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PhD THESIS

# **Research on the influence of some bioactive substances on seed germination, wintering and production in rapeseed**

(SUMMARY)

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## 1. INTRODUCTION

Globally, canola is the third most important oilseed crop in terms of area after soybean and cotton (FAOSTAT). It can be used as animal feed, human food or as a source of biofuel. In addition, the inclusion of canola in the crop structure (in set-aside) is an important element of good agricultural practice and has beneficial effects on soil fertility. In Europe, autumn canola cultivars are more widespread than spring ones. The highest yields of 3.3 - 4.3 t ha<sup>-1</sup> are obtained in Central and Western Europe. (FRIEDT et al., 2018).

Thanks to industry's interest in the crop, canola has become a strategically important crop. The incentive purchase price, the introduction of high-yielding varieties and cultivars and modern cultivation technologies have significantly increased the profitability of this crop. It can be seen that the areas under rapeseed have fluctuated greatly from one period to another. The most common cause of risk for crops under field conditions is the combination of several abiotic stress factors occurring simultaneously. Future research initiatives aimed at developing crop plants with improved tolerance to environmental conditions should focus on tolerance to a variety of stress conditions (especially those reflecting field environmental conditions) (MITTLER, 2006; SUZUKI et al., 2014).

Studies have confirmed the positive effects of soaking seeds in different bioactive solutions on germination processes as well as on early plant development. Most of these studies have been carried out in a controlled environment, such as growth chambers or greenhouses (FODORPATAKI et al., 2019; ZHU et al., 2021). When seeds are immersed, a physiological situation is created that stimulates germination and enhances uniform seedling sprouting (by altering hormones, metabolic activities, latency and membrane permeability) (JISHA et al. 2013; ABOUTALEBIAN and NAZARI 2017; AYMEN 2018; BOSE et al. 2018). Exogenous application of various bioactive substances to the leaf area and/or seed immersion stimulates biotic and abiotic resistance of plants by enhancing antioxidant defense system activity and osmoprotection as well as by controlling stress-related proteins. In addition, bioactive substances improve photosynthesis, nutrient uptake efficiency, hormonal changes in plants and also crop productivity and quality (WAQAS et al., 2019; ZULFIQAR, 2021).

## 2. AIM AND OBJECTIVES OF THE DOCTORAL THESIS

The main aim of the research is to improve the cultivation technology of canola, i.e. to increase the tolerance of the plants to abiotic stress, through treatments with bioactive substances applied to the seed and to the plants in different phenophases, in order to obtain favorable results in seed germination, better cold tolerance and, finally, an increase in the production capacity of the canola crop in the soil and climate conditions of the eastern Transylvanian Plain.

The research objectives are summarised below:

1. identification of bioactive substances and their physiological/biochemical effects used as pre-curing agents;
2. the establishment of their minimum effective concentrations;
3. improving the way treatments work/apply;
4. field verification of the effect of these treatments.

The figures below summarise the methods used in laboratory experiments (Fig 1) and field experiments (Fig 2).



Fig. 1. Outline of the organisation of laboratory experiments (original)

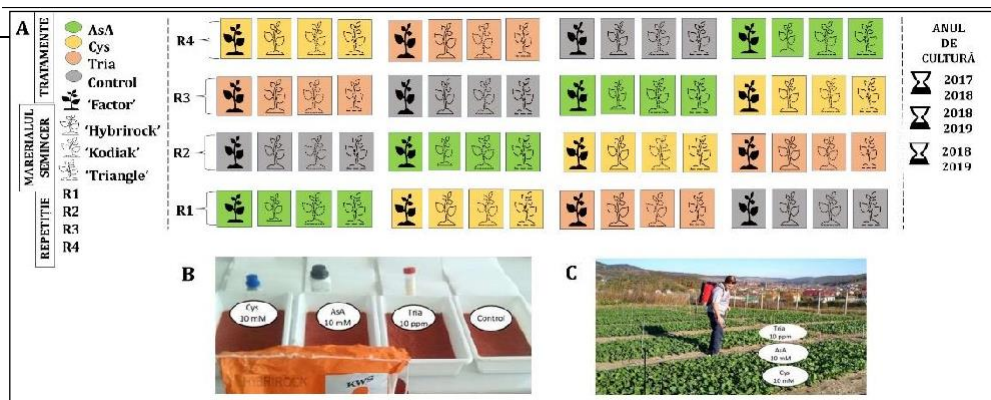


Fig. 2. Applied treatments and the used experimental design  
 (A) Experimental design: canola cultivars in treatments over three years of study;  
 (B, C) Aspects of seed and vegetation treatments (original)

### 3. THESIS STRUCTURE

The first part, the literature review, comprises chapters 1-2 and deals with general theoretical issues related to factors hampering the cultivation of autumn canola and research results on the use of bioactive substances.

The paper presents its personal contributions in chapters 3-6. The experiment is divided into two basic parts, one laboratory and one field. Chapter 3 describes the materials and methods used in the experiment, including the laboratory test methods and the organisation and implementation of the field experiments, as well as the statistical analyses. Chapter 4 contains the results of the laboratory and field experiments on bioactive substance treatments. Chapter 5 contains conclusions and recommendations, while Chapter 6 summarises the new scientific results of the work.

### 4. PERSONAL CONTRIBUTION

#### 4.1. Materials and working methods

In this study seeds from four cultivars of autumn canola (*Brassica napus* L.), (Factor- H1, Hybrirock- H2, Kodiak- H3, Triangle- H4), supplied by KWS Romania, were used.

The laboratory phase of the research was carried out using the educational infrastructure of the Faculty of Technical and Humanities Sciences Targu Mures (including the Study Centre Sfântu Gheorghe) of Sapientia University.

The effect of selected bioactive substances (ascorbic acid - AsA in concentrations of 1, 10, and 100 mM, L-cysteine - Cys in concentrations of 1, 10, and 100 mM and triacontanol - Tria in concentrations of 1, 10, and 100 ppm) on germination capacity, germination dynamics and biometric parameters of seedlings was examined.

Seeds were pretreated for 24 hours and then germinated under laboratory conditions. The aim of the research was to determine the lowest effective concentration.

After analysing the results from the first experiments, the concentration of triacontanol (1  $\mu\text{M}$ ) was changed, as the field results did not confirm the 10 ppm concentration as effective. The concentrations of ascorbic acid and cysteine were not changed. In these laboratory experiments we worked with two cultivars, due to rapid changes in the seed market, two cultivars were no longer available from the seed supplier. Further, in the second part of the laboratory experiments, we also performed various more detailed germination and physiological determinations and analyses on the selected biological material.

While monitoring the germination process, I focused on parameters such as germination percentage, mean germination time, germination index, germination uniformity and growth of germinated roots and plant stems.

Regarding vigour tests, we investigated the effect of treatments using the vigour tests recommended by the International Seed Testing Association (ISTA) (Seedling Growth After Germination Test, Cold Test, Accelerated Ageing Test and Electrical Conductivity Test).

In biochemical and physiological studies, we investigated the efficacy of treatment substances to counteract artificially applied cold treatments by monitoring proline levels, malondialdehyde (MDA) content, photosynthetic pigments and chlorophyll fluorescence parameters.

To test the laboratory results under field conditions, we conducted a three-year field study with treatments applied at the initially set concentrations (10 mM AsA and Cys and 10 ppm Tria) with four cultivars in two growing years and two cultivars in the last growing year. The bioactive substances were applied at three different times: seed treatment, foliar application in early autumn and early spring. Growth and yield parameters were recorded at different stages of plant development: autumn (early vegetative stage), spring and at harvest. The field experiments underlying the present PhD thesis were carried out in the period 2017- 2020 in the specific conditions of the agrofund located in the Experimental and Didactic Field of Sapientia Hungarian University of Transylvania, Faculty of Technical and Human Sciences, Târgu Mureş.

## **4.2. Results and discussion**

Statistical analyses were performed in PAST version 3.06 and SPSS version 27.

### **4.2.1. Results of laboratory experiments**

#### **Results of germination experiments**

In the literature, several studies confirm the stimulatory effect of ascorbic acid (AsA) (AFZAL et al., 2006; DRAGANIC and LEKIĆ 2012; ORTIZ\_ESPIN et al., 2017), cysteine (Cys) (NASIBI et al., 2016) and triacontanol (Tria) (PERVEEN et al., 2012; CHANDRA and ROYCHOUDHURY 2020; BHANDARI et al., 2021) on germination, but the concentrations of these substances differ greatly depending on the plant culture and the stressors

involved.

In the treatments applied, in most cases, we decided to use concentrations of 10 mM for AsA and Cys and 10 ppm for Tria. Our decision was confirmed by experiments on germination dynamics, where concentrations of 10 mM/ppm ensured faster germination in each case. After the first year of field experiment, we found that in the case of Tria, the concentration used (10 ppm) was not the most suitable, so we continued the research with a lower concentration of 1  $\mu$ M for Tria in the laboratory phase.

### **Germination results**

Not only the final germination percentage achieved is an important issue, but also other germination parameters such as mean germination time, germination index, germination velocity coefficient, germination timing index and germination uncertainty index, which are often used to assess the agronomic relevance of treatments (AL-AL-MUDARIS, 1998).

In general, it can be said that ascorbic acid (AsA) seed soaking gave the best results in the cultivar canola 'Factor' according to the indicators mean germination time (MGT), germination index (GI), germination velocity coefficient (CVG), germination rate index (GRI) and germination timing index (Z). In second place is immersion in triacontanol solution (Tria), while the results of L-cysteine (Cys) treatments are poorer compared to the control. 'Hybrirock' seeds responded better to Tria treatments, followed by AsA and Cys immersion.

### **Seed vigour results**

Testing the growth of young seedlings allows you to observe them on different days to determine which treatments accelerate germination and which produce faster growth. In this respect, for both cultivars, Tria proved to be the most effective treatment.

During Cold tests, germination capacity was reduced in each case. This is due to the essence of the method, as we test germination under stress conditions. The essence of the method is precisely to assess the degree of germination reduction, germination under stress conditions. Therefore, the benefit of the treatments applied to the material subjected to the cold test is an advantage. In each case the treatments succeeded in counteracting the cold effect.

During the accelerated ageing test, germination capacity decreased in all cases, especially in the cultivar 'Hybrirock'. The high humidity and high temperature used during accelerated ageing represent strong stress conditions for the seed, simulating physiological effects that occur during prolonged storage. The positive impact of treatments on seed vitality was also demonstrated during accelerated ageing trials, with greater emphasis on the cultivar 'Hybrirock' treated with AsA and Cys. In his observations BASKIN (1970) shows that accelerated ageing can also be used as an indicator of field behaviour, which is also supported by the results of the present thesis.

In the electrical conductivity tests, only the AsA treatment showed higher vigour in the 'Factor' cultivar compared to the control. However, treatments applied to seed samples may cast doubt on the test result. Twenty-four hours before each

measurement, samples were immersed in different solutions or in clean water, in which process the ions diffused through the seed tegument. This could have caused a disturbed diffusion of ions into the tested solution at the next immersion.

### **Results of biochemical and physiological determinations**

Leaf free proline as a biochemical marker of canola cultivars tolerance to cold stress increased proline production is closely related to osmotic stress.

Strong proline increase is usually observed in case of water stress. From the literature, it is known that its levels increase in response to stress in plants to fulfil its protective function (ASHRAF and FOOLAD 2007). Numerous studies have established a link between proline accumulation and stress tolerance (NAYYAR 2003).

We aimed to examine the level of proline accumulation in plants treated with AsA, Cys and Tria under cold stress conditions and to find a link between drought sensitivity and proline accumulation. Observing the variation of proline content by cultivar, we found that both canola cultivars had a significant increase in leaf free proline content under cold/osmotic stress due to treatment with the three bioactive substances.

In untreated plants, proline levels were low, and the increase in proline levels was more strongly stimulated by the treatments at the 'Factor' cultivar than in the 'Hybrirock' cultivar.

The 'Factor' cultivar is assumed to be less sensitive to cold lasting a week. Based on the effect on leaf proline content, it can be concluded that in the less cold-tolerant cultivar (Hybrirock), the three active substances significantly increase the accumulation of free proline in the presence of low temperatures.

### Membrane lipid peroxidation in leaves of canola plants exposed to cold stress

Malondialdehyde is the main marker used to monitor membrane lipid peroxidation, and the concentration of this marker is directly proportional to the degree of oxidative damage to cell membrane lipids. The degree of lipid peroxidation had significantly higher values in cold-stressed plants compared to control plants. At the same time, treatments with AsA, Cys and Tria could reduce the MDA concentration to the same value as the control group. Cold stress applied for one week had less effect on membrane damage by lipid peroxidation in 'Factor' than in 'Hybrirock'. Irrespective of the sensitivity of the cultivar, bioactive treatments significantly reduced the amount of toxic malondialdehyde.

### Concentration of chlorophyll and carotenoid pigments in leaves affected by cold stress

MAJIDI et al. (2015) draw attention to the fact that in Brassica species the change in chlorophyll content can be considered an essential element in assessing drought tolerance. The decrease in chlorophyll content occurs, not so much as a result of degradation, but rather as a result of inhibition of its synthesis (GARCÍA-VALENZUELA et al., 2005). According to our study, chlorophyll fluorescence measurements also showed that in two autumn canola cultivars low temperature caused a reduction in

photosynthetic activity, a finding also confirmed by DOGRU and ÇAKIRLAR (2020).

Chlorophyll-a and -b content and light-use efficiency were higher in cold-tolerant compared to sensitive oilseed rape. In plants considered more cold-sensitive (cultivar Hybrirock), a decrease in chlorophyll content was observed as a result of exposure to low temperatures, while in the more cold-tolerant cultivar (cultivar Factor) there was a slight increase. During cold exposure, AsA and Cys treatments reduced chlorophyll-a and chlorophyll-b content the most.

In our study, treatment with ascorbic acid (AsA) and cysteine (Cys) associated with the chilling effect resulted in a further decrease in total chlorophyll content (chlorophyll a+b) in cultivar 'Hybrirock' while in cultivar 'Factor' these two bioactive substances largely counteracted the decrease caused by the chilling effect, leading to chlorophyll contents closer to the control.

In the case of cold exposure, the increase in chlorophyll a/b ratio compared to the control was observed only in the case of cysteine (Cys) treatment in cultivar 'Factor'. Of the active substances used, cysteine had the least impact on the chlorophyll a/b ratio in the leaves of cold-treated plants.

Carotenoids play an important role primarily as photoprotectors in situations where environmental conditions are unfavourable and hinder the use of absorbed photon energy, and in such situations their quantity may increase as a defensive response to a potential photooxidative hazard.

Increased carotenoid levels upon cold exposure may be related to the higher low-temperature tolerance capacity of the 'Hybrirock' canola cultivar, and treatment with cysteine (Cys) reduced the photooxidative effect induced by low temperature, according to the results.

The results of the measurements show that the content of carotenoid pigments in the leaves of cultivar 'Factor' is about five times higher than in cultivar 'Hybrirock', which gives greater protection against the effects of oxidative stress in the photosynthetic apparatus of cultivar 'Factor'.

#### Chlorophyll fluorescence parameters induced in intact leaves of plants exposed to cold stress

In both cultivars, the values measured for the parameters Basic Fluorescence (Fo), Equilibrium Fluorescence (Fs) and Non-photochemical Chlorophyll Fluorescence Quenching (NPQ) are significantly higher in cold-exposed plants compared to control plants. To compensate for this effect, the applied treatments were effective to different extents.

The application of cysteine (Cys) reduced baseline chlorophyll fluorescence values just below the values recorded in control plants. In cultivar 'Factor' the increases in Fo values are more pronounced than in 'Hybrirock', indicating a higher cold sensitivity in photon energy accumulation.



Cysteine (Cys) and triacontanol (Tria) manage to maintain equilibrium fluorescence levels in the range of those recorded in control plants, completely cancelling out the effects of low temperature.

Due to the fact that the bioactive substances used (AsA, Cys, Tria) were able at low temperature to improve the degree of photochemical utilization of the absorbed light energy, a smaller amount of unused energy was converted into heat energy, thus due to the treatments, the non-photochemical quenching of chlorophyll fluorescence had much lower values under cold conditions.

In both cultivars the values measured for the parameters Fm (maximum fluorescence), Fm' (maximum modulated fluorescence), Fv/Fm (potentiation quantum yield),  $\Phi$ PSII (effective quantum yield) and Rfd (photosynthetic apparatus vitality index) are significantly lower in plants exposed to cold effects compared to control plants. To compensate for these effects, the applied treatments were effective to different extents. Following cold exposure electron transport in the acceptor region of PSII was slowed down and none of the bioactive substances used was able to restore the maximum fluorescence values (Fm) to the level characteristic of the control variant.

In cold-exposed canola plants, this stress is partially alleviated by treatments with ascorbic acid (AsA), cysteine (Cys) or triacontanol (Tria) in the case of cultivar 'Factor', and in 'Hybrirock' by treatments with AsA and Cys.

Cysteine (Cys) completely cancels the negative effect of low temperature on the Fv/Fm ratio (potential quantum yield). In the case of cultivar 'Hybrirock', the decrease in Fv/Fm is cancelled by all three treatments with natural bioactive substances. The decrease is more evident in 'Factor' (from 0.85 to 0.75). This suggests a more pronounced cold sensitivity of cultivar 'Factor' compared to 'Hybrirock'.

The total cancellation of the low temperature effect was only possible with the help of cysteine in the  $\Phi$ PSII parameter (effective quantum yield).

With regard to the photosynthetic vitality index (Rfd) of canola leaves, it can be seen that the cold treatment caused a substantial decrease, about half of the control values. Cysteine and triacontanol can prevent a decrease in the energy efficiency of photosynthesis when plants grow under low temperature conditions.

We found that 'Hybrirock' was less sensitive to the effects of cold, showed lower values on several parameters compared to 'Factor', but the treatments were able to reduce the negative effects to the same or similar level. Regarding the order of effectiveness of the treatment substances established for 'Factor', it is Cys, AsA, Tria, and for 'Hybrirock' it is Cys, Tria, AsA.

#### **4.2.2. Results of field experiments**

Studies in controlled environments have shown that pre-treatment of seeds and foliar treatments with various bioactive substances can stimulate germination, sprouting, uniform plant emergence, photosynthesis and nutrient uptake efficiency and can lead to increased productivity, plant numbers in the crop and quality (AFZAL et al.,

2006; DRAGANIC and LEKIĆ 2012; GENISEL et al., 2015; LUO et al., 2022). Only a few studies provide a comparative experimental perspective on the use of bioactive substances in field-grown autumn canola (AHMADI et al., 2016; RAZA et al., 2017; ZHU et al., 2021).

The aim of this study was to investigate the effects of seed soaking and foliar treatment with ascorbate (AsA), cysteine (Cys) and triacontanol (Tria) on growth and yield parameters of two/four cultivated canola cultivars (Factor, Hybrirock, Kodiak, and Triangle) under field conditions for three crop years under temperate-continental conditions in Transylvania (Târgu Mureş area).

Overall, the current work shows that seed treatment with 10mM ascorbic acid (AsA) and cysteine (Cys) can have beneficial effects on plant development, while their foliar application can increase silique number (NS), seed yield (SYP) and oil content (OC) of the canola cultivars studied.

Not all substances that have been tested in the laboratory have been successful in the field. In some cases, Tria (10 ppm) had no effect on plants or even a negative effect on important agricultural parameters such as estimated seed yield (ESY), seed/plant yield (SYP) and oil content (OC) could be observed.

The effects of AsA and Cys could be observed throughout the growing season for all four cultivars, while the effects of Tria were only visible on biometric parameters in autumn and spring, as well as on root parameters.

In general, 'Factor' and 'Hybrirock' responded better to the treatments than 'Kodiak' and 'Triangle'. In addition, the order of effectiveness of the treatments for 'Factor' is Cys, AsA and Tria, while for 'Hybrirock', the order is AsA, Cys and Tria, and in cultivars 'Kodiak', 'Triangle' the effect of AsA and Cys treatments is similar, followed by Tria effects.

Additional statistical tests on 'Factor' and 'Hybrirock' cultivars tested in all three growing years provided further information.

Factor analyses showed whether there was an interaction between treatment and crop year, i.e. treatment and cultivars. For these analyses, plant height at harvest (PHH) and seed oil content (OC) data were used because the other parameters did not meet the assumptions required for the two-way ANOVA test.

Statistical analysis confirmed that the treatment effect on plant height at harvest (PHH) was significantly influenced by weather conditions during the growing seasons.

When the effect of treatment and cultivar on PHH was examined, it was found that the treatment effect on PHH was not significantly influenced by the cultivar. In general, the bioactive substances had a positive effect on PHH compared to the control crops, but PHH was more dependent on weather conditions and cultivar than on the treatments applied.

According to factor analysis, the effect of treatment on seed oil content (OC) was significantly influenced by year and cultivar. In general, AsA and Cys had a significantly positive effect compared to control and Tria.

A PCA analysis was performed to determine the correlation between the hydrothermal index and mean monthly precipitation as environmental variables with the studied parameters. In case 'Factor', hydrothermal index and mean precipitation were positively correlated with PHH, seed yield per plant (SYP), estimated seed yield (ESY), silique mass per plant (WS), epigeic phytomass (PFW), shoot diameter (RND) and hypogeous phytomass (RWF). A negative correlation was observed between weather conditions and the parameters of seed oil content (OC), plant height in autumn (PHA), number of leaves per plant (NL), mass of 1000 grains (TSW) and silique number per plant (NS). For 'Hybrirrock', the parameters root neck diameter (RND), root fresh weight (RWF), root length (RL) and number of branches per plant in spring (NBS) were negatively correlated with mean rainfall and hydrothermal index, while the parameters seed yield per plant (SYP), estimated seed yield (ESY), silique mass per plant (WS), plant height at harvest (PHH) and phytomass epigee (PFW) were positively correlated.

#### **4.2.3. Economic analysis of the experimental variants**

An improvement in cultivation technology can be considered successful if the technological elements introduced have an economic justification.

On the basis of the estimated yields from the research field experiments, we made an estimate of the profitability, taking into account the purchase prices of the bioactive substances used in the treatment and the purchase price of canola on the world market.

There are remarkable production surpluses due to treatment effects. Following the AsA treatment, a yield surplus of  $0.80 \text{ t ha}^{-1}$  (1200 RON) can be observed in cultivars 'Factor', 'Hybrirrock' and 'Triangle'. Cys treatments were more effective in cultivars 'Hybrirrock', 'Kodiak' and 'Triangle', where a yield surplus of about  $2 \text{ t ha}^{-1}$  (3500 RON) can be observed. Tria had a smaller effect and was only more efficient on a smaller number of cultivars, so that the estimated yield increased by  $0.4 \text{ t ha}^{-1}$  (430 RON) on cultivars 'Kodiak' and 'Triangle' compared to the control group.

In the case of 'Factor' and 'Hybrirrock' cultivars, AsA treatment for 'Factor' and AsA and Cys treatments for 'Hybrirrock' yielded additional returns, taking the average of three crop years. Analysing the average two-year results for 'Kodiak' and 'Triangle', it can be seen that the Cys treatment was the most profitable for 'Kodiak', followed by the Tria treatment, and for 'Triangle', all three treatments made a positive difference in economic efficiency.

There are extremely high-income values for Cys treatment at Kodiak' or 'Hybrirrock', and there are extremely low, negative values for Tria treatment at 'Factor' and 'Hybrirrock'.

The economic analysis of the experimental variants therefore shows a specific link due to the interdependence between the factors analysed (bioactive substance, cultivar and crop year).

## 5. Conclusions and recommendations

### 5.1. General conclusions

It can be seen that seed treatment and foliar application of the bioactive substances used in these experiments can provide protection to plants against abiotic stress processes, but the mode of treatment administration, the stage at which they are applied (seed or foliar, on vegetation), and the type and degree of abiotic stress can greatly influence the effect induced by the substances used in the treatments.

There is a need for more and more detailed monitoring of the germination process and additional tests focusing on seed viability. These tests can provide a more comprehensive picture of the effects of treatments on seed germination and seedling development.

It is recommended to extend the range of biochemical and physiological investigations and determinations with various (in type and intensity) other stressors (salinity, drought, etc.) that more effectively mimic the environmental conditions that plants may encounter, in order to determine the stressors for which treatment substances can provide effective protection.

There is also a need for more research with an experimental design that includes more complex mathematical and statistical methods, which would allow more accurate confirmation of differences between treatment effects, while minimising the interference of climatic influence and cultivar used in interpreting the results.

There is also a need to extend the small-scale experiments to larger-scale experiments so that we can draw informed conclusions and recommendations on applicability and cost-effectiveness in crop production.

### 5.2. Recommendations

1. To improve the speed, uniformity and timing of germination, we recommend seed treatment with AsA (10 mM), Tria (1  $\mu$ M) and Cys (10 mM) for the cultivar 'Factor'. For the cultivar 'Hibrirock', treatments with Tria, then AsA and Cys were effective in this order.
2. In order to measure the positive effects of seed treatment on seed vigour, it is recommended to perform the Accelerated Ageing Test, Cold Test and Seedling Growth Tests.
3. The negative effects of low temperature can be reduced by treatments with Cys and AsA (10 mM), respectively Tria (1  $\mu$ M) in the case of cultivar 'Factor', while in the case of cultivar 'Hibrirock', treatments with Cys, Tria and AsA were effective in this order.
4. In field experiments, treatment in vegetation may be recommended. The order of efficacy of treatments for 'Factor' is Cys, AsA and Tria, while for 'Hibrirock', the order is AsA, Cys and Tria, and in cultivars 'Kodiak' and 'Triangle', the effect of AsA and Cys treatments is similar, followed by Tria. For boosting biometric (autumn and spring)

and root parameters, we recommend Tria treatment (10 ppm), and for longer duration and more parameters, the efficacy of AsA and Cys treatments is more visible.

5. When evaluating the effects of field treatments, the impact of weather conditions and variety/cultivar must be taken into account.
6. The application of seed and/or foliar treatments on young seedlings can be considered a cost-effective, low-input, environmentally friendly and sustainable method that can be easily implemented in large-scale cultivation technology.

## 6. Originality and innovative contributions of the thesis

1. The results of laboratory and, in particular, field experiments on the use of bioactive substances are particularly important in terms of practical feasibility.
2. To date, few studies have been conducted worldwide on the applicability of triacontanol and cysteine in plant breeding.
3. Such treatments and similar treatments with bioactive substances, applied on seeds or during the vegetative period, could be considered in the creation of new cultivars as a method of increasing the stress tolerance capacity of plants.
4. This line of crop technology improvement represents a promising prospect with applicability in production activity on farms in Romania and beyond.

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