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RESEARCH ON WHEAT SEED GERMINATION AS A FUNCTION OF TEMPERATURE, UNDER THE INFLUENCE OF TREATMENT WITH BIOSTIMULATORS AND AT DIFFERENT LEVELS OF FERTILIZATION

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Abstract

During 2020-2021, a bifactorial experiment was set up on the ARDS Caracal cernozome to study the influence of biostimulator seed treatment (Ympact and Kerafol applied in different doses) on different levels of fertilization. The seed used in the first testing year-2021 and that obtained in 2022 was tested in terms of germination at different temperatures (1°C, 5°C, 10°C, 15°C and 20°C) as well as in terms of the number of days required for germination depending on the factors presented above. The Glosa wheat seed germinated at all temperatures, distinctly and very significantly more than at 1°C. However, there were no differences between the other temperatures, each taken as a control. The number of days required for germination is very significantly reduced from one temperature to another. At 1°C, wheat requires 33 days to germinate, while at 20°C, only 4 days. For the NPK fertilized variant in autumn + ammonium nitrate and foliar in spring, germination of the seed obtained is very little different between temperatures of 5, 10, 15 and 20°C and between the biostimulator treatment variants.

Key words: *biostimulator, fertilization levels, germination, number of days required for germination, wheat.*

INTRODUCTION

Germination plays a substantial role in plant development, being the first stage of plant life. This process is easily influenced by environmental factors, which often have negative effects on germination, reducing plant growth, development and production (Moș et al., 2020). Seed quality is a particularly important aspect of wheat breeding technology, as uniform germination and high seedling vigour successfully contribute to the stability and productive performance of the crop (Vasilescu et al., 2019; Cardarelli et al., 2022). Seed germination is a contributing factor to high yield. Among abiotic factors, temperature is considered an important requirement for wheat germination, as it determines the amount of additional water and substrates needed for growth and development. Temperature is a factor that can alter germination because it can influence the rate of uptake of water and other substrates needed for growth and development (Wanjura and Buxtor, 1972; Essemine et al., 2002).
Temperature has a major impact on germination time (Guan et al., 2009). The effect of temperature on germination can be described as a scale with steps: minimum, optimum and maximum (Ostadian Bidgoli et al., 2018). The optimum temperature results in the most significant germination percentage (%) in the shortest time. Each germination stage has its own temperature; due to the complexity of the germination process, the temperature response may vary throughout the germination stages. Seed response to temperature is a function of cultivar, seed quality, time since harvest, and other factors (Dadach et al., 2015). Numerous studies have been conducted on temperature regulation of germination. Riley (1981) demonstrated that the energy status of cells and the behaviour of certain enzymes change with changing temperatures. ATP content and protein synthesis rate increase as temperature reaches the optimum and decrease as it goes in other directions (Riley, 1981). The antioxidant system greatly affected the germination of wheat seeds (Kunos et al., 2022).
Along with temperature, soil pH, water availability and soil moisture have the greatest influence on seed germination among abiotic factors (Rizzardi et al., 2009). Seed germination

reflects seed size, abundance and distribution in the seedbed (Cone and Spruit, 1983; Bentsink and Koornneef, 2008; McCormick et al., 2016).

Germination of wheat seeds occurs only when the seed dormancy period has been completed, which, as Matei (2003) shows, in varieties grown in Romania, averages between 40 and 60 days. However, there are also cultivars with very short dormancy (up to 10 days), varieties with medium dormancy (between 30 and 45 days) and varieties with long dormancy (more than 45 days).

Grain germination depends on the grain's ability to use its more efficient reserves (Buriro et al., 2011) and is a contributing factor to grain yield formation. Rapid and uniform germination is essential for better plant growth and higher yield (Lupașcu et al., 2020).

Seed germination begins with water uptake by the dormant dry seed and is achieved by root emergence as the embryo axis lengthens (Fu et al., 2021). An orderly sequence of morphogenetic and physiological mechanisms, including energy transfer, nutrient uptake, and physiological and biochemical changes, are part of this process (Bewley and Black, 1994). The water uptake of a seed occurs in three stages: phase I - a rapid initial uptake, phase II - a plateau, and phase III - an increase in water uptake, but accompanied by the initiation of germination (Manz et al., 2005). Phase I germination, called imbibition, results in the softening and swelling of the seed coat at or near an ideal temperature (Xue et al., 2021).

Ghițău and Donțu (2010) investigated under laboratory conditions the effect of applying biostimulants in different concentrations on germination (energy and germination capacity), root length and coleoptile in winter wheat, Boema variety. In all treatments there was no difference in germination energy and germination capacity. The highest germination percentage occurred with the biostimulants: BCO-4K - 96.12%, BCO-2K+zinc acetate - 94.5% germination; BCO-4DMA - 95.5% germination; BCO-2DMA+zinc acetate - 93.87% germination.

The study of the influence of factors: years (Y)/culture (C)/treatment with *Bacillus* spp., *Trichoderma harzianum* (T), conducted by Poštić and co-workers (2021) showed that the year factor, Y x C interaction as well as Y x T interaction were not significant ($p \geq 0.05$). Instead, cultivar and treatments as well as their interaction on all determined traits were significant ($p \leq 0.05$ to $p \leq 0.01$).

Another study showed data on the reaction of prospective lines and released varieties of common winter wheat (*Triticum aestivum* L.) to different temperature levels (4, 14, 22°C).

Based on important growth and development traits (germination, root length, stem length) the researchers calculated vigour index, variance (genotypic, phenotypic), coefficients of variation, broad heritability and genetic advantage of traits. It was concluded that there is an opportunity for the studied genotypes to be involved in breeding programmes to improve cold hardiness (Lupașcu et al., 2020).

A study to investigate the germination performance of wheat seed under different moisture conditions, temperatures and seed densities was conducted by Khaeim et al. in 2022. The objectives of this study were (1) to determine the optimum range of water quantity for germination based on the volume of water at one milliliter intervals and the amount of water as a percentage of the mass of 1000 grains; (2) to determine the effects of temperature and germination time of wheat seeds; (3) to investigate the effect of seed number and seedling density in a Petri dish on germination percentage and seedling viability under the same amount of water. The results showed that germination in different percentages can occur in a wide range of water amounts starting from 0.65 ml, which represents 75% of the mass of 1000 seeds, and that the optimum range for germination is 4.45-7.00 ml, which represents 525-825% of this.

For laboratory experiments, a density of maximum 15 wheat seeds per 9 cm Petri dish was recommended. In general, 20°C was the ideal temperature for seedling development. Germination has a wider range, from 20°C to 30°C. The recommendation is made especially for breeding projects, when the number of seeds is limited (Khaeim et al., 2022).

Wheat yield in Romania varied after 2010 from 2,659 to 4,888 kg/ha and was influenced by the genetics used by growers (variety or hybrid), soil and climatic conditions (soil, climatic factors, climatic accidents) and technological factors (mainly crop rotation, fertilization, tillage, seed quality and sowing conditions, weed, disease and pest control, harvest conditions) (Dumbravă et al., 2019).

MATERIALS AND METHODS

A two-factor experiment was set up in 2020 and 2021 using the 3 replicate sub-divided plots method. The factors studied were: factor A - fertilization level with 4 gradations: a1 -fertilized with NPK in autumn (N50P50K0); a2- fertilized with NPK in autumn + ammonium nitrate in spring (N140P50K0); a3-fertilized with NPK in autumn + foliar in spring (N73P50K0); a4- fertilized with NPK in autumn + ammonium nitrate and foliar in spring (N163P50K0) and factor B - seed treatment with biostimulator with 6 gradations: b1- untreated with fungicide and biostimulator; b2- treated with fungicide but untreated with biostimulator; b3- treated with fungicide and YMPACT 0. 7 l/t; b4- treated with fungicide and YMPACT 0.35 l/t; b5- treated with fungicide and KERAFOL 1 l/t; b6- treated with fungicide and KERAFOL 0.5 l/t.

The seed used to set up the experiment in the first year of testing (2020-2021) and the seed obtained in 2022 (crop year 2021-2022) was tested for germination and number of days required for germination according to the factors presented above at different temperatures (1ºC, 5ºC, 10ºC, 15ºC and 20ºC) - factor C. Basically it was a single experiment, but for the germination study, the seed came from 2 sources - the first sample from seed used at sowing and the second from seed obtained at harvest.

The variety used - Glosa, registered in 2005, was obtained at INCDA Fundulea from the complex hybrid combination Delabrad "S"/Dor "S"//Bucur, by individual selection. In Romania, of all the wheat varieties in production, the most widespread is the Glosa variety, which occupies about 35% of the total cultivated area (Radu, 2023), enjoying great success among farmers in all areas of the country, with a very high adaptability and production potential superior to previous varieties.

Germination was determined in a temperature-controlled growth chamber (Snijders Scientific).

After homogenization and repeated splitting of the seed sample, 3 x 100 grains were randomly counted. In a washed and disinfected plastic tray of dimensions 40 x 25 x 7, a smooth layer of cotton wool was placed on which the grains were placed, i.e. 100 grains spaced in 10 rows and 10 columns (equivalent to one repetition). The three replicates for each sample were placed in different trays and a volume of 300 ml of water was sprayed evenly over each tray and then covered with clear food wrap. The placement of the trays in the growth chamber was randomized. They were checked periodically.

Germination, indicated by the appearance of the primary root followed by the appearance of the coleoptile, was expressed as a percentage of the total number of germinated or ungerminated grains of the initial 100.

(Numbers of sprouted grains*100)/100

(Numbers of ungerminated grains*100)/100.

RESULTS AND DISCUSSIONS

In the year 2021, without involving the influence of treatment, it was observed that the wheat seed of the variety Glosa, germinated at all temperatures distinctly and very significantly more than at 1ºC. In contrast, there were no differences between the other temperatures, each taken as a control (Table 1).

In contrast, the number of days required for germination decreases significantly from one temperature to another. At 1ºC, wheat requires 33 days to germinate, while at 20ºC, only 4 days (Table 2). It should be pointed out that the lower number of days for germination (very significant decreases) is a positive aspect for the crop.

Table 1. Germination of wheat seed as a result of temperature - 2021

Temperature (ºC)	Germination (%)	Difference + semnificance			
		C1	C2	C3	C4
1ºC (C1)	90.6	0.0			
5ºC (C2)	95.6	5.0**	0.0		
10ºC (C3)	96.1	5.5***	0.5	0.0	
15ºC (C4)	98.1	7.5***	2.5	2.0	0.0
20ºC	97.5	6.9***	1.9	1.4	-0.6
LSD 5%	2.9				
LSD 1%	3.9				
LSD 0.1%	5.1				

Table 2. Number of days needed for germination depending on temperature - 2021

Temperature (ºC)	No germination days	Difference + semnificance			
		C1	C2	C3	C4
1ºC (C1)	33	0			
5ºC (C2)	18	-15ººº	0		
10ºC (C3)	10	-23ººº	-8ººº	0	
15ºC (C4)	5	-28ººº	-13ººº	-5ººº	0
20ºC	4	-29ººº	-14ººº	-6ººº	-1ººº
LSD 5%	0.2				
LSD 1%	0.3				
LSD 0.1%	0.4				

The low values of the limit differences are due to the homogeneity of the number of days needed for germination between repetitions.

The influence of fungicide + biostimulator treatment on germination, regardless of temperature, is not shown by statistical calculation (Table 3).

Table 3. Germination according to wheat seed treatment - 2021

Seed treatment	Germination (%)	Difference C
b1-untreated with fungicide and biostimulator	94.5	0.0
b2-treated with fungicide but untreated with biostimulator	96.8	2.3
b3-treated with fungicide and YMPACT 0.7 l/t	95.9	1.4
b4-treated with fungicide and YMPACT 0.35 l/t	95.3	0.8
b5-treated with fungicide and KERAFOL 1 l/t	96.1	1.6
b6-treated with fungicide and KERAFOL 0.5 l/t	94.9	0.4
LSD 5%	3.3	
LSD 1%	4.6	
LSD 0.1%	6.7	

The number of days required for germination, although spread over 13-15 days, was very significantly higher or lower depending on the seed treatment. Therefore, the biostimulator YMPACT, irrespective of dose, stimulated seed germination compared to both untreated variants, while KERAFOL, irrespective of dose, slowed germination (Table 4).

Table 4. Number of days needed for germination depending on seed treatment-2021

Seed treatment	No of days required for germination	Diff. C
b1-untreated with fungicide and biostimulator	14	0
b2-treated with fungicide but untreated with biostimulator	14	0
b3-treated with fungicide and YMPACT 0.7 l/t	13	-1ᵒᵒᵒ
b4-treated with fungicide and YMPACT 0.35 l/t	13	-1ᵒᵒᵒ
b5-treated with fungicide and KERAFOL 1 l/t	15	+1***
b6-treated with fungicide and KERAFOL 0.5 l/t	15	+1***
LSD 5%	0.2	
LSD 1%	0.3	
LSD 0.1%	0.5	

The influence of germination temperature x treatment interaction on seed germination is differentiated (Table 5).

While in the fungicide-treated and non-biostimulator-treated variants germination is significantly and distinctly significantly higher at all temperatures compared to that at 1ºC, in the YMPACT-treated variants, germination is not statistically different from that at 1ºC.

Table 5. Germination as a result of temperature x seed treatment interaction - 2021

Temperature (ºC)	Germination (%)					
	b1	b2	b3	b4	b5	b6
1ºC (C)	90.7	89.7	93.0	92.0	90.3	88.0
5ºC	90.3	99.7**	96.0	92.7	97.7*	97.0*
10ºC	96.7	97.3*	96.0	96.3	96.7	93.7
15ºC	98.3*	98.0*	98.7	97.0	98.3*	98.0**
20ºC	96.3	99.3**	95.7	98.3	97.3	98.0**
LSD 5%	7.2					
LSD 1%	9.6					
LSD 0.1%	12.5					

This suggests that YMPACT biostimulator, helps the seed to germinate at the same values regardless of temperature. The same cannot be said for KERAFOL, where regardless of the dose, the seed germinates better at 5 and 15ºC, and at half the dose at 20ºC.

As the graph below shows, the lowest germination amplitude is at 15ºC and the highest at 5ºC. Although the fungicide-treated but not biostimulator-treated (b2) variant at 3 of the temperatures (5, 10 and 20ºC) has the highest germination values, this is not necessarily statistically assured (Figure 1).

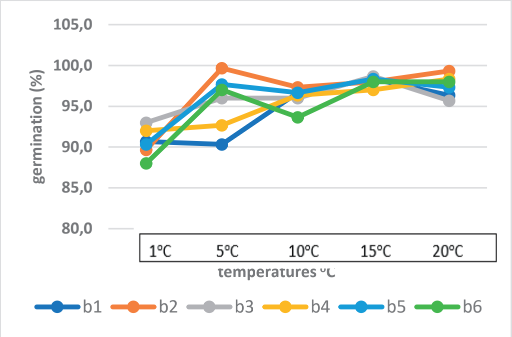

Figure 1. Germination amplitude at the same temperature based on seed treatment - 2021

In year 2022, the seed analysed came from the experiment located in the previous year and therefore the samples sum up all the factors studied, germination being done according to them. Consequently, it was observed that depending on the level of fertilization applied, the resulting seed germinated significantly, distinctly significantly and very significantly better than the variant in which only NPK complexes were administered in autumn (Table 6). This is due to the existing correlation between germination and mass of 1000 grains, the correlation coefficient r = 0.415 being significantly different at P 5% level (0.400). The larger the grain, the better it germinates.

In both variants where ammonium nitrate was applied in spring, germination was at the control level, regardless of the B-factor grading, i.e. the biostimulator did not influence germination. In contrast, in the absence of ammonium nitrate, YMPACT biostimulator at both doses highly significantly negatively influenced germination (only NPK in autumn) and the full dose - significantly negatively at NPK autumn + foliar in spring (Table 7).

Depending on the biostimulator treatment, the results showed that germination is reduced with statistical assurance in YMPACT treatment, both doses and KERAFOL treatment, 0.5 L/t dose.

Table 6. Germination as a function of fertilization in wheat - 2022

Fertilization	Germination (%)	Diff.+ Semnif. C	MMB
a1-fertilized with NPK in autumn (C)	94.8	0.0	36.61
a2-fertilixed with NPK in autumn + ammonium nitrate in spring	96.3	1.5*	37.19
a3-fertilixed with NPK in autumn + foliar in spring	96.7	1.9**	37.89
a4-fertilized with NPK in autumn + ammonium nitrate and foliar in spring	98.1	3.3***	36.95
LSD 5%	1.2		
LSD 1%	1.8		
LSD 0.1%	2.8		
Correlation coefficient germination - MMB			0.415

Table 7. Germination as a result of biostimulant treatment (Factor B) x fertilization (Factor A) and unilateral biostimulant treatment (Factor B) - 2022

Seed treatment	Germination (%)				
	a1	a2	a3	a4	average
Interactions	Treatment biostimulators (Factor B) x fertilization (Factor A)				Factor B
b1-untreated with fungicide and biostimulator	95.4	96.5	97.1	98.3	96.9
b2-treated with fungicide but untreated with biostimulator	94.8	96.6	97.0	98.0	96.6
b3-treated with fungicide and YMPACT 0.7 l/t	93.7^{ooo}	96.5	95.9_{o}	97.5	95.9^{oo}
b4-treated with fungicide and YMPACT 0.35 l/t	93.5^{ooo}	95.4	96.5	98.3	95.9^{oo}
b5-treated with fungicide and KERAFOL 1 l/t	95.6	97.3	97.7	98.6	97.3
b6-treated with fungicide and KERAFOL 0.5 l/t	95.7	95.7	95.7^{o}	97.9	96.3^{o}
LSD 5%	1.2				0.6
LSD 1%	1.7				0.8
LSD 0.1%	2.2				1.1

As in the previous year, germination is very significantly higher at all temperatures compared to the control, regardless of fertilization level (Table 8).

Table 8. Germination as a result of the interaction of temperature (Factor C) x fertilization (Factor A) and unilateral temperature (Factor C) - 2022

Temp (ºC)	Germination (%)				
	a1	a2	a3	a4	average
Inter actions	temp (Factor C) x fertilization (Factor A)				Factor C
1ºC (C)	89.9	92.4	92.1	93.5	92.0
5ºC	95.4 ***	97.6 ***	97.8 ***	98.6 ***	97.4 ***
10ºC	97.1 ***	97.8 ***	97.4 ***	99.5 ***	98.0 ***
15ºC	96.4 ***	97.2 ***	98.4 ***	99.7 ***	97.9 ***
20ºC	95.1 ***	96.6 ***	97.9 ***	99.2 ***	97.2 ***
LSD 5%	1.3				0.6
LSD 1%	1.7				0.9
LSD 0.1%	2.2				1.1

Seed obtained from the NPK-fertilized variety in autumn and ammonium nitrate-fertilized variety in spring required a significantly longer germination time compared to the control-NPK variety in autumn (Table 9).

Table 9. Number of days needed for germination depending on fertilization level - 2022

Fertilization	No days required germination	Dif+ Semnificance C
a1-fertilized with NPK in autumn (C)	16.6	0.0
a2-fertilized with NPK in autumn + ammonium nitrate in spring	17.7	1.1*
a3-fertilized with NPK in autumn + foliar in spring	17.1	0.5
a4-fertilized with NPK in autumn + ammonium nitrate and foliar in spring	16.5	-0.1
LSD 5%	1.0	
LSD 1%	1.4	
LSD 0.1%	2.3	

Interaction of biostimulator treatment (Factor B) x fertilization (Factor A) and unilateral biostimulator treatment does not influence the number of days needed for germination. The germination process occurs at the same time regardless of the variant differentiated by the level of fertilization from which the analysed seed originated (Table 10).

Irrespective of the level of fertilization, the number of days required for germination is very significantly lower at all temperatures tested than at 1ºC. As in the previous year, the number of days needed to germinate was very significantly lower at all temperatures compared to the control, regardless of fertilization level (Table11). They decreased from 47 days needed for germination at 1ºC to 4 days needed for germination at 20ºC.The values of the limit differences showed that the number of days required for germination decreased with statistical assurance from one germination to another.

Table 10. Number of days needed for germination depending on biostimulator treatment (Factor B) x fertilization (Factor A) and unilateral biostimulator treatment (Factor B) interaction - 2022

Seed treatment	No. days required for germination				
	a1	a2	a3	a4	average
Interacțiuni	Treatment biostimulators (Factor B) x fertilization (Factor A)				Factor B
b1-untreated with fungicide and biostimulator	16.7	16.8	17.1	16.5	16.8
b2-treated with fungicide but untreated with biostimulator	16.6	16.7	17.2	16.5	16.8
b3-treated with fungicide and YMPACT 0.7 l/t	16.7	16.9	17.3	16.5	16.8
b4-treated with fungicide and YMPACT 0.35 l/t	16.7	16.5	17.0	16.5	16.7
b5-treated with fungicide and KERAFOL 1 l/t	16.7	16.5	17.2	16.5	16.7
b6-treated with fungicide and KERAFOL 0.5 l/t	16.6	16.5	17.0	16.5	16.7
LSD 5%	1.0				0.5
LSD 1%	1.4				0.7
LSD 0.1%	1.8				0.9

Table 11. Number of days needed for germination as a result of the interaction of temperature (Factor C) x fertilization (Factor A) and unilateral temperature (Factor C) - 2022

Temperature (ºC)	No. days required for germination				
	a1	a2	a3	a4	average
Interactions	Temperature (Factor C) x fertilization				Factor C
1ºC	46.7	46.5	48.8	46.0	47.0
5ºC	17.4^{ooo}	17.4^{ooo}	18.0^{ooo}	17.6^{ooo}	17.6^{ooo}
10ºC	10.2^{ooo}	10.3^{ooo}	9.6^{ooo}	10.0^{ooo}	10.0^{ooo}
15ºC	5.0^{ooo}	5.0^{ooo}	5.0^{ooo}	5.0^{ooo}	5.0^{ooo}
20ºC	4.0^{ooo}	4.0^{ooo}	4.0^{ooo}	4.0^{ooo}	4.0^{ooo}
LSD 5%	1.0				0.5
LSD 1%	1.4				0.7
LSD 0.1%	1.8				0.9

CONCLUSIONS

The lowest germination amplitude is at 15ºC and the highest at 5ºC.

The number of days required for germination, although spread over the range 13-15 days, was very significantly higher or lower depending on the seed treatment. Thus, the biostimulator YMPACT, regardless of dose, stimulated seed germination compared to both untreated variants, while KERAFOL, regardless of dose, hindered germination. At 1ºC, wheat took 33 days to germinate, while at 20ºC, only 4 days. The results showed that the lower number of days for germination (very significant decreases) is a positive aspect for the crop.

In the NPK fertilized variant in autumn + ammonium nitrate and foliar in spring, the germination of the obtained seed is very little differentiated between temperatures of 5, 10, 15 and 20ºC, as well as between the biostimulator treatment variants.

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