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EFFECT OF K, NA, MG AND CA IONS CONTENT IN SUGAR BEET SEED PERICARP AND PEG TREATMENT ON THE RESISTANCE OF THE SEEDLINGS TO *APHANOMYCES COCHLIOIDES* DRECH.

ABSTRACT

In previous investigations it was observed that the high content of Mg in sugar beet seed pericarp increased the resistance of seedlings to *A. cochlioides* Drech. The experiments conducted in 1991 - 95 aimed at verification of this fact. Beet seed pricarp was enriched with mineral elements in field conditions using different fertilization of stecklings. In laboratory conditions seeds washed twice in water were soaked in salt solutions ($MgSO_4$, Mg $(NO_3)_2$, KCl) or PEG 6000. Washing sugar beet seeds with water without any additional protection favours infection of the emerging seedlings with *A. cochlioides*. The mean results obtained from field experiments show that the lowest number of dead seedlings and significantly the highest number of survived ones were observed in the seeds washed and soaked in PEG. Similar results were found using KCl. The question still remains whether the protective effect is of a specific or osmotic character. The results confirmed a possibility of seedling protection in the above described way.

Key words: *Aphanomyces cochlioides*, black root rot, sugar beet

INTRODUCTION

The soil fungus *Aphanomyces cochlioides* Drech., commonly known as a causal agent of the black root rot of sugar beet seedlings occurs in almost all regions of sugar beet cultivation (Chicuo *et al.* 1982, Narita 1984, Kimber 1993, Payne *et al.* 1994, Senoo *et al.*1997, Smith 1997). The disease caused by *A. cochlioides* does not appear untill the seedlings are well established (Rush 1992) and it affects also mature plants. The most spectacular effect is observed during the period from germination till the primary cortex shedding (Osińska 1984). It is well known that *A. cochlioides* is not the only reason for development of black root rot of the sugar beet seedlings. However, within the microflora of the seedlings showing symptoms of black root rot, this fungus accounts for 10% to 88%

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of the affected plants (Uchino and Hanazawa 1981). A control of the fungus is not the only possible way of preventing the disease. The oldest known ways of protecting the plants are agrotechnical ones e. g.: crop rotation (Szymczak-Nowak 1986), where rye, oats or maize are considered the best forecrops. (Matsuzaki et al 1982). Damping off of the seedlings was increased at high soil pH, high nitrogen and low magnesium concentration (Szymczak-Nowak 1990), associated with porous soil structure which could be reduced by compacting the soil with a roller after sowing (Narita 1984). The most effective nitrogen content in soil was 60-120 mg/l, whereas the contents of both potassium and phosphorus in soil had no effect on plant susceptibility. The contents of magnesium in the pericarp (Podlaski 1990) and that of magnesium and calcium in soil (Szymczak-Nowak 1993) increased tolerance to the disease. Also previously the authors found that washing of the seeds with water made the seedlings less tolerant to the black root rot disease. As the resistance to the infection with *A. cochlioides* is conditioned polygenicly (Coe and Schneider 1966), it is not easy to achieve satisfactory results in the resistance breeding. Recent biotechnological approach is promissing since the first transgenic plant has been obtained with the radish gene, responsible for the production of a natural fungicide of protein character to control the pathogen (Long 1995).

The present paper aimed at verification of the hypothesired effect of chemical composition of seed pericarp on infection of sugar beet seedlings by *A. cochlioides*.

MATERIAL AND METHODS

The trials were conducted in 1991-1995. Sugar beet seeds of Jastra variety were the object of the experiment, which included:

A). Production of, sugar beet seeds, enriched with K, Na, Mg, Ca ions or PEG.

B). Isolation of the pure *A. cochlioides* Drech. culture,

C). Evaluation of the infection degree.

Production of sugar beet seeds of diversified content of chemical compounds in the pericarp

There were three different approaches applied - in the field conditions

I. diversified fertilization of the stecklings

II. different time of seed harvest

III. in the laboratory conditions soaking in the seeds washed twice with water in the chemical compounds solutions.

In 1991 the stecklings of Jastra variety were fertlized with four different fertlizing levels:

combination 1. a control plot fertlized with N 140 kg × ha^{-1}, P 100 kg × ha^{-1} and K 180 kg × ha^{-1};

combination 2 - K dose increased to 180 kg × ha^{-1} and additionally N 80 kg × ha^{-1} applied in the rosette stage;

combination 3 - the same level of fertilization as in combination 1 plus Mg 15 kg × ha^{-1} in the form of kieserite and additionally the stecklings were sprayed twice with $MgSO_4$ solution before and after flowering;

combination 4 - the double fertilisation dose as in combination 1;

combination 5 - the same level of fertilization as in control plots. The stecklings from combination 5 were harvested either when the seeds on the lower part of a seedstalk became brown (combination 5a) or later, when almost all seeds were brown (combination 5b). Each combination was done in four replications. Quality of seeds was evaluated by the standard methods. The cation contents in water extracts of ground pericarp was determined as described earlier (Podlaski and Chrobak 1984) using atomic spectrophotometer.

In 1993-1995 in the laboratory conditions the seeds were washed twice with water at 20°C, dried at room temperature and soaked for 24 h in the following solutions:11.5% KCl (osmolarity - 3.5 MPa), 20% Mg $(NO_3)_2$ (osmolarity - 6.7 MPa), 20% $MgSO_4$ and polyethyleneglycol (PEG) 6000. The concentrations applied did not affect germination ability of the seeds (after 14 days). For better evaluation of seed quality the seeds were sown in the sterilized sand moistened up to 60% of field water capacity. After 1, 2 and 3 days the cylinder-shaped samples of the sand were taken. The height of the cylinder was 1 cm and its diameter - 1.5 cm. The samples were taken in such a way that the seed was in the very centre of the cylinder. The samples were dried and the seed was separated from the sand. Then the seeds and the sand were resaturated with water and the contents of cations in the extracts were examined with atomic spectrophotometer.

Isolation of pure *A. cochlioides* Drech. culture.

Each year after beet harvest soil samples from the fields of Plant Breeding Stations in Straszków, Śmiłów, Polanowice, Więcławice and Klecina were taken for isolation of various types of *A. cochlioides*. In the Straszków laboratory the fungus cultures were isolated by the trap method, using sugar beet as a host plant. After 2-3 weeks the spores of *A. cochlioides* became visible under a microscope. The pure culture was grown on the dextrin-potato agar, and several times transplanted under microscope control. The isolated mycelium was transferred to the dextrin-potato liquid medium for 72 h. The culture toxicity was tested after washing three times with water.

Evaluation of seedling infection.

This was carried out both in the greenhouse and in the field. For the greenhouse trials 100 seeds from each combination were sown in 6 replications in the pots filled with the mixture of sand and compost (4:1). The seeds were dressed with Oxafun T and were inoculated with water suspension of *A. cochlioides* zoospores, directly on seeds. The infection degree was examined every 3-4 day. A final evaluation was done one month after the emergence. The results are presented as mean degree of seedling infection and in percent of the infected and healthy plants during 30 days of vegetation. The mean degree of infection was calculated as a mean weight, i. e. a total of the products of the share of the dead, highly infected, moderately infected, low infected and the healthy seedlings multiplied by a factor score and divided by 100. The factor score was as follows: 1 - dead seedlings, 3, 5, 7 - highly, moderately and low infected seedlings respectively; 9- healthy seedlings. The percentage of infected seedlings was related to the number of emerged plants.

In the field trials 100 seeds from each combination were sown in 6 replications. The seeds were inoculated with water suspension of *A. cochlioides* zoospores in the same way as in the greenhouse trials. Infection was evaluated 1 month after plant emergence.

RESULTS

Table 1.

The results of the laboratory evaluation of the sugar beet seeds obtained in the course of the field trial

Combinations	Germination ability [%]		Pieper's coefficient [days]	
	after 4 days	after 14 days	after 4 days	after 14 days
1.	60.8ab	94.5a	3.6b	4.4b
2.	48.2b	92.8a	3.8a	4.7a
3.	51.0b	93.2a	3.7b	4.5b
4.	63.8a	94.0a	3.5b	4.2b
5.a	40.6c	85.0b	4.1a	5.0a
5.b	59.1ab	95.5a	3.5b	4.3b
Average	53.9	92.5	3.7	4.5
$LSD_{\alpha=0,05}$	10.2	6.1	0.3	0.4

Means followed by the same latter do not differ significantly
Combinations as it is given in Material and Methods

Laboratory evaluation of seeds quality obtained from all combinations of field experiments (Table 1) proved a significantly lower germination ability and a higher Pieper coefficient for the seeds from the earlier harvest (combination 5a). The differences in the quality of the seeds from the plots of all other combinations after 14 days of germination, were insignificant. The content of cations in the water extracts from the ground

Table 2.
The contents of K, Na, Mg and Ca ions in water extracts from the ground pericarp of the sugar beet seeds obtained in the field trial

Combinations	Content of ions [$\times 10^{-6}$ mol $\times$ g^{-1} of pericarp]			
	K	Na	Mg	Ca
1.	300.5b	115.2c	10.2c	4.1ab
2.	451.6a	140.2b	12.1bc	4.1ab
3.	300.6b	125.1b	25.1a	2.0c
4.	310.2b	120.2b	12.8bc	3.8b
5.a	472.5a	170.5a	16.2b	5.1a
5.b	391.4ab	105.1c	11.4c	3.8b
Average	371.1	129.4	14.6	4.0
$LSD_{\alpha=0,05}$	115.1	20.5	4.1	1.2

Means followed by the same latter do not differ significantly
Combinations as it is given in Material and Methods

pericarp (Table 2) proved that the pericarp of the seeds from the plots fertilized with a higher dose of K (combination 2) and from combinations 5a, i. e. the seeds harvested earlier contained significantly higher level of K ions than from other experiments. A significantly higher level of Mg ions was found in the pericarp of the seeds from the plots additionally fertilized with Mg (combination 3). As shown in Table 3 emergence of the seedlings from the plots fertilized with different doses of N, P., K varied significantly. The seeds from the earlier harvest (combination 5a) showed the lowest emergence and the lowest percentage of healthy seedlings, while the highest percentage of healthy seedlings were from the control plots, from the plots fertilized with Mg (combination 3), and from the seeds harvested later (combination 5b). Quality of the seeds with the pericarp enriched with mineral salts under laboratory conditions

Table 3.
The consecutive effect of different fertilization of stecklings in the field on seedlings emergence and the degree of the infection with *A. cochlioides*, evaluated as a rate in relation to the number of seedlings emerged. The greenhouse trial

Combinations	Emergence [%]	Share of seedlings [%]		
		Healthy	Dead	Infected
1.	83.3	80.6a	10.7c	8.7
2.	89.4a	72.1b	19.1a	8.7
3.	88.3ab	80.8a	10.2c	9.0
4.	86.2b	76.1ab	15.1abc	8.8
5.a	80.1d	70.5b	20.2a	9.3
5.b	85.1bc	79.1a	11.2b	9.7
Average	85.4	76.5	14.4	9.0
$LSD_{\alpha=0,05}$	2.4	4.7	6.1	ns

Means followed by the same latter do not differ significantly
Combinations as it is given in Material and Methods

Table 4.
The quality of the sugar beet seeds washed twice in water and then soaked in the salt solutions or PEG 6000

Seed treatment	Germination ability [%]		Pieper Coefficient after 14 days [days]	Emergence (greenhouse) [%]
	After 4 days	After 14 days		
1. Control	81.1b	95.3	3.5a	85.9b
2. Washed twice	93.0a	95.3	2.5b	91.3a
3. Soaked in KCl	75.9d	93.5	3.6a	88.9ab
4. Soaked in $Mg(NO_3)_2$	77.0c	93.7	3.4a	87.9b
5. Soaked in $MgSO_4$	75.0d	92.1	3.4a	91.0ab
6. Soaked in PEG 6000	64.5e	90.0	3.5a	89.9ab
Average	77.8	93.3	3.3	89.2
$LSD_{\alpha=0.05}$	1.6	ns	0.5	3.2

Table 5.
Effect of the laboratory treatment of the sugar beet seeds on the content of K, Na, Mg and Ca ions in 1g of seed after 1, 2, 3 days of germination in the sand. Average from 3 years

Seed treatment	Contents of ions [$\times 10^{-3}$g $\times$ 1 g of seeds^{-1}]											
	K			Na			Mg			Ca		
	Germination after [days]											
	1	2	3	1	2	3	1	2	3	1	2	3
1. Control	1.2d	0.8c	0.5d	0.3b	0.2c	0.2b	1.5a	1.2b	1.0b	2.6a	1.4a	1.2c
2. Washed twice	1.1e	0.4e	0.2e	0.1c	0.1d	0.1c	1.0d	0.7e	0.6d	0.7d	0.9c	0.8e
3. Soaked in KCl	1.8a	2.4a	2.0a	0.6a	0.6a	0.5a	0.8e	1.4a	1.2a	1.4c	0.7d	0.5f
4. Soaked in $Mg(NO_3)_2$	1.5b	0.9b	0.7c	0.6a	0.4b	0.2b	1.1c	0.7e	0.6d	1.9b	1.3b	1.5a
5. Soaked in $MgSO_4$	1.4c	0.7d	0.8b	0.7a	0.4b	0.2b	1.2b	1.0d	0.8c	2.0b	1.4a	1.4b
6. Soaked in PEG 6000	1.2d	0.9b	0.8b	0.2b	0.1d	0.1c	1.0d	1.1c	0.6d	0.8d	0.9c	0.9d
Average	1.4	1.0	0.8	0.4	0.3	0.2	1.1	1.0	0.8	1.6	1.1	1.1
$LSD_{\alpha=0,05}$	0.08	0.04	0.06	0.11	0.08	0.05	0.07	0.05	0.04	0.11	0.08	0.08

(Table 4) confirmed a well known fact that washing of the seeds with water increased germination rate and the Pieper coefficient after 4 days. Should be noted that soaking of the washed seeds in the solutions indicated in Table 4 neither affect germination ability and Pieper coefficient after 14 days, nor greenhouse emergence of the seedlings.

Table 5 presents a time course of changes in the contents of K, Na, Mg and Ca per 1 g of sugar beet seeds following 1, 2 and 3 days of germination in the sterilized sand. The lowest content of the ions was in the pericarp of washed seeds. The slowest changes of ion content was noted in the seeds soaked in polyethylene glycol. This leaking suggests that PEG hinders the process of leaking the ions out of the pericarp. Consequently the lowest was the amount of mineral elements penetrated to the seed-bed from the seeds treated with PEG (Table 6). As it can be seen K ion enriched in seeds soaked in KCl was the most mobile cation and penetrated in large quantity to the seed bed. Similarly soaking the seeds in either $MgSO_4$ or Mg $(NO_3)_2$ led to an increased Mg content in the seed bed. The applied seed treatment applied had a great impact on the tolerance of the seedlings to the infection in the greenhouse conditions (Table 7). Seeds twice washed with water showed the highest susceptibility The number of healthy seedlings (in relation to the total number of emerged seedlings) was significantly highest in case of the seeds soaked in PEG and also, what seems to be worth noting, in KCl solution. The smallest number of healthy seedlings emerged from the seeds washed twice with water. High number of dead seedlings from the seeds soaked in $MgSO_4$ or in Mg $(NO_3)_2$ seems to be even more surprising. The mean results from the two years of

Table 6.

Leakage of the ions from the pericarp of sugar beet seeds to the seed sand bed after 1, 2 and 3 days of germination. Average from 3 years

Seed treatment	Contents of ions [$\times 10^{-6}$ mol $\times$ 1 g of sand^{-1}]											
	K			Na			Mg			Ca		
	Germination time [days]											
	1	2	3	1	2	3	1	2	3	1	2	3
1. Control	6.6b	7.2b	8.0b	3.8a	3.2a	3.0a	1.1b	1.5c	2.0c	2.2d	4.8d	5.6e
2. Washed twice	1.4e	1.3d	0.9f	1.2c	0.4e	0.2d	0.5c	1.0d	1.2e	1.1e	4.9d	5.8d
3. Soaked in KCl	21.4a	19.8a	16.0a	1.9b	2.6b	3.0a	1.0b	1.4c	1.5d	4.4c	7.7c	8.0c
4. Soaked in $Mg(NO_3)_2$	1.6d	1.4cd.	1.2e	0.9d	0.8d	1.2b	9.9a	9.5b	8.0b	7.7b	8.8b	10.0a
5. Soaked in MgSO4	2.6c	1.5c	1.6c	0.8e	0.8d	1.2b	10.0a	11.1a	9.0a	8.0a	9.0a	9.0b
6. Soaked in PEG 6000	1.2f	1.3d	1.4d	0.8e	1.0c	0.9c	0.2d	0.6e	1.5d	1.1e	3.8e	4.9f
Average	5.8	5.4	4.8	1.6	1.5	1.6	2.5	4.2	3.9	4.1	6.5	7.2
$LSD_{\alpha=0,05}$	0.07	0.10	0.15	0.08	0.10	0.10	0.14	0.11	0.12	0.14	0.11	0.07

experimentation in the field (Table 8) show that the lowest number of dead seedlings and significantly the highest number of survived ones were observed in the seeds washed and soaked in polyethylene glycol. The number of the survived seedlings from the control experiment is significantly higher than that from the seeds twice washed with water, similar to the number of the seedlings derived from the seeds soaked in KCl and Mg salts.

Table 7.
Effect of diversified sugar beet seeds treatment on the infection degree of the seedlings by *A. cochlioides*. The results of the greenhouse trials conducted in 1993-1995

Seed treatment	1993			1994			1995			1994-1995 Average		
	Average infection	The rate of seedlings [%]:		Average infection	The rate of seedlings [%]:		Average infection	The rate of seedlings [%]:		Average infection	The rate of seedlings [%]:	
		Healthy	Dead		Healthy	Dead		Healthy	Dead		Healthy	Dead
1.Control.	5.6b	25.2b	12.1ab	6.0ab	8.1b	73.0a	6.8ab	35.1b	41.7b	6.4ab	21.6b	57.4a
2. Washed twice	4.7c	12.5c	12.0ab	5.5b	5.8b	76.3a	6.5b	33.6b	50.8a	6.0b	19.7b	51.8a
3. Washed twice. soaked in KCl	6.1a	38.9a	11.9ab	6.4a	13.7ab	68.4a	7.2a	43.9a	35.2ca	6.8a	28.8a	52.1a
4. Washed twice. soaked in $Mg(NO_3)_2$	6.0ab	41.0a	17.8a	6.2a	10.1b	70.2a	6.9ab	37.2b	41.4b	6.6a	23.6b	55.8a
5. Washed twice. soaked in $MgSO_4$	-	-	-	5.8ab	8.2b	66.5ab	7.0ab	37.5b	43.9b	6.4ab	22.8b	55.2a
6.Washed twice. soaked in $MgSO_4$+KCl	6.0ab	35.2a	10.2b	-	-	-	-	-	-	-	-	-
7. Washed twice. soaked in PEG 6000	-	-	-	6.2a	15.9a	56.3b	7.3a	43.2a	43.2b	6.8a	29.6a	49.8a
$LSD_{\alpha=0.05}$	0.45	8.10	6.20	0.60	5.50	11.2	0.50	4.50	4.50	0.45	4.80	8.10

Table 8.
Effect of diversified sugar beet seeds treatment on the infection degree of seedlings by *A. cochlioides*. The rates of the survived and dead seedlings in relation to the total number of the seedlings emerged. The results of the field trials, Straszków 1994-1995

Seed treatment	1994		1995		1994-1995 Average	
	The rates of seedlings [%]					
	Survived	Dead	Survived	Dead	Survived	Dead
1. Control	17.8b	82.2ab	20.3b	79.7ab	19.0b	81.0ab
2. Washed twice	10.3c	89.7a	16.7bc	83.3ab	13.5d	86.5a
3. Washed twice and soaked in KCl	18.1b	81.9b	17.2bc	82.8ab	17.6c	82.4ab
4. Washed twice and soaked in $Mg(NO_3)_2$	15.2bc	82.6ab	16.6bc	83.4ab	15.9cd.	83.0ab
5. Washed twice and soaked in $MgSO_4$	16.1b	83.9ab	15.0c	85.0a	15.6cd	84.4ab
6. Washed twice and soaked in PEG 6000	23.5a	76.5b	25.2a	74.8b	24.4a	75.6a
$LSD_{\alpha=0,05}$	5.10	7.60	4.50	10.10	3.90	9.10

DISCUSSION

The obtained results have proved that a proper seed treatment may increase resistance of the seedlings to *A. cochlioides*. The seedlings emerged from the seeds washed with water are the most susceptible to the infection. K and Mg ions could be effectively introduced into the sugar beet seed pericarp both in the field and laboratory conditions. Data on sugar beet resistance to *A. cochlioides* in artificially infested soil as well as fungicide control of this disease are quite numerous (Payne and Williams 1990. Tezuka *et al.* 1995. Uchino *et al.* 1996. Asher and Payne 1988). However there is no information on introducing ions into sugar beet seed pericarp. Yokosawa *et al.* (1988) suggested that zoospores *A. cochlioides* are attracted to hypocotyls of sugar beet seedlings by the endogenous nitrate and chloride ions. In the present study the seeds containing nitrate ions (Mg $(NO_3)_2$) were among those showing the highest infection degree in the greenhouse conditions. Also in the field experiments these seeds produced the lowest number of healthy seedlings. This is consistent with the observations of Yokosawa *et al.* (1988). In contrast to his results the percentage of healthy seedlings from the seeds soaked in KCl is high in both the greenhouse and field conditions. In the greenhouse as well as in the field experiments the lowest percentage of healthy seedlings and the highest number of dead seedlings were from the washed seeds. This fact is worth discussing since the washed seeds are not supposed to contain ions that could be attractants for *A. cochlioides* zoospores. The question still remains whether the protective effect of inorganic compounds in the pericarp is of a specific or osmotic character. The fact that the effect of polyethylene glycol is very similar to or even better than that of mineral salts could rather speak for an osmotic effect. Hence the assumption that the decrease of the osmotic or/and water potential in the immediate environment of the emerging seed results in hampering of *A. cochlioides* development and hinders seedlings infection.

CONCLUSIONS

1. Washing of sugar beet seeds with water without any additional protection favours infection of the emerging seedlings with *A. cochlioides* Drech.
2. Some inorganic chemical compounds ($MgSO_4$. Mg $(NO_3)_2$. KCl) as well as PEG introduced into the sugar beet pericarp resulted in the increase of the resistance to the infection with *A. cochlioides* Drech.

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