RESEARCH REGARDING THE INFLUENCE OF SOME RHIZOGENOUS PRODUCTS AND OF THE WETTING TIME ON THE GERMINATION OF SEEDS AND GROWTH OF PEPPER SEEDLINGS

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Abstract

In Romania, the pepper culture is established exclusively by planting seedlings, which is why more attention must be paid to this technological link. For peppers, the germination of the seeds is slower, but to increase the germination capacity this experiment was carried out. As biological material, the Alexandru variety was used, 3 rhizogenous products for seed wetting, respectively, Raiza 0.2%, Keramin 0.25% and Kerafol 0.25% and 2 time cycles for seeds wetting, 6 h and 12 h, compared with non-wet seeds and seeds wet only in water 24 h. It was found that the best results regarding seed germination were recorded after wetting the seeds with 0.25% Keramin, for 12 hours and after wetting the seeds in water 24 h. Biometric features of the seedlings were more influenced by treating the seeds with 0.25% Keramin for 6 hours. Also, all the rhizogenic products led to better results compared to the non-wet seeds and to almost similar results as the seeds wet in water for 24 h.

Key words: germination, growth, pepper, seedlings.

INTRODUCTION

Pepper is a very important vegetable plant that is cultivated within different culture systems and is globally consumed under different forms. It is well known as one of the richest vegetable in Vitamin C, the red pepper having107-154 mg/100 g fresh product (Martinez et al., 2005).

Pepper has a high content of capsaicin, especially the hot pepper, which is why it is highly used specific cuisines such as Asian, Indian, Mexican and South American.

In addition, due to capsaicin, hot pepper has anti-septic and anti-inflammatory properties and protects the human body against rheumatoid diseases and conditions, (Krishna, 2003).

It is a species with slow growth of plants, so it is recommended to use biofertilizers for the intensification of physiological processes and the growth of the seedlings from the first stages (Vlahova and Popov, 2014). Due to slow seed germination, to large periods of time between sowing and plant emergence and to uneven plant emergence, stimulation of pepper seeds' germination is a necessity. For this, pre-germination treatments are applied, with positive effects on the germination percentage and time, such as seed wetting through various methods and with different wetting agents (Lutts, 2016). Pre-germination treatment through osmosis (osmo-priming) of the pepper and tomato seeds reduces the time period until plant spring and ensures a better uniformity of the spring even when temperatures are below the optimum level (Giuliani et al., 1992; Rogers, 1989). Osmotic pressure, temperature and length of the treatment depend on the species (Bradford et al., 1990). Studies show that different seed wetting techniques enrich the germination for pepper seeds. This way, Kaewduangta et al., 2016, shows that seeds wet with osmotic solutions (osmo-priming) have germinated better that the ones soaked in water (hydro-

priming) and among those, the ones soaked in solution with PEG 6000 for 14 days have recorded a higher germination percentage and index (germination speed). Moreover, treating the seeds though osmo-priming leads to obtaining higher and longer seedlings, with an increased number of roots compared to using other treatments. Soaking pepper seeds with germination between 66% and 98%, in a solution with KNO₃ 2%, for 4 days at 20°C led to an increased germination percentage of the seeds that had a lower germination capacity (Ermis et al., 2016), which makes it possible to use older seeds for which the germination is lower. Treating pepper seeds with GA₃ 0.01 mg/l anticipates the germination process with several days compared to the untreated seeds 1995). Treating the seeds with (Hoza. gibberellic acid associated with mechanical scratching of the tegument and using the gibberellic acid led to an increase in the physiological potential of the studied pepper genotypes regarding the germination speed and percentage (Guadalupe et al., 2019). Treating the pepper seeds, California Wonder variety, with cu nanochitine, chitosa or hidropriming significantly reduced the average germination time compared to untreated seeds (Samarah, N.H., et al., 2016). Similar results were obtained for corn, for which the chitosan solution reduced significantly the average germination time, demonstrating that chitosan can be a new treatment applied to the pepper seeds (Shao et al., 2005; Guan et al., 2009). Treating the seeds with warm water (47°C-49°C, 50°C-52°C and 53°C-55°C) for 30, 45 and 60 min in vitro, showed that for a water temperature of 50-52°C, and a 30 min hydration, seed germination is very good and the obtained seedlings are vigorous and qualitative (Singh et.al., 2019). The treatment of cereal seeds with collagen hydrolyzate determined the increase of their speed and germination rate, stimulated the increase of the seed content in gibberellic acid and the amount of biomass of the seedlings (Epure et al., 2018). Other researchers (Hanieh et al., 2013) observed that by treating the pepper seeds with salicylic acid of 0.1 and 0.5mM concentrations for 24 h the seed germination percentage and plant growth rate were improved, especially for the 0.5mM concentration. Wetting pepper seeds

with different chemical products (NaCl 50 mM, CaCl₂ 50 mM, ABA 100 μ M and others) for 24 h, at 25 ± 2°C, with moderate stir, increases the seed germination percentage and induces to the obtained plants tolerance against various types of stress compared to the seeds soaked in water at 40°C or untreated (Yadav et al., 2011).

MATERIALS AND METHODS

Research was conducted within the experimental field of USAMV Bucharest, Faculty of Horticulture, inside a greenhouse specialized for producing vegetable seedlings, during March-April 2018. The experiment was organized with 2 variable factors, one being the wetting product and the other the wetting time of the seeds.



Figure 1. USAMV Bucharest greenhouse

The purpose of the research was to establish the wetting duration for the pepper seeds and the influenced of the used product on seed germination. The biological material used was the long pepper variety Alexandru, semi-late variety, productive, with elongated-conic fruits, a red colour when matured and average weight of 80-120 g. Three products were used to stimulate seed germination, as detailed below: Raiza, product with rhizogenous effect. contains oligopeptides, polypeptides, oligosaccharides, polysaccharides, polyamines, vitamins and seaweed extract (Ascophyllum nodosum). It is totally water soluble and enriched with free amino acids (10.7%) and total nitrogen (N) 4%. It stimulates root growth and the capacity to absorb water and nutrients. Kerafol contains a complex of hydroisolated proteins and activators that improve plant growth and increase the cellular division speed and plant growth speed, phenomenon that is also met when the product is used for seed wetting. Kerafol contains amino acids 28%, out of which 24% are free amino acids, 5.7% total nitrogen (N) out of which 4.3% organic nitrogen, 3.1% soluble potassium oxide (K_2O) and 14% carbon organic.

Keramin has complex composition. а respectively free amino acids 14%, total nitrogen (N) 3.7% out of which organic nitrogen (N) 1.9% and ammonial nitrogen (N) 1.8%, potassium oxide (K₂O) 6%, copper (Cu) 0.07%, copper chelated with EDTA 0.07%, iron (Fe) 0.10%, iron chelated with EDTA 0.10%, manganese (Mn) 0.05%, zinc (Zn) 0.07%, and zinc chelated with EDTA 0.07%. Functions like a stimulator for seed germination, by influencing the cellular growth and division and the growth of plants, roots and meristematic tissues.

The pepper seeds were soaked in different products for different durations, as detailed in Table 1.

	Table 1. Experimental scheme				
nt	Wetting	Wetting			

Variant	Wetting	Wetting duration
	product	(h)
V1	Not soaked	0
V2	Water	24
V3	Kerafol 0.25 %	6
V4	Kerafol 0.25 %	12
V5	Keramin 0.25 %	6
V6	Keramin 0.25 %	12
V7	Raiza 0.20 %	6
V8	Raiza 0.20 %	12

The experiment was organized in subdivided parcels, with 3 replications each of 15 plants/replication. Seed soaking was made within plastic bottles of 500 ml, the quantity of solution or water being equal for all variants, respectively 300 ml. The quantity of seeds used was 8 g, one gram of seeds for each variant. The seeds were introduced into the bottles containing the solutions and stored within the warm greenhouse until the end of the wetting period, then removed and sowed immediately in plastic crates, on substrate of wet peat Kekkila. When the first real leaf emerged, the seedlings were transplanted into alveolar pallets with 45 alveoli. During seedling production, the temperature was maintained between 22°C and 26°C during the day and between 16-18°C during the night, repeated irrigation procedures

were applied to maintain the substrate wet and 2 phyto-sanitary treatments were applied, one with Dithane M45 0.2% and one with Topsin 70 WDG 0.1%.

When the seedlings reached the plating phase, determinations were made regarding plant height and root length by measurements, number of leaves, weight of the aerial part, stem diameter with callipers and root system volume with the graded cylinder. The interpretation of the results was made through variant analysis.

RESULTS AND DISCUSSIONS

As a result of the measurements made, it was noted that soaking pepper seeds in rhizogeneous solutions, regardless of the product and wetting duration, determined a higher germination percentage than for the untreated seeds (Table 2). Pepper responded well to seed soaking with rhizogeneous products for 12 h compared to soaking the seeds in the same products for 6 h.

Thus, during day 6 since sowing, the best results were obtained for the seeds soaked in Keramin 0.25%, Raiza 0.2% and Kerafol 0.25%, the germination being 61 %, 59 % and 58 %. For the seeds soaked for 6 h, the germination percentage was between 50% and 56%, the best product being Keramin 0.25 %. Eight days after sowing, seed germination recorded the same tendency as for the 6-day milestone; at the end of the germination period, meaning 10 days after sowing, the highest germination percentage was obtained for the seeds treated with Keramin 0.25%, for 12 h, respectively 90%.

All products stimulated seed germination. Soaking the seeds in water for 24 h had a positive effect and similar to the effect of Raiza 0.25%, respectively 82%. The lowest percentage of plant emergence was recorded for the untreated seeds (Table 2). The results were statistically ensured.

By graphically representing the influence of the wetting products (Figure 2), it was observed that the best results were obtained when using the products Keramin and Kerafol for all 3 moments of measuring the germination percentage.

Variant	Wetting product	Wetting	Day		
		duration (h)	6 th	8 th	10 th
V1 (Mt)	Not soaked	0	32 Mt	55 Mt	76 Mt
V2	Water	24	43N	76 ***	82N
V3	Kerafol 0.25 %	6	54*	67***	89*
V4	Kerafol 0.25 %	12	58*	68 ***	87*
V5	Keramin 0.25 %	6	56*	70 ***	88*
V6	Keramin 0.25 %	12	61**	80 ***	90**
V7	Raiza 0.20 %	6	50 N	62 *	85N
V8	Raiza 0.20 %	12	59 N	69 ***	84N
DL 5%			20.13	6.54	9.84
DL 1%			27.97	9.05	13.68
DL 0.1%			38.91	12.60	19.03

Table 2. Dynamics of plant emergence for pepper seeds depending on the used product and wetting duration (%)



Figure 2. Influence of the wetting product on the seed germination (%)

Different wetting duration of the pepper seeds demonstrated that it influences the germination percentage, compared to the untreated seeds (Figure 3).



Figure 3. Influence of wetting time on the seed germination (%)

Analyzing the seedlings obtained from seeds soaked before sowing, compared to the untreated seeds, it was noted that the used products influenced also the plant growth during the first life phases, as per the description of those products (Table 3).

Soaking the seeds in Keramin 0.25% for 6 and 12 h had a greater influence on the pepper seedling growth than the other wetting products and durations. Thus, plant diameter at parcel was 3.8 mm for seeds soaked for 12 h and 3.7 mm for seeds soaked for 6 h. Similar results were obtained also for the seedlings obtained from seeds treated with Kerafol 0.25%, meaning 3.4 mm for soaking for 6 h and 3.7 mm for 12 h. Wetting the seeds with Raiza 0.2%, for 12 h, had the same results as soaking the seeds with water for 24 h, respectively 3.2 mm.

The seed wetting stimulated the growth of the roots; however, among the used products there were no significant differences, but for the plants obtained from untreated seeds the growth was weaker.

The volume of the root system varied between 2.1 cm³ for plants obtained from untreated seeds and 2.7 cm³ for those obtained from seeds treated with Keramin 0.25% and Raiza 0.2%, for 6 and 12 h. Also, the weight of the roots was influenced by the treatment, especially by Keramin 0.25% and Raiza 0.2%, for 6 and 12 h.

Variant	Wetting product	Wetting	Stem	Seedling	Number	Root	Root	Weight of
		duration	diameter at	height	of	length	volume	aerial part
		(h)	parcel (mm)	(cm)	leaves	(cm)	(cm^3)	(g)
V1 (Mt)	Not soaked	0	2.4 Mt	11.7 Mt	5.3 Mt	11.4 Mt	2.1 Mt	2.9 Mt
V2	Water	24	3.2***	15.4***	6.8***	12.1***	2.7**	3.9 ***
V3	Kerafol 0.25 %	6	3.4***	15.5***	6.3***	12.2***	2.5 N	3.7***
V4	Kerafol 0.25 %	12	3.7***	15.1***	7.2***	12.3***	2.6 *	3.7***
V5	Keramin 0.25 %	6	3.5***	16.9***	7.1***	12.9***	2.7**	4.3***
V6	Keramin 0.25 %	12	3.8***	16.9***	7.5***	12.7***	2.7**	4.2***
V7	Raiza 0.20 %	6	3.4***	16.8***	7.4***	12.5***	2.7**	4.1***
V8	Raiza 0.20 %	12	3.2***	17.7***	7.4***	12. 6***	2.7**	3.8***
DL 5%			0.16	1.02	0.47	0.31	0.40	0.40
DL 1%			0.23	1.42	0.66	0.43	0.56	0.56
DL 0.1%			0.32	1.97	0.92	0.60	0.77	0.77

Table 3. Seedling characteristics for pepper plants

The results were statistically ensured through variant analysis. The effect of the wetting products was manifested also during the root growth phase.

The best results being obtained as a result of using rhizogeneous products (Figure 4). Wetting duration influenced seedling growth, especially soaking the seeds for 6 h and 12 h (Figure 5). Between the morphological parameters of the seedlings were shown the correlation. Thus, between the height of the plants and the number of leaves there was a direct correlation, with a correlation coefficient $r^2 = 0.8219$ (Figure 6).



Figure 4. Influence of wetting product on seedling growth



Figure 5. Influence of wetting duration on seedling growth



Figure 6. Correlation between height of plant and number of leaves

Between the height of the plants and the volume of the root a strong positive correlation was determined, with a high correlation coefficient, $r^2 = 0.8542$ (Figure 7).



Figure 7. Correlation between height of plant and number of root volume

Between the height of the plants and the weight of the aerial part there was also a positive and strong correlation, with a high correlation coefficient, $r^2 = 0.7778$ (Figure 8).



Figure 8. Correlation between height of plant and weight of aerial part

CONCLUSIONS

Research regarding wetting pepper seeds with rhizogenous products for 6 and 12 h showed that pepper reacted well and the seed germination process was improved. It was noticed that when soaking the seeds with Keramin 0.25% for 12 h. The germination was of 61% for the 6th day since sowing, compared to untreated seeds, which after the same time period germinated only at 32%. This showed a very good germination speed. The increased being of 52.45% compared to the control variant. Better results, compared to the control, were also obtained for soaking the seed with Kerafol 0.25% and Raiza 0.2%, for 6 and 12 h. Soaking the seed with water for 24 h led to an increase in germination of 7.3%, compared to the untreated seeds. The wetting products influenced the growth of the seedlings, element was determined by the seedling that characteristics. The seedlings obtained from seed soaked in rhizogenous products were more vigorous, fact determined by a higher root diameter (3.2-3.8 mm), seedling height (15.5-17.7 cm),a higher number of leaves (6.3-7.4),root volume (2.5-2.7 cm³) and weight of aerial part (3.7-4.3 g). It was observed that by soaking the seeds in water for 24 h better results could be obtained regarding seed germination and also growth of the seedlings, compared to the untreated seeds.

REFERENCES

- Epure D.G., C. F. Cioineag, M. Becheritu, C. Gaidau, E. Stepan, M. Gîdea (2018). Use of biofertilizant based on collagen hydrolysate for cereal seed treatment, *AgroLife Scientific Journal*, 7(1), 48-55.
- Gagliardi B., J. M. Filho (2011). Assessment of the physiological potential of bell pepper seeds and relationship with seedling emergence. *Revista Brasileira de Sementes*, 3(1) Londrina.
- Giuliani D., Nuvoli S., Pardosi A., Tognoni F. (1992). Tratamenti pregerminativi di semi di pomodoro e peperone. *Colture protette*, 6, 73-78.
- Guadalupe J. S., J. Alcalá-Rico, A. López-Benítez, M. E. Vázquez-Badillo, D. Sánchez-Aspeytia, S. Rodríguez-Herrera, M. Á. Pérez-Rodríguez, F. Ramírez-Godina, (2019). Seed Physiological Potential of *Capsicum annuum* var. *glabriusculum* Genotypes and Their Answers to Pre-Germination Treatments. *Agronomy*, 9(6), 325.
- Guan. Y.J., Hu. J., Wang. X.J., Shao C.X. (2009). Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *Journal of Zhejiang University* Science B, 10, 427-433.
- Hanieh A., D. Mojtaba, Z. Zabihollah, A. Vahid (2013). Effect of Pre-sowing Salicylic Acid Seed Treatment on Seed Germination and Growth of Greenhouse Sweet Pepper Plants. *Indian Journal of Science and Technology*, 6, 3868-3871.

- Hoza G., Popescu V., Neata G. (1995). Influența giberelinei GA₃ asupra germinării semințelor de ardei *in vivo. Lucr.şt.USAMV Bucureşti. seria B*, XXXVIII, 35-39.
- Kaewduangta W., Phirayot K., Ponlawat U. (2016). Improved gemination and vigour of Sweet Pepper (*Capsicum annuum* L.) seeds by hydro- and osmopriming. *Azarian Journal of Agriculture*, 3, 70-75.
- Krishna A. (2003). Capsicum, the genus *Capsicum*. Taylor and Francis Group, London and New York; CRC Pres London. https://doi.org/10.1201/9780203381151.
- Lutts S., P. Benincasa, L. Wojtyla, S. Kubala, S. R. Pace. K. Lechowska, M. Quinet, M. Garnczarska (2016). Seed priming: New comprehensive Approaches for an old empirical technique. New Challenges in Seed Biology, chapter 1-7.
- Martínez S., M. López, M. G. Raurich, A. Bernardo Alvarez (2005). The effects of ripening stage and processing systems on vitamin C content in sweet peppers (*Capsicum annuum L.*). International Journal of Food Sciences and Nutrition 56(1), 45-51.
- Rogers B.A., Pike L.M. (1989). Preplant seed treatments for improving earliness and uniformity in germination of pepper seeds for transplant production. *Journal Horticultural Society Rio Grande*, 42, 25-31.
- Samarah N.H., Wang H., Welbaum G.E. (2016). Pepper (Capsicum annuum) seed germination and vigour

following nanochitin. chitosan or hydropriming treatments. *Seed Science. & Technology*, 44(3), 1-15.

- Shao C., Wen-Jian S., Wei-Min H. (2005). Effects of seed priming with chitosan solutions of different acidity on seed germination and physiological characteristics of maize seedling. *Journal of Zhejiang* University Agriculture and Life Science, 31, 705-708.
- Singh S., N. K. Bharat, H. Singh, S. Kumar, S. Jakhar, N. Vijay (2019). Effect of hot water treatment of seeds on seed quality parameters and seedling growth parameters in bell pepper (*Capsicum annuum*). *Indian Journal of Agricultural Sciences*, 89(1), 33-137.
- Sıtkı E., E. Ozden, E. S. Njie, I. Demir (2016). Pretreatment Germination Percentages Affected the Advantage of Priming Treatment in Pepper Seeds. *American Journal of Experimental Agriculture* 13(1), 1-7.
- Vikas Y. P., M. Kumari, Zakwan A. (2011). Seed Priming Mediated Germination Improvement and Tolerance to Subsequent Exposure to Cold and Salt Stress in Capsicum. *Research Journal of Seed Science.* 4, 125-136.
- Vlahova Velselka, Popov Vladislav (2014). Impact of biofertilisers on vegetative grouth and leave gasexchange of pepper seedlings (*Capsicum annuum* L.) in organic farming. *Agrolife Scentific Journal*, 3(1), 156-162.