

GERMINATION AND VIGOUR OF KOHLRABI SEEDS SUBJECTED TO PRIMING IN THE PRESENCE OF *Alternaria brassicicola* (Schw.) Wiltshire

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Abstract. Priming is a treatment applied for seed enhancement. *Brassica* vegetable seeds are commonly affected by *Alternaria brassicicola*. The changes in germination and vigour of kohlrabi (*Brassica oleracea* L. var. *gongylodes* L.) seeds subjected to two priming methods in the presence of *A. brassicicola* were investigated. The following treatments were applied: uninoculated non-primed seeds (control), seeds inoculated with *A. brassicicola* ($2 \cdot 10^4$ spores ml^{-1}), uninoculated and inoculated seeds hydroprimed for 1–2 days, and uninoculated and inoculated seeds osmoprimed for 1–4 days. The percentage of germinating seeds, germination capacity, the percentages of diseased seedlings and dead seeds were determined. The speed of germination, tetrazolium staining index, and electrical conductivity described seed vigour. The presence of *A. brassicicola* on/in seeds was confirmed on CW semiselective agar. Osmopriming was conducive to penetration of *A. brassicicola* into kohlrabi seeds. The presence of the fungus had no influence on the percentage of germinating seeds, but a significantly lower germination capacity and a higher percentage of diseased seedlings and dead seeds were observed after inoculation. Non-primed inoculated seeds germinated faster than uninoculated ones. Despite the presence of *A. brassicicola*, osmotic priming improved seed vigour as measured by the speed of seed germination and electrical conductivity. The results of the tetrazolium vigour test showed its limited usefulness for seeds heavily infected with *A. brassicicola*.

Key words: *Brassica oleracea* var. *gongylodes*, seed enhancement, seed health, seed quality

INTRODUCTION

Priming is a widespread commercial tool used to improve seed performance over a range of environmental conditions. During this treatment seeds are exposed to restricted water availability under controlled conditions to allow some physiological processes of germination to occur, but germination itself is not completed [McDonald 2000].

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Priming influences germination rate and synchrony, percentage of seed germination, seedling vigour and growth [Pill 1995]. There are several techniques of seed priming. The simplest technique is hydropriming, which comprises soaking or misting seeds in water and redrying them before they complete germination. It minimizes the use of chemicals and avoids discarding materials that may be undesirable in the environment [McDonald 2000]. Osmotic priming consists of presoaking seeds in an osmotic solution, usually a salt or polyethylene glycol (PEG), in order to control their water uptake and prevent radicle protrusion. However, some researchers have observed an increase in seed infestation with fungi after seed priming [Biniek and Tylkowska 1987, Tylkowska and Biniek 1996, Nascimento and West 1998, Janas et al. 2000, Dorna et al. 2001, Tylkowska and van den Bulk 2001, Wright et al. 2003, Jensen et al. 2004].

Alternaria brassicicola (Schw.) Wiltshire is a very important seed transmitted pathogen of brassicas (*Brassica oleracea* L.) [Wu et al. 1979, Tylkowska and Bereśniewicz-Dudała 1988, Richardson 1990, Humpherson-Jones 1992, Sivapalan and Browning 1992]. Infected lots have been characterized by a low seed germination capacity, low seedling vigour, as well as pre- and post-emergence damping off. The fungus is mostly located on the seed surface [Tylkowska and Bereśniewicz-Dudała 1988].

The purpose of this study was to investigate the changes in germination and vigour of kohlrabi (*B. oleracea* L. var. *gongylodes* L.) seeds subjected to two priming methods in the presence of *A. brassicicola*.

MATERIALS AND METHODS

Seeds of kohlrabi infested with *A. brassicicola* to a low degree, were used in the study. The percentage of seeds infested with the fungus in a sample was only 5.0% and surface disinfection removed it.

The seeds were subjected to the following treatments:

- uninoculated seeds (control),
- seeds inoculated with *Alternaria brassicicola*,
- uninoculated seeds hydroprimed for 1 day (HPK 1),
- uninoculated seeds hydroprimed for 2 days (HPK 2),
- uninoculated seeds primed osmotically for 1 day (OPK 1),
- uninoculated seeds primed osmotically for 2 days (OPK 2),
- uninoculated seeds primed osmotically for 3 days (OPK 3),
- uninoculated seeds primed osmotically for 4 days (OPK 4),
- inoculated seeds hydroprimed for 1 day (HP 1),
- inoculated seeds hydroprimed for 2 days (HP 2),
- inoculated seeds primed osmotically for 1 day (OP 1),
- inoculated seeds primed osmotically for 2 days (OP 2),
- inoculated seeds primed osmotically for 3 days (OP 3),
- inoculated seeds primed osmotically for 4 days (OP 4).

Seeds in each treatment were at the beginning surface disinfected by rinsing with 70% ethyl alcohol followed by soaking in 1.5% solution of sodium hypochlorite

(NaOCl) for 3 min. and triple rinsing with sterile distilled water to remove the propagules of *A. brassicicola* from seed coat.

Disinfected seeds were inoculated with *A. brassicicola* by soaking in a suspension containing $2 \cdot 10^4$ spores in 1 ml. They were shaken (Laboratory shaker type 358S, Elpin+, Poland) at amplitude 3 and speed 150 rpm in the spore suspension (10 ml g^{-1}) for 10 min. After inoculation the seeds were drained off on a sieve, placed between blotting paper and dried in laminar air over night.

Uninoculated and inoculated seeds were hydroprimed or primed osmotically in polyethylene glycol 8000. For hydropriming, seeds were placed in 100 ml flasks and 500 μl of distilled water per 1 g of seeds was added. Then flasks were sealed with a parafilm and an aluminium foil and incubated in darkness at 20°C for 1 and 2 days. Afterwards, the seeds were placed in semi-open Petri dishes and dried back at 20°C and 45% relative humidity for 48 h to equilibrium moisture content. For osmotic priming, 50 seeds were placed in 9 cm diameter Petri dishes on 4 blotters moistened with 5 ml PEG 8000 solution of the osmotic potential of -1.5 MPa. The dishes were sealed with a parafilm and incubated for 1, 2, 3 and 4 days in darkness at 20°C. After priming, the seeds from each replicate were washed separately under the tap water for 5 min. and next rinsed three times in sterile water to remove PEG. Afterwards, the seeds were surface dried with blotting paper, placed in semi-open Petri dishes and dried back in the same way like after hydropriming.

The determination of seed moisture content was carried out on two replicates of 1 g from each treatment. The seeds were dried at 105°C for 5 h and the moisture content was calculated based on the difference between seed weight before and after drying.

The incidence of *A. brassicicola* was confirmed on 400 seeds from each treatment. They were placed on the surface of the CW semiselective agar medium [Wu and Chen 1999] in 9 cm diameter Petri dishes, 10 seeds per a dish, and then incubated at 24°C in darkness for 10 days. For the determination of inner infection before being placed on the medium, half of them was surface disinfected with 1% solution of sodium hypochlorite (NaOCl) for 10 min. followed by rinsing thoroughly with sterile distilled water and drying between blotting paper. Determination of the fungus was based on the appearance of its colonies and sporulation.

Germination test was conducted at 20°C in darkness on 4 replicates of 50 seeds from each treatment. After 10 days of incubation, the percentages of normal seedlings (germination capacity), diseased seedlings and dead seeds were evaluated [ISTA 2003a].

Calculation of speed of germination, tetrazolium test and electrical conductivity test were applied to evaluate seed vigour. Speed of germination was determined on 4 replicates of 50 seeds from each treatment. The seeds were incubated under the same conditions as described for germination test. Germinating seeds, i.e. showing a visible root protrusion through the seed coat, were counted daily until no new germs occurred and removed from Petri dishes. T_{50} (time to 50% of the total number of germinating seeds) and mean germination time values were calculated.

The tetrazolium test was conducted on 2 replicates of 50 seeds from each treatment. Evaluation of the appearance of embryos and the degree of their staining was made under stereoscopic microscope (Zeiss, Germany) at the magnification 32.5–50 \times [Hampton and TeKrony 1995, ISTA 2003b].

The TTC vigour index was calculated according to the following formula

$$I_{TTC} = [(nx1) + (nx2) + (nx3)] : N,$$

where:

I_{TTC} – TTC vigour index

1, 2, 3 – a degree of embryo staining

n – number of embryos belonging to a given degree of their staining

N – total number of analysed embryos

The higher the I_{TTC} value, the higher the vigour of seeds.

The integrity of cell membranes, determined by deteriorative biochemical changes and/or physical disruption, can be considered the fundamental cause of differences in seed vigour which are indirectly determined as electrolyte leakage during conductivity test [Hampton and TeKrony 1995]. In electrical conductivity test three replicates of 1 g seeds from each treatment were soaked in 20 ml of double-distilled water and incubated at 25°C for 4 h with occasional stirring. The supernatant was collected and electrical conductance of the seed leakage was measured using a conductivity meter (Radelkis, Hungary).

SeedCalculator version 2.1 software [Jalink and van der Schoor 1999] was applied to calculate speed of seed germination. All results regarding seed health, germination and vigour were evaluated by means of variance analysis followed by the Duncan's multiple range test.

RESULTS AND DISCUSSION

The moisture of hydroprimed seeds reached 37.6% for uninoculated and 37.3% for inoculated seeds (tab. 1). Osmotic priming resulted in an increase in moisture content up to 35.9 and 35.8% for uninoculated and inoculated seeds, respectively. Those moisture levels were sufficient to initiate germination processes [McDonald 2000].

The total level of seed infestation with *A. brassicicola* for inoculated non-primed seeds was much higher than that for inoculated primed seeds (fig. 1). Probably many fungal spores which started to germinate during hydro- and osmopriming did not survive two day period of drying after the treatments. Increased sensitivity of germinating spores is a common phenomenon associated with the shift from a dormant to an active stage of growth and the production of novel nucleic acids and proteins [Rotem 1998]. Moreover, a large portion of the externally located inoculum could be removed during seed washing after osmopriming. In case of inoculated non-primed seeds the pathogen was located only on their surface (fig. 1). A tendency to an increase in the internal seed infection was observed during osmopriming, whereas only a few hydroprimed seeds were found to be colonized by the fungus after their disinfection (fig. 1). It could be concluded that unlike hydropriming, osmotic priming was conducive to penetration of *A. brassicicola* into kohlrabi seeds. The similar phenomenon was observed previously for carrot seeds naturally infested with *A. alternata*, *A. dauci* and *A. radicina* [Tylkowska and van den Bulk 2001].

Table 1. Moisture content of the primed kohlrabi seeds

Tabela 1. Wilgotność kondycjonowanych nasion kalarepy

Treatment Traktowanie	Moisture content Wilgotność %
Uninoculated seeds (control) Nasiona nieinokulowane (kontrola)	6.1
Inoculated seeds Nasiona inokulowane	5.4
HPK ¹ 1	37.8
HPK ¹ 2	37.6
OPK ² 1	34.7
OPK ² 2	35.1
OPK ² 3	35.4
OPK ² 4	35.9
HP ³ 1	37.0
HP ³ 2	37.3
OP ⁴ 1	34.9
OP ⁴ 2	35.0
OP ⁴ 3	35.5
OP ⁴ 4	35.8

¹ uninoculated seeds hydroprimed for 1 and 2 days, respectively – nasiona nieinokulowane hydrokondycjonowane odpowiednio przez 1 i 2 dni

² uninoculated seeds primed osmotically for 1, 2, 3 and 4 days, respectively – nasiona nieinokulowane osmokondycjonowane odpowiednio przez 1, 2, 3 i 4 dni

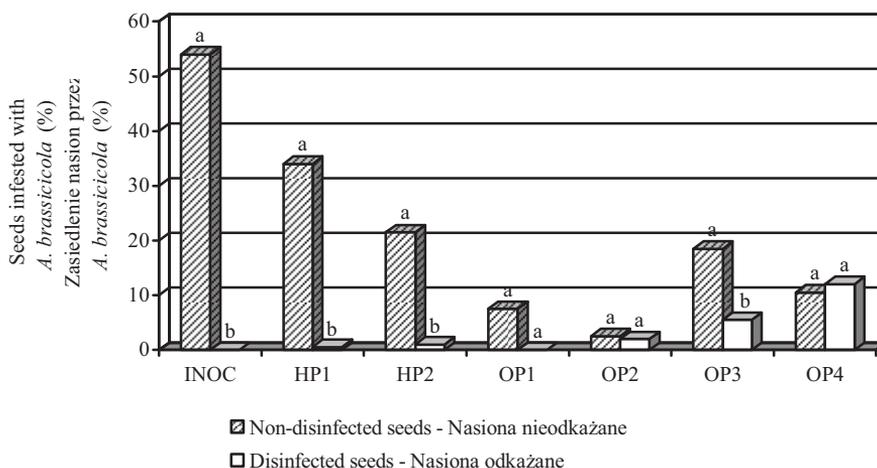
³ inoculated seeds hydroprimed for 1 and 2 days, respectively – nasiona inokulowane hydrokondycjonowane odpowiednio przez 1 i 2 dni

⁴ inoculated seeds primed osmotically for 1, 2, 3 and 4 days, respectively – nasiona inokulowane osmokondycjonowane odpowiednio przez 1, 2, 3 i 4 dni

Priming of uninoculated seeds affected neither the percentage of germinating seeds nor germination capacity nor the presence of diseased seedlings and dead seeds (tab. 2). The presence of *A. brassicicola* had no influence on the percentage of germinating seeds. On the contrary, a significantly lower germination capacity and a higher percentage of diseased seedlings and dead seeds were noticed after inoculation with the fungus. This may suggest that the presence of the fungus does not affect adversely the initiation of germination and the infection takes place at the later stages of this process as has been shown for carrot seeds inoculated with *A. radicina* [Tylkowska 1991]. The percentage of germinating seeds decreased after osmopriming of inoculated seeds for 4 days. This may be connected with a deeper location of *A. brassicicola* in the osmotically primed seeds. On the other hand, germination capacity increased as a result of osmotic priming applied to the seeds inoculated with *A. brassicicola*, whereas the percentage of diseased seedlings and dead seeds decreased. It can be explained by the considerable reduction in the total seed infestation after this treatment.

Seed moisture content after hydropriming was even higher than that after osmopriming, but in general the treatment did not improve seed vigour. The decrease in the electrical conductivity to a much lower degree as compared with that for osmotic priming was only observed (tab. 3). At the same time, osmopriming of uninoculated seeds im-

proved their vigour. After this treatment seeds germinated more rapidly and electrical conductivity decreased. As compared with non-primed inoculated seeds and osmo-primed seeds an increase in the TTC index was observed after 3–4 days osmotic priming inoculated seeds (tab. 3). Physiological basis of the TTC vigour test refers among other factors to the reduced staining in the tissues damaged by diseases [Powell 2005]. However, the presence of a fungus may lead to the difficulties in interpretation of the results because living mycelium also gets stained, and thus influences the I_{TTC} value.



INOC – inoculated non-primed seeds – INOC – nasiona inokulowane niekondycjonowane
 HP1, HP2 – inoculated seeds hydroprimed for 1 and 2 days, respectively – nasiona inokulowane hydrokondycjonowane odpowiednio przez 1 i 2 dni
 OP1, OP2, OP3, OP4 – inoculated seeds primed osmotically for 1, 2, 3 and 4 days, respectively – nasiona inokulowane osmokondycjonowane odpowiednio przez 1, 2, 3 i 4 dni
 For each treatment, the different letters indicate significant difference between values according to the Duncan's test at $\alpha = 0.05$ – Dla każdego sposobu traktowania, różnymi literami oznaczono wartości różniące się istotnie na poziomie $\alpha = 0,05$, według testu Duncana

Fig. 1. Effects of priming kohlrabi seeds inoculated with *Alternaria brassicicola* on the incidence of the fungus

Rys. 1. Wpływ kondycjonowania nasion kalarepy inokulowanych *Alternaria brassicicola* na występowanie tego grzyba

Priming operates to enhance seed quality by a combination of processes that may include cellular repair and improved membrane integrity, decreased seed exudation, enhanced mobilization of seed protein, lipid and starch as a result of activation or synthesis of key enzymes, osmotic adjustment and increase in radicle turgor, advanced embryo development and increased potential for oxidative phosphorylation and ATP accumulation [Pill 1995, McDonald 2000]. Lower electrical conductivity readings following osmo-priming could be a consequence of repairing processes taking place within seed cell membranes. In addition, these beneficial effects might be due to the rinsing removing solutes from the seeds during the priming procedure. As a practical result, primed seeds often perform better in disease-infested soils because of decreased electrolyte

leakage and faster germination rate, which both reduce the window of opportunity for fungal attack [Osburn and Schroth 1998].

Table 2. Effects of the kohlrabi seed priming on the germination parameters

Tabela 2. Wpływ kondycjonowania nasion kalarepy na parametry kiełkowania

Treatment Traktowanie	Germinating seeds Kielkujące nasiona %		Germination capacity Zdolność kiełkowania %		Diseased seedlings and dead seeds Siewki chore i nasiona martwe %	
Uninoculated seeds (control) Nasiona nieinokulowane (kontrola)	81.5	a-d	66.5	ab	9.5	ef
Inoculated seeds Nasiona inokulowane	84.0	a-c	41.5	e	46.5	a
HPK 1	71.5	d	65.0	ab	13.0	de
HPK 2	73.0	cd	58.0	a-d	14.0	de
OPK 1	87.5	a	66.0	ab	7.0	f
OPK 2	81.0	a-d	60.0	a-c	16.0	de
OPK 3	85.5	ab	69.0	a	8.0	ef
OPK 4	87.0	a	60.0	a-c	21.5	cd
HP 1	70.0	d	43.5	e	45.0	a
HP 2	69.5	d	51.0	c-e	36.5	ab
OP 1	75.0	b-d	48.5	de	29.5	bc
OP 2	79.0	a-d	61.0	a-c	23.5	b-d
OP 3	77.5	a-d	57.0	b-d	30.5	bc
OP 4	71.5	d	66.0	ab	28.5	bc

For the explanation see table 1 – objaśnienia podano pod tabelą 1

Means in columns followed by the same letters are not significantly different according to the Duncan's test at the level $\alpha = 0.05$ – Średnie w kolumnach oznaczone takimi samymi literami nie różnią się istotnie na poziomie $\alpha = 0,05$, według testu Duncana

Little is known about the physiological differences between primed and non-primed seeds and the processes occurring during different priming methods. Gallardo et al. [2001] found that priming of *Arabidopsis* seeds led to synthesis and degradation of different proteins that occur during germination. In their study they compared osmotic priming in PEG with imbibition in water. The abundance of certain heat shock proteins (HSPs) increased during osmopriming, whereas their level declined rapidly during hydropriming. The presence of these HSPs might ensure proper folding of other proteins because of their purported chaperone activity, and thus act in protecting the seeds. On the other hand, catalase activity increased, especially during hydropriming, presumably to alleviate oxidative stress occurring during germination.

Non-primed inoculated seeds germinated more rapidly than uninoculated ones (tab. 3). This might be a positive effect of mechanical and enzymatic activity of *A. brassicicola* as well as growth regulators produced by the fungus [Poapst et al. 1979, Suri and Mandahar 1984, Yao and Koller 1995, Berto et al. 1997, Bochenek et al. 2000]. Inoculation with *A. brassicicola* induced a reduction in seed vigour as measured by the TTC index. On the other hand inoculation with the fungus resulted in the de-

Table 3. Effects of kohlrabi seed priming on seed vigour

Tabela 3. Wpływ kondycjonowania nasion kalarepy na ich wigor

Treatment Traktowanie	Speed of germination Szybkość kiełkowania				TTC index TTC indeks		Electrical conductivity Przewodnictwo elektryczne $\mu\text{s}\cdot\text{g}^{-1}\cdot\text{cm}^{-1}$	
	T_{50} days – dni ¹		MGT days – dni ²					
	Uninoculated seeds (control) Nasiona nieinokulowane (kontrola)	1.60	ab	2.43	a	2.64	b	771.7
Inoculated seeds Nasiona inokulowane	1.26	b-d	1.81	bc	2.22	c	500.0	d
HPK 1	1.60	a-c	2.31	ab	2.63	b	656.7	b
HPK 2	1.82	a	2.55	a	2.64	b	616.7	c
OPK 1	1.36	b-d	1.98	bc	2.49	b	179.0	e
OPK 2	0.55	ef	1.38	cd	2.51	b	137.0	g
OPK 3	0.23	gh	0.94	d-f	2.63	b	137.0	g
OPK 4	0.38	h	0.95	d-f	2.65	b	125.0	g
HP 1	1.41	b-d	2.05	b	2.58	b	473.7	d
HP 2	1.22	cd	1.77	bc	2.24	c	500.0	d
OP 1	1.02	de	1.23	de	2.49	b	174.0	ef
OP 2	0.79	fg	1.15	de	2.65	b	134.0	g
OP 3	0.33	gh	0.93	ef	2.84	a	132.7	g
OP 4	0.15	h	0.58	f	2.68	ab	144.0	fg

¹ time to germination of 50% of the total number of germinating seeds – czas potrzebny do wykiełkowania 50% nasion z ogólnej liczby nasion kiełkujących

² mean germination time – średni czas kiełkowania

For the other explanations see table 1 and 2 – Objaśnienia podano pod tabelą 1 i 2

crease in electrical conductivity. This could be explained, at least partly, by the procedure applied before inoculation, i.e. soaking and rinsing the seeds, which might have effected in removing compounds possessing electrical conductivity properties.

CONCLUSIONS

1. Inoculation of kohlrabi seeds with *A. brassicicola* significantly decreased germination capacity and increased number of diseased seedlings and dead seeds, whereas had no effect on the percentage of germinating seeds.

2. Osmopriming was conducive to penetration of the fungus into kohlrabi seeds.

3. Despite the presence of *A. brassicicola*, osmotic priming improved seed vigour as measured by the speed of germination and electrical conductivity.

4. The results of the tetrazolium vigour test showed its limited usefulness for seeds heavily infected with *A. brassicicola*.

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KIEŁKOWANIE I WIGOR NASION KALAREPY PODDANYCH KONDYCJONOWANIU W OBECNOŚCI *Alternaria brassicicola* (Schw.) Wiltshire

Streszczenie. Kondycjonowanie nasion jest zabiegiem poprawiającym ich jakość. Nasiona warzyw kapustnych są powszechnie zasiedlone przez grzyb *Alternaria brassicicola*. W pracy analizowano wpływ dwóch metod kondycjonowania na kiełkowanie i wigor nasion kalarepy (*Brassica oleracea* L. var. *gongylodes* L.) inokulowanych *A. brassicicola*. Badano nasiona nieinokulowane i niekondycjonowane (kontrola), nasiona inokulowane *A. brassicicola* ($2 \cdot 10^4$ zarodników ml^{-1}), nasiona nieinokulowane i inokulowane hydrokondycjonowane przez 1 i 2 dni oraz nasiona nieinokulowane i inokulowane osmokondycjonowane przez 1–4 dni. Określano liczbę nasion kiełkujących, zdolność kiełkowania oraz procent siewek chorych i nasion martwych. Wigor nasion oceniano na podstawie szybkości ich kiełkowania, testu tetrazolinowego oraz testu konduktometrycznego. Zasiedlenie nasion przez *A. brassicicola* badano na półselektywnej agarowej pożywce CW. Osmokondycjonowanie sprzyjało penetracji nasion przez patogen. Obecność grzyba w/nasionach nie miała wpływu na procent kiełkujących nasion, ale znacząco zmniejszała ich zdolność kiełkowania, zwiększając jednocześnie liczbę siewek chorych i nasion martwych. Pomimo obecności patogena kondycjonowanie osmotyczne poprawiało szybkość kiełkowania nasion i wyniki testu konduktometrycznego. Wyniki testu tetrazolinowego wykazały jego ograniczoną przydatność do badania wigoru nasion porażonych w dużym stopniu przez *A. brassicicola*.

Słowa kluczowe: *Brassica oleracea* var. *gongylodes*, uszlachetnianie nasion, zdrowotność nasion, jakość nasion