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IMPACT OF PRESOWING SOAKING OF AMARANTH SEEDS IN SOLUTIONS OF GROWTH REGULATORS PART I. EFFECT OF SOME GROWTH REGULATORS ON SEED GERMINATION CAPACITY

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Abstract. The effect of some methods for the presowing improvement of seeds of amaranth of the cultivar Rawa (*Amaranthus cruentus* L.) on their sowing value was assessed in laboratory conditions. The results of seed soaking in solutions of commercial preparations were examined, where the active substance was synthetic growth regulators – auxin and giberelin. The experiments were conducted in 2010-2012 at the time preceding sowing seeds in the field (in April each year). It was indicated that under laboratory conditions germination energy and capacity of amaranth seeds of the cultivar Rawa depends to a much larger degree on germination temperature than on choosing the preparation in which sowing seeds are soaked, and germination capacity at 25ºC can be regarded as quite satisfactory in all the treatments. Soaking amaranth seeds in solutions of bioregulators turned up to be effective, although only in definite conditions.

Key words: amaranth, germination, growth regulators, seed soaking

INTRODUCTION

Rest can be defined as any time of temporary slowing down or stopping the growth of creative tissues. Seeds in the state of rest do not germinate, in spite of potentially favourable conditions for germinating. Three types of seed dormancy are distinguished – (1) resulting from the external determinants of germination (2) determined by physical and biochemical factors occurring inside the seeds, but provoked by external factors, and – (3) resulting from internal physiological state of the germ, independent on the surrounding conditions. Seed dormancy of most species can be primary (inborn) or secondary. Primary dormancy is connected with natural maturing of seeds. In many species such a state is induced by the germination temperature that is improper for them, or not meeting the light conditions creating favourable conditions for germination. This

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is the first type dormancy (1). Inborn dormancy is also provoked by the processes of physical and mechanical nature, connected mainly with the hardness of the seed cover (difficult access of oxygen and water to the inside of the seed), as well as the presence of abscisic acid in it (inhibiting the effect of giberelins). Thus these are the case of the second type dormancy (2). Inborn dormancy can also simply result from immaturity of seeds – the third type dormancy (3). Secondary dormancy occurs in not germinating seeds, in which the stage of absolute dormancy already passed and they are able to germinate. It is thought that this property is evolutionary adaptation of seeds of many species for stopping the germination process in unfavourable thermal, light, water or salinity conditions. This phenomenon is probably provoked by ABA and can be reversed, as long as there are conditions that favour germination [Baskin and Baskin 2004]. Since the lack of readiness of seeds to germinate leads to unevenness of emergences, a number of methods for presowing seed processing was developed in order to stimulate them for better germination and emergences of seedlings in the shortest time possible and in a wide range of environmental conditions [Mayer and Poljakoff-Mayber 1989, Powell 2009, Jisha *et al.* 2013].

Seed dormancy is a quite common phenomenon. Farnsworth [2000] claims that nearly 67% of the 195 species of 143 genera of tree plants, perennials and annual plants she mentions show various types of seed dormancy. Although there is not the genus *Amaranthus* (amaranth) on the list, the literature reports cited below indicate that the problem of seed dormancy of different amaranth species has been analysed many times.

The genus *Amaranthus* derives from South America and consists of 75 species which are widespread in the world nowadays [Sauer 1993]. From the biological point of view, the seeds of amaranth are fruits, small nuts, in which the proper seed is surrounded with the hard pericarp. Such structure of fruits favours their durability, where in conditions of their natural environment plants sometimes have to wait for long months for suitable subsoil moisture to germinate. According to the report of Mexican scientists [Itúrbide and Gispert 1994], even in the climatic conditions of the amaranth natural habitat, plant emergences are sometimes difficult, although seed germination proceeds very fast – only in a few days. Amaranth seedlings, however, are very small and the soil moisture in the surface layer determined their survival.

Attempts to explain the reasons for low germinating capacity of amaranth seeds were made on different species. The studies included species classified as pseudo-cereals: *A. cruentus*, *A. hypochondriacus* and *A. caudatus*, as well as the vegetable and fodder species – *A. mangostanus* (syn. *A. tricolor*). Their tendency to cross both among themselves and with wild species was observed [Brenner 1995]. As there is a large genetic similarity between various species of amaranth, wild forms growing over amaranth crops are often model in experiments over the dormancy of seeds of this kind, hence the studies of stimulating germination of *A. albus*, *A. blitoides*, *A. hybridus*, *A. palmeri*, *A. powelli*, *A. retroflexus*, *A. rudis*, *A. spinosus*, *A. tuberculatus*. In Poland two amaranth cultivars are grown on a small scale: Rawa, representing the species *A. cruentus,* and Aztek – *A. hypochondriacus*. Of the above-mentioned wild species, those commonly occurring in our country include *A. retroflexus*, but also frequently: *A. albus*, *A. blitoides* and *A. hybridus* [Mirek *et al.* 2002].

Almost all the methods known from agricultural practice were used in experiments over increasing the germinating capacity of seeds of the mentioned species. They included treatments affecting an increase in permeability of the seed cover: soaking seeds in water [Musa *et al.* 2014], in the alcohol solution of $CaCl_2$ [Colmenarez de Ruiz

and Bressani 1990], in the sulfuric acid solution [Soomarin *et al.* 2010], by cooling [Zharare 2012], as well as the action of alternating magnetic field to seeds [Dziwulska--Hunek *et al.* 2009]. There was used hydropriming with the water solution of KNO_3 and KH_2PO_4 [Tiryaki *et al.* 2005], and even – ionized radiation [Aynehband and Afsharinafa 2012]. The effectiveness of osmo-conditioning seeds of the species in question was also tested using solutions of polyethylene glycol (PEG) [Pill and Evans 1994, Moosavi *et al.* 2009, Sun *et al.* 2011].

In the studies dealing with the analysis of seed germinating conditions the most attention is focused on biochemical processes occurring in seeds. Hence broad literature concerning relations between the concentration of growth hormones, particularly in the light of relations between abscisic acid and giberelic acid [Mayer and Poljakoff-Mayber 1989, Steckel *et al.* 2004, Ashraf and Foolad 2005, Kucera *et al.* 2005, Kępczyński *et al.* 2006, Powell 2009]. The mentioned authors not only confirm the opinion about the importance of ABA as a growth regulator with the effect inhibiting germination and antagonistic to the effect of GA3, but also turn attention to the significance of the temperature at which the germination process of seeds of many species occurs, including amaranth. The role of auxin in the process of germination is still under discussion. Mayer and Poljakoff-Mayber [1989] as well as Maransari and Smith [2014] cite a number of works where the authors suggest that the favourable effect of IAA manifests in cooperation with GA3, and applied separately to the seeds – is weak. Moscova [2012] reports that she observed a several-percent increase in the germinating capacity of amaranth seeds under the influence of prior treating them with IAA solution. Similar observations were made by Kim *et al.* [2006] as a result of experiments with rice and by Guangwu and Xuwen [2014] – in experiments over germination of Masson's pine growing in China.

Most the above-mentioned methods for presowing treatment of amaranth seeds provoked the expected results (except for their exposure to the effect of gamma radiation). The most spectacular effects were given by the use of various treatments to seeds stored for several years, overwintering in soil, exposed to the salt stress, that is – those which have naturally weakened germination capacity [Sznigir and Kępczyński 2009, Moosavi *et al.* 2009, Moscova 2012]. Ashraf and Foolad [2005] warn, however, that artificially stimulated seeds after sowing have to find favourable water conditions, since the process of germinating cannot be already stopped. .

Evolutionary adaptation of amaranth seeds protecting them against germination in unfavourable thermal and water conditions poses a threat to obtain the even and sufficient emergences at the cultivation sowing time of amaranth grown in Poland (in the middle of May at the latest), when the soil temperature is still relatively low. Thus at dry weather, the emergences of small and shallowly placed seeds last even two weeks. The impossibility of applying herbicides creates favourable conditions for early weed infestation on a plantation, which results in uneven plant density and, more importantly, it prevents the raw material from obtaining the equal standard.

The authors of cited studies of the effect of growth hormones on seed germination were interested in the course of the germinating process itself and they do not mention the application of the study results in practice. They used chemically pure preparations in their experiments, not commonly available for the producers. However, as the results of many studies are promising, taking up the present study, it was assumed that it is worth testing their effect in field conditions using commercial preparations containing growth hormones. Therefore in the present studies an attempt was made to assess the

effect of presowing processing of amaranth seeds using two preparations available on the market, trying to specify the conditions of their practical application in seed production of the selected amaranth cultivar.

MATERIAL AND METHODS

Laboratory experiments were conducted over 2010-2012 at the time preceding sowing seeds in the field (in April each year). The experiments aimed to examine the effects of soaking the seeds in solutions of commercial preparations, containing synthetic substances with the character of growth regulators – auxins and giberelins. The subject of this study was the seeds of amaranth (*Amaranthus cruentus* L.) of the cultivar Rawa.

The experimental factors and treatments were: (1) the duration of soaking the seeds, 8, 16 and 24 hours, (2) commercial preparations, Pol-Gibrescol 800 SP at a concentration of 0.03% (a.s. gibberellic acid GA_3), Betokson Super 025 SL at a concentration of 2.0% (a.s. 2-naftoxyacetic acid NAA), a mixture of both preparations in the given concentrations, dry untreated seeds – the control treatment.

Soaking was performer on Petri dishes lining with 2 layers of filter paper copiously moistened with previously prepared solutions of the preparations, at 15°C and in the light. After the right time interval, the seeds were rinsed on a sieve under running water, placed on filter paper for drying in the open air at the room temperature for 24 hours. Petri plates were used for seed germinating. The substrate was prepared of chemically pure filter paper. The seeds were placed on Petri dishes, 100 seeds on each. Prepared Petr dishes were placed in incubators with controlled temperatures of 15 and 25ºC and left for germinating. The substrate humidity was 60%. All determinations were made in 4 replications.

The laboratory analysis of seed germination was made in accordance with the standard PN-R-65950, 1994. Calculations included the number of seeds forming normal seedlings (normally germinating), laboratory germinating energy after 4 day, laboratory germinating capacity after 7 and after 14 days.

Statistical analysis of the results of laboratory experiments was made separately for individual combinations for which the percentage of normally germinating seeds were compared in pairs with the appropriate percentage of seeds from the control treatment, separately for each time, measurement and separately for germination at 15 and 25ºC. The Student-t test for two structure coefficients was applied [Stanisz 2006]. Statistically different results for combinations with processed seeds in result tables were marked with boldface.

RESULTS

In laboratory conditions, the germinating energy and capacity of amaranth seeds of cultivar Rawa subjected to soaking in solutions of bioregulators was to a much greater degree depended on the temperature in which they germinated than on the methods of their treatment (Table 1). Germination capacity at 25ºC can be regarded as quite satisfactory in all the treatments, and the untreated seeds showed it in a slightly lower or similar degree to the conditioned ones. The results of the analysis of germinating

capacity conducted at 15ºC are considerably worse. After 4 days of incubation, the control seeds showed on average ten times weaker germination than those kept at 25ºC at the same time, and the fastest germinating seeds, initially soaked in solutions of GA_3 – four times lower. After fourteen days of the experiment, the percentage of germinated, previously soaked, seeds at 15ºC constituted only a half of the percentage of the seeds germinating at the higher temperature, and only30% of the control seeds. The use of solutions of GA_3 were more effective than of synthetic auxin, and a mixture of both regulators stimulated seed germination to a slightly greater degree at 15ºC.

Table 1. Germination energy (after 4 days) and capacity (after 7 and 14 days) of amaranth seeds depending on the method of their conditioning as compared with the control treatment (mean from 2010-2012)

Tabela 1. Energia (po 4 dniach) i zdolność kiełkowania (po 7 i 14 dniach) nasion szarłatu w zależności od sposobu ich kondycjonowania w porównaniu z obiektem kontrolnym (średnio z lat 2010-2012)

Preparation and concentration Preparat i stężenie	Conditioning time Czas kondy-cjonowania h	Germination temperature – Temperatura kiełkowania					
		15ºC			25ºC		
		percentage of seeds germinating after, % udział nasion kiełkujących po upływie					
		4 dni/days	7 dni/days	14 dni/days	4 dni/days	7 dni/days	14 dni/days
Pol-Gibrescol 800 SP 0.03%	8	**14**	**25**	**34**	59	63	66
	16	**17**	**29**	**36**	57	65	67
	24	**15**	**28**	**38**	54	60	64
Mean for preparation – Średnio dla preparatu Pol-Gibrescol 800 SP		**15**	**27**	**36**	57	**63**	66
Betokson Super 025 SL 2.00%	8	8	21	33	61	67	69
	16	7	18	30	53	57	60
	24	8	21	30	56	59	61
Mean for preparation – Średnio dla preparatu Betokson Super 025 SL		7	20	31	57	61	63
Pol-Gibrescol 800 SP 0.03% Betokson Super 025 SL 2.00%	8	**17**	**32**	**46**	67	73	75
	16	9	**27**	**40**	61	65	67
	24	9	**25**	**39**	55	59	62
Mean for preparation mixture Średnio dla mieszaniny preparatów		12	**25**	**38**	61	66	69
Control – dry seeds Kontrola – nasiona suche		6	13	20	53	57	66

results of conditioning significantly different from the control treatment were marked with boldface – drukiem pogrubionym oznaczono wyniki kondycjonowania istotnie statystycznie różne od obiektu kontrolnego

The time of soaking the seeds depended to some degree on the kind of preparation. For Pol-Gibrescol, slightly better effects were observed after treating the seeds for 16 hours, whereas the use of a mixture of preparations and only the preparation Betokson Super 025 SL gave the best results after 8 hours of the treatment duration. The process of conditioning using the preparation Pol-Gibrescol 800 SP statistically significantly increased the germinating capacity of amaranth seeds at 15ºC.

In comparison with the treatment where amaranth seeds were soaked in solutions of the preparation Pol-Gibrescol, treating the seeds with the preparation Betokson Super 025 SL, particularly at 15ºC, did not give such good effects.

The number of germinating amaranth seeds increased along with prolonging the process of soaking, and the dynamics of an increase in the number of germinating seeds was also dependent on the temperature at which the experiment was conducted. Already after 4 days, the proportion of seeds germinating at 25ºC was several times higher than at 15ºC. On average in the three-year period and at the germination temperature of 15ºC an increase in the number of seeds germinating between the measurement after 4 and 7 days was at least twofold, whereas at 25ºC it was on average no more than 11%. Prolongation of time of seed germination by next 7 days brought further increase in the germination capacity index by 33 to 55% in seeds germinating at the lower temperature and by 3 to 16% at the higher temperature (Table 2).

Table 2. Multiple of increase in percentage of seeds germinating along with extending of conditioning time depending on the method of their conditioning, as compared with the control treatment (mean from 2010-2012)

Tabela 2. Wielokrotność zwiększenia udziału nasion kiełkujących wraz z wydłużaniem czasu kondycjonowania w zależności od sposobu ich kondycjonowania w porównaniu z obiektem kontrolnym (średnio z lat 2010-2012)

Preparation and concentration Preparat i stężenie	Germinating temperature – Temperatura kiełkowania			
	15ºC		25ºC	
	relation of percentage of seeds germinating at later time to the earlier time – stosunek udziału nasion kiełkujących w terminie późniejszym do wcześniejszego			
	7 to 14 days 7 do 4 dni	14 to 7 days 14 do 7 dni	7 to 14 days 7 do 4 dni	14 to 7 days 14 do 7 dni
Pol-Gibrescol 800 SP 0,03%	1.80	1.33	1.11	1.05
Betokson Super 025 SL 2,00%	2.86	1.55	1.07	1.03
Pol-Gibrescol 800 SP + Betokson Super 025 SL	2.08	1.52	1.08	1.05
Control treatment – Obiekt kontrolny	2.17	1.54	1.08	1.16

DISCUSSION

When discussing the published results, it is most difficult to take a stance on the amount of IAA and GA_3 doses adopted by other scientists, since in the present study solutions of commercial preparations were used, not suitable chemically pure substances. The concentration of preparations was also highly diversified: e.g.. Kim *et al.* [2006] used GA_3 at a concentration of 10 μM per 50 ml for hormonal regulation of rice germination, whereas Zharare [2012] does not report in what volume of water he diluted 100 μM GA_3 in his experiment on the germination stimulation of seeds of two amaranth species (*A. hybridus* and *A. retroflexus*). It is possible that the mentioned authors used also other concentrations of hormonal substances, which is not mentioned. In the present study, in fact germination of seeds of the cultivar Rawa was tested in several concentrations of solutions of commercial preparations (for Pol-Gibrescol: from 0.01 to 0.04%, and for Betokson from 1 to 3% and all the combinations of those

concentrations in mixtures). Only those which gave the best results were mentioned in this work.

The results of the present study confirmed the statements of the cited authors that soaking amaranth seeds both in water and in solutions of different substances for a period longer than 8 hours does not give a significant increase in seed germination capacity.

Presented results are completely in accordance to the results of all the studies concerning the effect of temperature on the process of amaranth seed germination. High thermal requirements of this kind of species are stressed, and some authors even tested responses to temperature that never occurs in Polish conditions. Dziwulska-Hunek and Kornarzyński [2009] report that the seeds of amaranth cv. Rawa achieved the highest germination capacity at 45ºC. Cristaudo et al [2007] note that within the temperature range 30-40ºC, the germination capacity of amaranth seeds can increase up to 95-100%. According to Steckel *et al.* [2004] and Tiryaki *et al.* [2005], the temperature most favourable for germinating of both cultivated and wild species of amaranth amounts to 20-25ºC.

Germination dynamics of processed and control seeds decreased along with prolonging of the time of conditioning both in the present study and in those described in the cited literature [Kępczyński *et al.* 2006, Kim *et al.* 2006, Moosavi *et al.* 2009, Adeniyi and Obatolu 2014]. Leon *et al.* [2007] observe that the effects of germination stimulation in amaranth are provoked by the effect of exogenous GA_3 and become apparent only in seeds in the state similar to the natural stopping dormancy. Therefore this treatment is not completely effective. That is probably why in all studies germination ability tested in laboratory conditions did not exceed 80%, irrespective of the method for seed processing. Only in the mentioned study by Cristaudo *et al.* [2007] we can find a statement that the germination capacity of amaranth seeds can be increased to as much as 100%. It is notable that the importance of the effect of substances used for seed processing decreased in a higher germination temperature, which was also confirmed by the results of the present study. Very satisfying increase in the germination ability of amaranth seeds was recorded by Musa *et al.* [2014] using only hydropriming at temperature 28ºC.

It is worthy to note a good result of combined use of the preparations Pol-Gibrescol 800 SP and Betokson Super 025 SL, in solutions in which amaranth seeds were soaked for 8 hours. Each year this combination of experimental factors best induced seed germination. This suggests emerging of the effect of auxin, as a hormone supporting the effect of GA_3. Similar conclusions can be found in the report by Guangwu and Xuwen [2014].

The results of the study quoted in the work, as well as the review of literature reports, indicate a high sensitivity of amaranth to the ambient temperature during the germination stage, and this probably to the greatest extent provoke difficulties in achieving good, even relatively early emergences of seeds of this species in field conditions in Poland. However, a delay of the sowing time so that it would fall in a warmer period is not recommended, since the amaranth growing period lasts long. Many authors claim that the results of examination of the laboratory seed germinating capacity not necessarily are confirmed in the field conditions [Ashraf and Foolad 2005, Grzywacz and Orzeszko-Rywka 2007]. This refers particularly to seeds with difficult germination, even after prior treatment improving their germination capacity, as it occurs in the case of amaranth.

CONCLUSIONS

1. In laboratory conditions, the germination energy and capacity of amaranth seeds of the cultivar Rawa much more depend on the germination temperature than on the kind of preparation in which seeds are soaked. Germination capacity at 25ºC can be regarded as satisfactory, irrespective of the method for seed material treatment.

2. Effective stimulation of amaranth seed germination at 15ºC is induced by soaking them for 8h in 0.03% water solution of Pol-Gibrescol, as well as in a mixture of preparation solutions at concentrations 0.03% Pol-Gibrescol and 2% Betokson 025 Super SL.

3. At the temperature similar to those prevailing during amaranth seeds germination in the field (15ºC), the process of germination in laboratory conditions lasts long and does not exceed 50% of seeds, which can explain the unevenness and prolonging of amaranth emergences in production conditions.

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WPŁYW NASTĘPCZY PRZEDSIEWNEGO MOCZENIA NASION SZARŁATU UPRAWNEGO W ROZTWORACH REGULATORÓW WZROSTU. CZĘŚĆ I. WPŁYW WYBRANYCH REGULATORÓW WZROSTU NA ZDOLNOŚĆ KIEŁKOWANIA NASION

Streszczenie. Oceniano wpływ wybranych sposobów przedsiewnego uszlachetniania nasion szarłatu krwistego odmiany Rawa (*Amaranthus cruentus* L.) na ich wartość siewną w warunkach laboratoryjnych. Badano skutki moczenia nasion w roztworach preparatów handlowych, w których substancją aktywną były syntetyczne regulatory wzrostu – auksyna i giberelina. Doświadczenia prowadzono w latach 2010-2012 w terminie poprzedzającym siew nasion w polu (w kwietniu każdego roku). Wykazano, że w warunkach laboratoryjnych energia i zdolność kiełkowania nasion szarłatu odmiany Rawa w większym znacznie stopniu zależy od temperatury kiełkowania niż od doboru preparatu, w którym moczone są nasiona siewne, a zdolność kiełkowania przy temperaturze 25ºC uznać można za zupełnie zadowalającą we wszystkim obiektach. Zabiegi moczenia nasion szarłatu w roztworach bioregulatorów okazały się skuteczne, choć w tylko w określonych warunkach.

Słowa kluczowe: kiełkowanie, moczenie nasion, regulatory wzrostu, szarłat uprawny

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