Studii şi Cercetări	Martie 2019	Biologie	28/1	55-58	Universitatea"Vasile Alecsandri" din Bacău

SEED TREATMENTS TO IMPROVE SEED GERMINATION PARAMETERS OF SOME VEGETABLE SPECIES

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Key words: *vegetables crops, alternative treatments*

INTRODUCTION

The attack of different diseases and pathogens is among the factors that affect the germination of seeds and subsequently the growth and development of the seedlings, finally altering the vigour and resistance of the plants. Treating seeds in suspensions of beneficial microbial agents, in solutions of different natural compounds or salts improves their viability and germination, stimulates plant growth and protection against pathogens, and finally increases crop production. So far, different microorganisms have been studied ex. *Bacillus* spp., *Pseudomonas fluorescens*, *Trichoderma* spp. for seed immersion of different species, and a beneficial effect on germination percentage, seedling emergence time, early flowering, early maturation and production increasing as well as protection against soil pathogens were found (Chowdhury, 2000; Asaduzzaman et al., 2010; Mastouri et al., 2010; Hanci, 2014). Other studies have shown that the microorganisms combined with different natural substances are much more effective for seed fortification and seedling growth.

The genus *Trichoderma* is often found in soil microflora of various ecosystems, such as agricultural fields, forests, wetland areas in all climatic zones. Root colonization by *Trichoderma* strains leads to root growth and nutrient uptake and utilization, increased production, and increased resistance to abiotic and biotic factors. Field crop productivity can increase up to 30% after treating seeds or soil with different *Trichoderma* species (Benitez et al., 2004). The purpose of this study was to evaluate the effect of applying seed treatments with different *Trichoderma* strains and salicylic acid on germination rate and germination rate in seeds of varieties of beans, onions and peppers.

MATERIAL AND METHODS

Biologic material

Seeds of pepper (Cardinal and Regal varieties), bean (Lidia variety) and onion (Albă de Buzău and Aurie de Buzău varieties) were obtained from Research and Development Station for Vegetables Buzău, Buzău County, Romania. All seeds were sterilized by soaking in 2% sodium hypochlorite solution (NaClO) for 2 minutes, then in 70% ethanol for 2 minutes and finally washed for three times by sterile distilled water. After that the seeds were air dried in a sterile chamber 24-28h.

Preparation of Trichoderma inoculum

Trichoderma strains (Td85 and Tal12) which have been used in this experiment were obtained from the collection at the Research and Development Institute for Plant Protection. The strains were grown in Potato Dextrose Agar (PDA) plates and incubated for 10 days at 28°C to obtain abundant sporulation. Spore suspensions of *Trichoderma* sp. were prepared by scraping the spores from cultures using 15 mL distiled water and after added Tween 80, 0.1% solution to reduce surface tension and facilitate spore release. The concentration of spore suspension was counted using haemocytometer and was adjusted to two concentration 10^7 spores/mL and 10^8 spores/mL. Seeds from the species studied were soaking 4 or 6 hours.

Seed germination test

Three replications of 15 seeds each were used, placed in Petri dishes on sterile filter paper moistened with sterile distilled water equivalent to 2.5 times the paper weight (ISTA, 2009). The seeds were incubated at 25°C, in the dark for 7-14 days depending on the species. The seeds of the control variant were not soaked in water. The sterilized seeds were immersed in the fungal suspensions of two *Trichoderma* strains with two different spore concentrations. (10^7spores/mL and 10^8 spores/mL) and salicylic acid solutions in two concentrations 50mg/L (V1) and 100mg/L (V2), different periods of time (3 and 6 hours). The germination percentage and rate (number of days for complete germination) were calculated.

RESULTS AND DISCUSSIONS

The two fungal strains used in this study were selected based on the proven properties of plant growth stimulation and antifungal activity against soil pathogens. The present studies have shown comparative performances in the technique of immersion of vegetable seeds with solutions on germination. The time of 3 hours immersion of the

seeds in suspension of *Trichoderma* or salicylic acid was more suitable for seed germination. The time of 6 hours had a negative effect on the germination parameters for all the studied seed species. Spore concentration of 10^7 spores/ml of microbial suspensions increased seed germination, while the spore concentration of 10^8 spores/ml inhibited germination in all studied species. The effect of the two *Trichoderma* strains suspensions on the germination of the pepper onions and beans seeds in the laboratory conditions are presented in tables 1, 2 and 3.

The onion seeds, variety Albă de Buzău, did not germinate in any of the variants, treated with the fungal suspensions and control. The physiological or genetic deficiencies of the variety could be among the causes. Treatment of the Aurie de Buzău onion variety with the two *Trichoderma* strains led to a significant increase in the germination percentage compared to the control. The seeds germination rate of the of this variety was 70% when applying the Td85 strain and 60% when applying the Tdal12 strain, compared to the control that germinated 30%. The germination rate was faster in the case of treatment with the Td85 strain compared with the Tdal12 strain.

These results are in agreement with those obtained by Hanci et al. (2014) who found that *Trichoderma* suspension of $2x10^6$ spores/ml concentration determined a positive effect on onion seed germination (92%). Studies by Dorna et al. (2005) showed that treating onion seeds with suspensions of *Trichoderma koningii* and *T. harzianum* did not affect germination speed or germination capacity. The results of Celar and Valic (2005) showed that several *Trichoderma* strains (*T. ningii, T. viride, T. longibrahiatum*) have negative effects on the germination but also on the germination speed of the onion seeds.

Peppermint seeds, the Carmin variety, had a higher germination when have been treated with microbial suspension of Td85 (86%) compared with those treated with Tdal12 (75%). Similar results were obtained in the case of the long pepper Regal, which had 80% germination when the seeds were treated with Td85, compared with 65% germination after the suspension soaked in Tdal12.

The bean variety responded similarly when treating with the two fungal strains studied, achieving the germination of 100% after 4 days. All immersion variants of the applied seeds were effective in all the analysed vegetable species, determining the early germination, with 8 days earlier in peppers (compared to 14 days), 6 days earlier onion (compared to 12 days) and 5 days earlier on beans (compared to 7 days) compared to untreated control.

Of the two *Trichoderma* strains used, Td85 determined a higher germination percentage compared to the Tdal12 strain in both pepper and onion seeds.

Plates in which the seeds were inoculated with suspensions of the two *Trichoderma* strains maintained moisture longer than untreated ones. This could be an important finding, *Trichoderma* strains would play a role in resistance to drought-like abiotic factors.

The results in this study show that salicylic acid increases the germination rate for all varieties of the studied species, except the white onion variety, Albă de Buzău. Of the two concentrations of salicylic acid used, 50mg/L and 100mg/L, the second had conclusive results compared with the first one that inhibited germination. Seeds of the long pepper Regal variety had the highest percentage of germination (93%) and the highest rapid germination rate when treated with salicylic acid. The pepper seeds, Carmin variety, had 90% germination after treatment with salicylic acid compared to the untreated control (33%). The seeds of both pepper varieties started to germinate after 4 days in the variants with salicylic acid.

The seeds of Lidia bean treated with salicylic acid started to germinate at 2 days, completing their germination at 4 days. Very good germination (97%) was also observed in the control variant after 4 days, but the roots were shorter than the treated variant. The results in this study are in line with those of Gharib & Hegazi (2010) who found that percentage and germination rate for beans treated with salicylic acid were improved, at the optimum temperature of 25°C and at the lower temperature of 15°C as well. Also, the seeds of the Aurie de Buzău onion variety treated with salicylic acid started to germinate after 3 days and recorded a germination rate of 95%.

Table 1. Germination potential of some vegetables treated with Trichoderma Td85 suspension

Species / Variety	% germination		Germination speed	
	Untreated control	Td 85	Untreated control	Td85
Pepper (Carmin)	40	86	8.2	7.2
Long pepper (Regal)	33	80	8.1	6.0
Beans (Lidia)	96	100	5.1	4.0
Water onion (Aurie de Buzău)	26	70	7.3	5.2
White onion (Alba de Buzău)	0	0	0	0

Table 2. Germination potential of some vegetables treated with Trichoderma Tdal12 suspension

Specia /Soi	% Germination		Germination speed	
	Untreated control	Tal12	Untreated control	Tal12
Pepper (Carmin)	33	75	9.8	9
Long pepper (Regal)	33	65	9.5	8.5
Beans (Lidia)	96	100	5.5	5
Water onion (Aurie de Buzău)	33	60	9	8.1
White onion (Alba de Buzău)	0	0	0	0

Table 3. Germination potential of some vegetables treated with salicylic acid

Specia /Soi	% Germination		Germination speed	
	Untreated control	Salicylic acid	Untreated control	Salicylic acid
Pepper (Carmin)	33	90	8.1	6.2
Long pepper (Regal)	26	93	8.5	6.1
Beans (Lidia)	97	100	5.01	3.02
Water onion (Aurie de Buzau)	20	95	7.2	4.6
White onion (Alba de Buzău)	0	0	0	0

CONCLUSIONS

The germination of vegetable seeds was influenced by immersion time, solutions concentration and immersion agent. Immersion of seeds in *Trichoderma* suspensions with a concentration of $1x10^7$ spores/mL favoured germination while concentrations greater than $2x10^8$ spores/mL had inhibiting effects. All the immersion variants of seeds were effective in all the analyzed vegetable species, inducing early germination. Immersion of seeds with solutions of different compounds can be successfully used to improve the percentage and speed of germination for seeds of peppers, onions and beans.

ABSTRACT

Obtaining quality seedlings from vegetable species is essential for field crop productivity. In most cases, the low seed germination capacity is an impediment to achieving this goal. Treating seeds with different solutions of natural compounds or suspensions of beneficial microorganisms for improving seed germination parameters is an ecological and viable solution in the field. The present study presents results obtained in vitro regarding the effect of seed treatments with *Trichoderma* strains and salicylic acid on germination parameters (percentage and rate) for three species of vegetables, beans, onions and peppers.

The experiments confirmed the positive effect of both salicylic acid and *Trichoderma* treatments on the germination percentage and rate for seeds of all species studied compared to the control variants. *Trichoderma* spores treatments with $1x10^7$ spore/mL concentration gave the best results.

ACKNOWLEDGEMENTS

This study was carried out in the project funded by the Ministry of Research and Innovation, CCCDI-UEFISCDI, number PN-III-P1-1.2 PCCDI-2017-0659 / *Innovative technologies for reducing the negative impact of climate change on legume crops* (LEGCLIM), within PNCDI III.

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