

CASE REPORT

A Case Study on the use of hydrogen peroxide as an agent for radish seeds Germination Promotion

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Abstract

The Plant growth regulation substances (PGRS) are of great demand in modern agriculture. Among numerous valuable properties, many PGRS possess an ability to promote seed germination through the increase in germination rate (GR). Hydrogen peroxide (HP) is believed to be a modest, cheap, and effective PGRS if used properly. The aim of this study was to investigate HP's ability to increase GR if it is used for seed pre-sowing treatment. Three options (water treatment, treatment with 3% HP for 5 minutes and for 15 minutes) were included in the greenhouse study with radish seeds (var. Saksa). As a result, it was found that HP treatments were statistically significantly inferior to water treatment in terms of GR. The longer the duration of the seeds exposition to HP was the higher GR suppression was recorded. Therefore, preliminary results testify that HP is not suitable as a pre-sowing treatment for germination promotion. Further studies are required to support or decline the preliminary outcome.

Keywords: Germination rate, Growth regulation substance, Pre-sowing treatment, Statistical processing, Suppression of germination.

Introduction

Plant growth regulation substances (PGRS) are chemical compounds of synthetic or natural origin aimed to enhance plant growth and development, promote germination, strengthen plant's immunity to diseases, improve their resistance and tolerance to biotic and abiotic adverse stress factors. In recent decades, PGRS occupied their place in cultivation technology of most crops at the same parity with mineral fertilizers and plant protection substances. Up to date, it is difficult to imagine intensive agro technology with no application of PGRS. The latter has proved their efficiency and economic reasonability for numerous field, vegetable, fruit crops. Major effects of PGRS include increased leafage and green biomass, improved growth parameters, higher adaptability, and, in most cases, lesser extent of infestation with insects and diseases under slight but statistically significant yield increase (Lavrynenko et al., 2011; Lykhovyd et al., 2020). Most PGRS provide the highest efficacy under the optimal water supply and fertilization regimes.

Apart from direct use for strengthening the plants in the period of their growth and development, some PGRS are applied as a pre-sowing treatment to improve initial growth and enhance germination quality. Besides, some PGRS possess disinfecting properties that benefit to obtaining vigorous crops. Rational pre-sowing treatment is crucial for the improvement of sowing qualities of seeds (Honchar & Shcherbakova, 2016). Most modern PGRS, available on the global market, are represented by mono- or complex compounds containing beneficial strains of bacteria, phyto enzymes, amino acids, micronutrients, vitamins, etc. (Bahan et al., 2020a). Properly used, they allow diminish the use of mineral fertilizers and agents for plant protection during the growing period (Havryliuk, 2017).

At the same time, one must admit that apart from new PGRS, common wisdom of plant producers has already adopted some regular chemical compounds to satisfy the needs for pre-sowing treatment. For example, KMnO_4 , boric acid, CuSO_4 , etc. were

used by our forebears as pre-sowing disinfectants for the seeds of cucumbers, tomatoes, and other vegetable crops long time before the development of modern fungicides. There is also an evidence for the use of lemon acid, ZnSO_4 , ashes, aloe juice as alternative pre-sowing treatments (Shulhina, 2015). These substances appear to be affordable, but their efficacy is not scientifically supported enough. Besides, it is a well-known scientific fact that different crops (even within a relatively close group by origin) react differently to the same PGRS, so it is necessary to provide scientific evidence of a concrete PGRS efficacy applied on a concrete crop (Bahan et al., 2020b).

Current scientific knowledge suggests oxygen treatment to be one of the most effective pre-sowing treatments (Zinevych, 1985). Its technology embraces holding of seeds at the temperature of $+20...25^\circ\text{C}$ in the water, which is under continuous inflow of oxygen, for 18-24 hours. Such a technology usually results in improved germination.

However, the technology of oxygen treatment of seeds is not a wide-spread one due to its low availability to most farmers and high costs. Our hypothesis is that hydrogen peroxide (H_2O_2) could to some extent replace classic oxygen treatment, as far as it is known that this chemical compound will release water and oxygen after a contact with seed surface (Ramsden, 1985). Therefore, the goal of this preliminary study was to find out whether HP is a suitable alternative to more common pre-sowing PGRS in terms of germination promotion.

Materials and Methods

The study of HP effect on germination rate (GR) was carried out in greenhouse conditions. Radish (*Raphanus sativus* var. radicula Pers.) seeds (Saksa variety, Ukrainian selection) were used as a biological material. The seeds were treated before sowing in the standard universal soil substrate "EcoFlora" with water (control), 3% HP for 5 minutes (HP-1), and 3% HP for 15 minutes (HP-2). GR was accounted manually 14 days after sowing, and calculated as a ratio (1):

$$GR = \frac{NGS}{NSS} \times 100\%$$

Where NGS is a number of germinated seeds, pcs; NSS is a number of sown seeds, pcs.

The study was conducted in four replications without randomization. Standard deviation (SD) and analysis of variance (ANOVA) at $\alpha=0.05$ were performed by the common statistical methodology (Bronstein & Semendiayev, 1986; Ushkarenko et al., 2014). Significance of the difference between the studied options was assessed by the value of the least significant difference ($\text{LSD}_{0.05}$). The difference is statistically significant only if it exceeds the value of $\text{LSD}_{0.05}$. Simple linear regression analysis was performed by the standard calculation procedure with further graphical presentation in MS Excel 365 spreadsheets.

Results

The results of the study are presented in the Tab 1 and in the Fig 1. It was found out that there is no significant difference between HP treatments; while the control was significantly superior over both HP treatments in terms of GR (the difference is greater than $\text{LSD}_{0.05}$ value). Regression analysis testify about statistically significant trend (R^2 is 0.871) to decrease of GR in radish seeds with longer exposition of them to HP. Thus, 3% HP possesses inhibitory activity on radish seeds, resulting in germination suppression by 6.25 for HP-1 and 7.50% for HP-2 treatments, respectively.

Table 1 Germination rate (GR) of radish seeds depending on the exposition to 3% hydrogen peroxide (HP)

Treatment option	Exposition to 3% hydrogen peroxide, min	GR*, %	SD, %	± to control, %	$\text{LSD}_{0.05}$, %
Control	0	77.00 ^a	37.34	–	
HP-1	5	70.75 ^b	14.17	-6.25	2.45
HP-2	15	69.50 ^b	14.8	-7.5	

Note: – The same letters mean no statistically significant difference between the treatments.

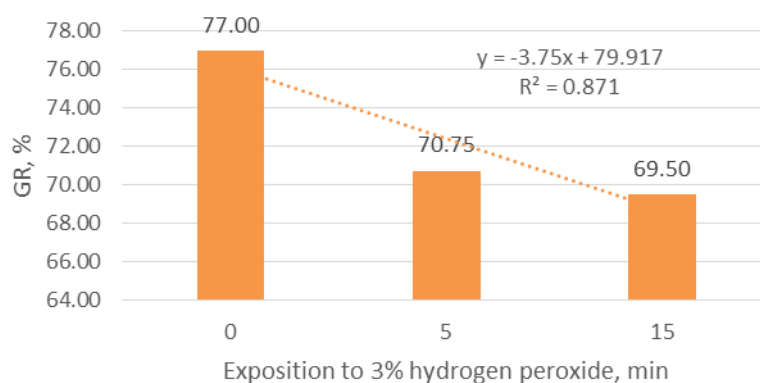


Figure 1 Germination rate (GR) of radish seeds depending on the exposition to 3% hydrogen peroxide (HP).

Discussion

It is an established scientific fact that HP plays an important role as a signal molecule in the cycles of seed maturation and germination (Bewley et al., 2013). HP accumulation has been found during imbibition and at point of seed germination (Kubala et al., 2015). Besides, the agent is considered to be a signal hub for the regulation of seed dormancy and germination processes, and it can also cooperate with phyto enzymes (Corbineau et al., 2014). HP is also an important chemical compound regulating the processes of seed ageing, and its dormancy period. Therefore, it is highly important to improve scientific knowledge on physiological and agrotechnological effects of this chemical agent on seeds of various crops, especially, in terms of germination rate regulation (Sameer et al., 2020).

However, HP treatment effects on seed germination are not thoroughly studied in agricultural science; therefore, this subject remains relevant and provides a great field of scientific research. Until now, there are several scientific works dedicated to HP and seed germination. The work by Raad (2013) suggests HP to be a common fungicide and algicide, while its effect on seed germination is to be determined. Raad (2013) claims that low concentration (one teaspoon of 3% hp per one cup of water solution) HP treatment of snap pea seeds resulted in faster growth of roots, while the increase in concentration of the agent resulted in worsening of initial seeds germination and plant growth, as well as in our study.

In the work by (Ishibashi et al. 2008) we can find the statement that HP is usually considered as a toxic compound in seed physiology science (McDonald, 1999), while some reports could be found in support of the thesis that this agent, if applied exogenously for seed treatment, improves to some extent germination of some plants in the dose-dependent manner, as Raad (2013) claimed in the above-mentioned study (Chien & Lin, 1994; Fontaine et al., 1994). Other scientists also discovered the ability of HP to ameliorate initial plant growth and enhance GR in such crops as wheat, rice, barley (Neredo et al., 1998) (Ishibashi et al. 2008) revealed that HP indeed enhanced GR and this effect differed by the studied wheat varieties.

As far as current scientific evidence testifies, there is still no common point in scientific community regarding the effects of HP on seed GR. The results of currently available studies are limited to just a few crops, and little variation in the treatment options of the agent application is another limitation factor. The statements of different scientific groups are contradictory, making it impossible to come to a common point. So, the subject is still open to research and scientific debate, requiring more scrupulous studies to draw any scientifically substantiated conclusions.

Conclusions

The results of this study revealed inhibitory activity of 3% HP on radish seeds germination. Preliminary outcome suggests that HP should not be used in pre-sowing period. More scientific evidence is required to support or decline the results of current research. Further studies with seeds of other crops are to be continued to enlarge scientific evidence.

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