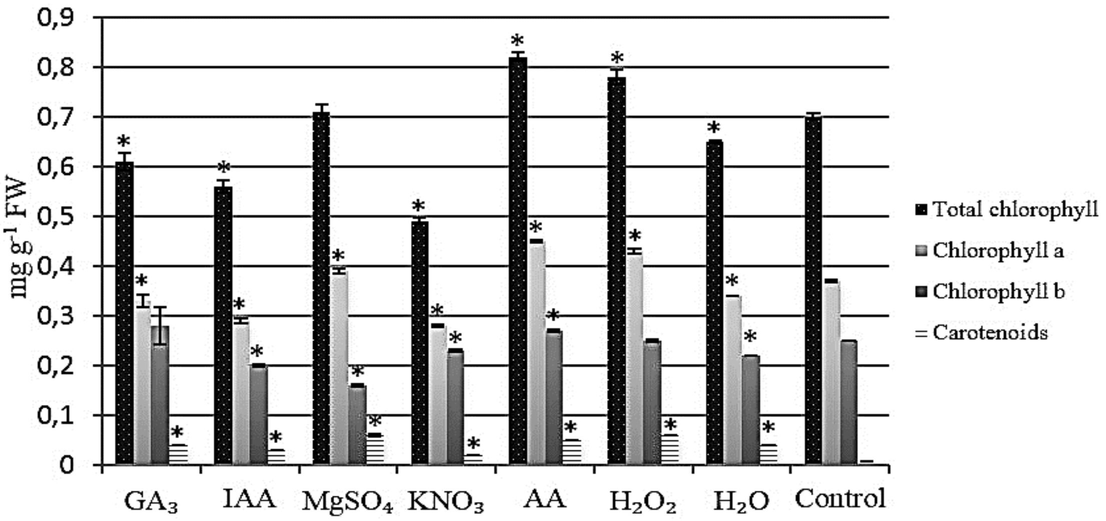

Figure 1. Influence of priming on concentration of photosynthetic pigments of basil (*O. basilicum* cv. 'Genovese'). An asterisk (*) indicates a statistically significant difference ($p \leq 0.05$) of the applied treatments compared to the control based on the LSD test.

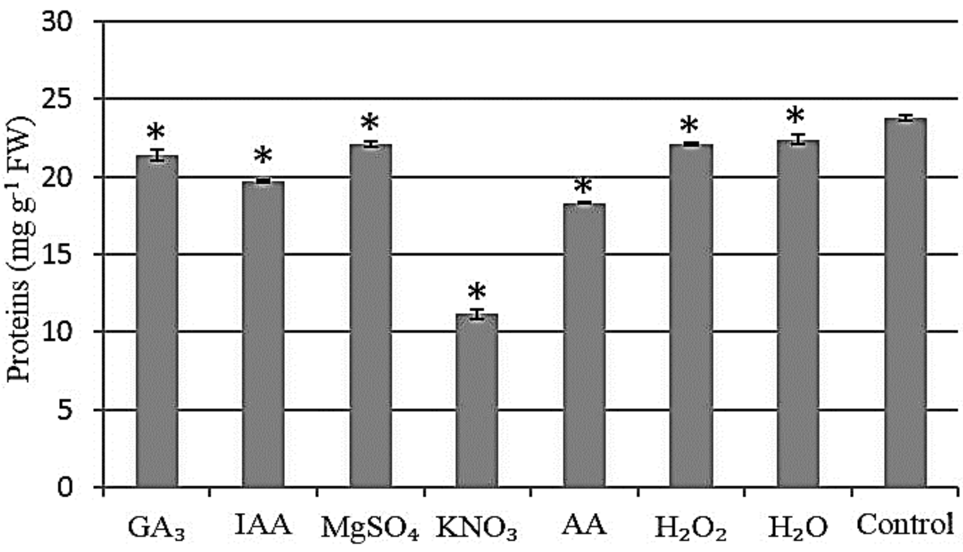

Figure 2. Influence of priming on concentration of soluble proteins of basil (*O. basilicum* cv. 'Genovese'). An asterisk (*) indicates a statistically significant difference ($p \leq 0.05$) of the applied treatments compared to the control based on the LSD test.

CONCLUSION

In the last few decades, great attention has been paid to the cultivation of basil, as a very valuable medicinal, aromatic and spice plant across the region. In that context, this study is designed to examine the impact of different priming methods that would contribute to improving the quality of basil seeds, which directly affects their efficient productivity. The obtained results suggest that the application of appropriate priming agents improves both, seed and seedling performance. The most favorable effects for all tested parameters were achieved by treatment with H_2O_2. Priming with this agent can contribute to better performance of *O. basilicum* cv. 'Genovese' and could be applied as a standard method in basil cultivation.

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