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EFFECT OF OSMOPRIMING ON GERMINATION, VIGOUR AND LOCATION OF FUNGI IN *ZINNIA ELEGANS* SEEDS

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Abstract

Seven samples of naturally infected zinnia (*Zinnia elegans*) seeds (achene), unprimed and osmoprimed in polyethylene glycol (PEG) solution, were studied using germination and vigour tests. The tests were performed for disinfected and non-disinfected seeds. Location of inoculum in the seeds was determined with two histopathological methods: mycological analysis of non-disinfected and disinfected seeds and component plating. Priming improved germination capacity and decreased the percentage of diseased seedlings. The treatment significantly improved seed vigour. Total seed infestation increased after priming, both in non-disinfected and disinfected seeds. Before priming fungi mainly infested outer layers of seeds, but inner infection was higher after priming.

Key words: fungi, location of inoculum, osmopriming, seed germination, seed vigour, zinnia seeds

Introduction

For the economic production of plants, especially in cellular plates, high level of uniform seedling emergence is essential. This can be achieved only by using high quality seeds, i.e. those which germinate quickly and uniformly, are not susceptible to climatic and storage stresses and give highly vigorous seedlings. Osmotic priming of seeds has been shown to be an effective method to improve seed quality by increasing speed and uniformity of germination (Bradford 1986). Polyethylene glycol (PEG) of 8000 molecular weight is a commonly used seed priming compound. However, a side effect, i.e. an increased seed infestation with fungi has been observed after priming in PEG solution (Nascimento and West 1998, Tylkowska and Biniek 1996, Tylkowska and van den Bulk 2001, Zhao et al. 2004).

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Zinnia (*Zinnia elegans*), an annual ornamental plant, has been cultivated commonly worldwide for cut flowers and flowerbeds. According to many authors *Alternaria zinniae* seems most important fungal seed-borne pathogen of zinnia plants, causing spotting of the petals, foliage and stems, and rotting of the roots (Dimock and Osborn 1943, Richardson 1990, Łacicowa et al. 1991, Palacios et al. 1991, Wu and Yang 1992). Łacicowa et al. (1991) reported that zinnia seeds produced in Poland were commonly infested with *A. alternata*, *A. zinniae*, *Botrytis cinerea*, *Fusarium* spp. and *Penicillium* spp. Prior experiment of the senior author (Łacicowa et al. 1979) showed that *A. zinniae*, *Fusarium culmorum*, *F. solani*, *F. oxysporum* and *Sclerotinia sclerotiorum* were in most cases responsible for severe damages of zinnia plants in the field.

Viability of pathogens in seeds depends on many factors such as host plant species, morphology of seed and stage of its development, type, amount and location of inoculum, presence of antagonistic microorganisms and storage conditions (Agarwal and Sinclair 1987). Most of the seed-borne species may be transmitted on the surface of the seeds, e.g. *A. alternata*, *Bipolaris sorokiniana, F. avenaceum*, *F. culmorum*, *Stemphylium botryosum* and *S. consortiale* (Neergaard 1977). Gambogi et al. (1976), studying behaviour of seed-borne *A. zinniae*, found that superficial seed infestation usually caused diseases on plants after emergence, however, deeply seated fungi could affect the true seed, leading to pre-emergence death of seedlings.

Location of inoculum in the seed may be determined with several methods, but comparison of percentage of non-disinfected and disinfected seeds and component plating are applied most commonly (Maden et al. 1975, Mathur et al. 1975, Sinha and Khare 1977, Ranganathaiah and Mathur 1978, Singh 1983, Singh et al. 1993).

The experiments were conducted to study the influence of osmopriming on germination, vigour and location of pathogenic and saprotrophic fungi in zinnia seeds.

Materials and methods

Experiments were carried out on seven samples of zinnia seeds, cv. 'Golden Dawn' (330196/289 – sample I), cv. 'Jowita' (530/64/13135/149 – sample II), cv. 'Kirke' (530/64/13135/139 – sample III), cv. 'Orys' (430/64/3433/799 – sample IV), cv. 'Red Man' (330/196/225 – sample V), cv. 'Scarlet Flame' (530/64/13135/122 – sample VI), and cv. 'Talia' (530/64/1315/129 – sample VII), obtained from Seed Company (CNOS Przedsiębiorstwo Nasiennictwa Ogrodniczego) in Poznań.

Osmopriming

Seeds were primed for five days in darkness at 20°C. Fifty seeds were placed in each 9-cm-diameter Petri dish on four layers of blotter moistened with 5 ml of polyethylene glycol (PEG 8000, Sigma-Aldrich Co.) solution of osmotic potential of –1.0 MPa. To obtain the solution, 284 g of PEG was dissolved in 1 kg of sterile water (Michel and Kaufmann 1973). The Petri dishes were sealed with parafilm.

After priming, seeds from each replicate were washed separately under tap water and rinsed three times in sterile distilled water to remove PEG. Then, they were surface dried with blotting paper and placed in semi-open Petri dishes at 20°C and 45% relative humidity for 24 h to equilibrium moisture content.

Seeds germination and vigour

Primed and unprimed seeds of each sample were surface sterilised with 1% aqueous solution of sodium hypochlorite (NaClO) for 10 min, followed by three rinses in sterile distilled water and drying with sterile blotting paper. Six replicates of 50 seeds from each treatment were placed in Petri dishes containing six layers of moistened blotters and incubated in the darkness, at 20°C. Percentage of normal seedlings (germination capacity at the first and final count) and abnormal seedlings (deformed and diseased) were determined after four and 10 days according to the ISTA rules (International… 2006).

To characterize seed vigour, six replicates of 50 seeds were incubated under the same condition as in the previous test. Radicle protrusion was scored daily for 10 days. The germination rates, characterising seed vigour, i.e.: T_1 – time to 1% of total number of germinating seeds (G_{max}), MGT – mean germination time, and $U_{75–25}$ – time between 25% and 75% of G_{max}, were evaluated using statistical program SeedCalculator 2.1 (Jalink and van der Schoor 1999).

Location of fungi in seeds

Two separate experiments were performed to determine the location of fungi in unprimed and primed zinnia seeds.

Mycological analysis of non-disinfected and disinfected seeds

Osmoprimed and unprimed seeds of each sample were disinfected with 1% aqueous solution of NaClO for 10 min, and then three times rinsed with sterile distilled water and dried with sterile blotting paper. Two hundred seeds (20 seeds per Petri dish) from each treatment were tested with the standard deep-freeze blotter method. The seeds were incubated for 24 h at 20°C in darkness, then transferred to –20°C for 20 h and subsequently incubated at 20°C under alternating cycles of 12 h NUV light and 12 h darkness for eight days. The fungi were identified on the basis of their growth and sporulation using stereomicroscope and a compound microscope (Machado et al. 2002, Mathur and Kongsdal 2003). The same analysis was performed for non-disinfected seeds. Location of fungi was determined on the ground of differences in their incidence on disinfected and non-disinfected seeds.

Component plating

For disinfection osmoprimed and unprimed seeds of each sample were soaked with 1% aqueous solution of NaClO for 10 min, and then three times rinsed with

sterile distilled water. From each sample 100 unprimed and 100 primed seeds were tested. Each seed was dissected aseptically under a stereomicroscope and all components, i.e: pericarp, integuments with endosperm and embryo were placed on potato dextrose agar medium (PDA, Scherlau Chemie, Spain) in 9 cm diameter Petri dish, two sectioned seeds per dish. Streptomycine at 100 ppm was added to the medium to prevent the development of bacteria. Petri dishes were placed at 20°C under alternating cycles of 12 h NUV light and 12 h darkness for 10 days. The fungi grown around separate parts of seeds were identified on the basis of their growth and sporulation visible under a stereomicroscope and a compound microscope (Machado et al. 2002, Mathur and Kongsdal 2003).

Statistical analysis

The results obtained were evaluated by analysis of variance followed by Duncan's multiple range test (Kala 2002).

Results

Seed germination and vigour

The results obtained varied depending on the treatment method and tested sample. Significant differences were observed in germination capacity of non-disinfected seeds of samples II, III, V and VI, which increased after priming. Disinfection of primed seeds lowered the germination capacity of samples II, V and VII, and increased the number of deformed seedlings in samples II, V and VII. At the same time, the treatment increase germination capacity of sample I and II. Seed priming in most samples decreased significantly percentage of diseased seedlings. However, this effect was observed after disinfection only in sample I (Table 1).

Seed priming considerably affected speed of germination, regardless of sodium hypochlorite treatment. Generally, T1 and MGT values were lower for osmoprimed seeds than unprimed seeds, even if the observed differences were not statistically significant. Disinfection of primed seeds had positive effect on uniformity of germination ($U_{75–25}$). However, after priming, non-disinfected seeds of sample I and III germinated less uniformly than unprimed seeds (Table 2).

Mycological analysis of non-disinfected and disinfected seeds

The following fungi were identified in tested seeds: *Acremoniella atra*, *Acremonium strictum*, *Alternaria alternata*, *A. zinniae*, *Aspergillus niger*, *Botrytis cinerea*, *Cladosporium* spp., *Colletotrichum* spp., *Epicoccum purpurascens*, *Fusarium* spp., *Gonatobotrys simplex*, *Mucor* sp., *Papulaspora* sp., *Penicillium* spp., *Phoma* sp., *Rhizopus nigricans*, *Trichothecium roseum* and *Ulocladium* spp. Among them *A. alternata*, *A. zinniae*, *Cladosporium* spp. and *Fusarium* spp. were occurring the most frequently.

Table 1

Effects of priming and disinfection on seed germination (%)

Sample	Non-disinfected seeds		Disinfected seeds	
	unprimed seeds	primed seeds	unprimed seeds	primed seeds
Germination capacity at first count				
I	21.7 a	26.3 a	27.3 a	43.3 b
II	36.0 a	49.7 b	15.3 a	27.0 a
III	24.0 a	52.3 b	11.7 a	19.7 b
IV	26.0 a	26.0 a	20.0 b	10.7 a
V	17.3 a	40.7 b	21.3 a	28.0 a
VI	25.0 a	44.3 b	25.7 a	30.7 a
VII	32.0 a	38.0 a	23.3 b	14.3 a
Germination capacity at final count				
I	22.7 a	26.3 a	34.3 a	44.3 b
II	37.7 a	52.0 b	22.0 a	27.3 b
III	29.0 a	54.3 b	31.0 a	29.7 a
IV	28.3 a	34.3 a	25.7 b	14.0 a
V	41.3 a	53.7 b	69.3 b	50.3 a
VI	26.0 a	45.7 b	34.3 a	32.3 a
VII	41.3 a	46.3 a	40.7 b	24.0 a
Deformed seedlings				
I	0 a	1.0 a	0.7 a	4.0 a
II	27.3 b	2.3 a	0.3 a	10.7 b
III	0 a	2.0 a	3.3 a	7.7 a
IV	0 a	3.2 b	0.3 a	3.7 a
V	11.7 a	18.0 b	11.7 a	31.7 b
VI	0 a	4.3 a	1.0 a	3.0 a
VII	4.0 a	4.3 a	2.7 a	20.0 b
Diseased seedlings				
I	70.7 b	49.7 a	56.7 b	44.7 a
II	25.0 a	36.7 a	63.3 a	49.3 a
III	65.3 b	37.7 a	54.7 a	52.3 a
IV	61.0 b	48.3 a	56.3 a	60.0 a
V	33.7 b	24.0 a	8.0 a	14.3 a
VI	63.7 b	36.7 a	56.0 a	56.7 a
VII	44.3 b	27.7 a	43.3 a	42.0 a

Means in the same row, separately for non-disinfected and disinfected seeds, followed by the same letter are not significantly different at $\alpha = 0.05$ level according to Duncan's multiple range test.

Table 2

Effects of priming and disinfection on seed vigour (days)

Sample	Non-disinfected seeds		Disinfected seeds	
	unprimed seeds	primed seeds	unprimed seeds	primed seeds
		T_1		
I	0.69 b	0.01 a	0.71 b	0.00 a
II	0.75 a	0.23 a	0.06 a	0.00 a
III	0.75 b	0.16 a	0.68 b	0.09 a
IV	0.70 b	0.12 a	0.19 a	0.04 a
V	0.44 a	0.15 a	0.35 b	0.00 a
VI	0.46 b	0.07 a	0.41 b	0.15 a
VII	0.81 b	0.00 a	0.63 b	0.00 a
		MGT		
I	1.52 b	1.00 a	1.72 b	0.33 a
II	1.22 b	0.98 a	1.20 b	0.35 a
III	1.86 b	1.56 a	2.08 b	0.63 a
IV	1.50 b	0.97 a	1.74 b	0.69 a
V	0.92 a	1.52 a	1.41 b	0.41 a
VI	1.33 b	0.71 a	1.62 b	0.66 a
VII	1.42 b	0.37 a	1.31 b	0.36 a
		U_{75-25}		
I	0.61 a	1.10 b	0.96 b	0.42 a
II	0.34 a	0.60 a	1.10 b	0.49 a
III	0.77 a	1.22 b	1.17 b	0.83 a
IV	0.68 a	0.73 a	1.33 b	0.61 a
V	1.65 a	1.21 a	0.82 b	0.54 a
VI	0.61 a	0.56 a	0.97 a	0.41 a
VII	0.46 a	0.50 a	0.54 a	0.39 a

T_1 – time to 1% of total number of germinating seeds, MGT – mean germination time, U_{75-25} – time between 25% and 75% of total number of germinating seeds.

Means in the same row, separately for non-disinfected and disinfected seeds, followed by the same letter are not significantly different at $\alpha = 0.05$ level according to Duncan's multiple range test.

Infestation of the seeds with these fungi was usually higher after priming than before the treatment (Table 3). The percentage of seeds infested with *A. alternata* increase significantly in samples I, IV, V and VII. Remarkable increase of *A. zinniae* seed infection was observed in samples III, V and VI. Only in sample II, infection of seeds with the pathogen decreased significantly from 63.5% to 47.5%. In samples I, II, VI and VII, the percentage of seeds infested with *Cladosporium* spp. increased after priming. Significant increase of *Fusarium* spp. seed infestation was observed in six from seven tested samples.

Table 3

Effects of priming and disinfection on the incidence of fungi (%)

Sample	Non-disinfected seeds		Disinfected seeds	
	unprimed seeds	primed seeds	unprimed seeds	primed seeds
Alternaria alternata				
I	49.0 a	63.0 b	48.0 a	50.5 a
II	62.5 a	66.0 a	54.5 a	58.0 a
III	84.0 a	83.0 a	74.0 a	72.5 a
IV	57.5 a	67.5 b	45.5 a	57.5 b
V	79.0 a	89.0 b	64.5 a	80.5 b
VI	66.0 a	81.5 a	62.5 a	65.5 a
VII	71.5 a	84.5 b	50.5 a	61.0 a
Alternaria zinniae				
I	64.0 a	71.5 a	46.0 a	63.5 a
II	63.5 b	47.5 a	42.5 a	42.5 a
III	47.5 a	58.5 b	34.5 a	52.5 b
IV	59.5 a	55.0 a	46.5 a	47.5 a
V	0.5 a	10.5 b	0.5 a	7.5 b
VI	45.0 a	56.5 b	40.0 a	50.5 b
VII	14.5 a	14.0 a	10.0 a	12.5 a
Cladosporium spp.				
I	0 a	2.5 b	0 a	3.0 b
II	20.0 a	41.5 b	9.5 a	9.0 a
III	47.5 a	45.0 a	38.5 b	17.0 a
IV	35.0 a	32.5 a	2.5 a	13.5 b
V	9.0 a	7.0 a	4.0 a	1.0 a
VI	29.5 a	53.0 b	25.0 a	16.5 a
VII	34.0 a	61.0 b	27.0 a	18.5 a
Fusarium spp.				
I	25.5 a	44.0 a	9.5 a	20.0 b
II	51.5 a	73.5 b	50.5 a	70.0 b
III	19.0 a	79.5 b	14.0 a	40.5 b
IV	63.5 a	87.5 b	33.5 a	44.0 b
V	12.0 a	64.0 b	9.0 a	47.0 b
VI	40.5 a	68.5 b	31.5 a	20.5 a
VII	68.0 a	83.0 b	53.0 a	56.0 a

Means in the same row, separately for non-disinfected and disinfected seeds, followed by the same letter are not significantly different at $\alpha = 0.05$ level according to Duncan's multiple range test.

Surface disinfection caused decrease in seed infestation. At the same time, negative effect of priming on seed health was detected in most cases. Statistically significant increase of seed infestation with *A. alternata* was noted in samples IV and V, with *A. zinniae* in samples III, V and VI, with *Cladosporium* spp. in samples I and IV, and with *Fusarium* spp. in samples I–V. In sample III, the percentages of seeds infested with *Cladosporium* spp. decreased after priming from 38.5% to 17%.

Summing up, total external and internal infestation of seeds with both pathogenic and saprotrophic fungi increase after priming.

Component plating

After mycological analysis of particular parts of unprimed seeds, both pathogenic and saprotrophic fungi were detected in higher percentage in outer layers of the seeds, mainly in pericarp, integument and endosperm. After priming all the fungi penetrated inner tissues, especially embryo. In embryos of unprimed seeds of samples II and V *A. zinniae* was not detected, but after priming the pathogen was observed in 4% (sample II) and 1% (sample V) of embryos. *Cladosporium* spp. were not present in embryos of unprimed seeds of samples III, V and VI, but were detected in 33%, 2% and 7% of embryos of primed seeds, respectively. In embryos of unprimed seeds of samples I, V and VI *Fusarium* spp. were not detected, although after osmopriming 5% (sample I) and 9% (samples V and VI) embryos infestation with these fungi was observed (Table 4).

Total infestation of seeds after priming increased from 70–96% to 99–100% in tested samples (Table 5).

Discussion

The effects of osmopriming on germination, vigour and location of fungi in zinnia seeds were determined in present study.

The results obtained varied depending on the seed lot and treatment. In general osmopriming improved germination of the seeds but disinfection many a time negatively influenced this parameter.

Low germination capacity was observed for all tested seed lots. It seems possible that it was connected with seed infestation with fungi. Wu and Yang (1992) reported that *A. zinniae* can restrain germination of zinnia seeds. In general, osmopriming improved the parameter, however, after disinfection a decrease of germination capacity was frequently noted.

Bradford (1986) described osmopriming as an effective method to improve seed quality by increasing speed and uniformity of germination. That took confirmation in presented results, which showed that zinnia seed priming had positive effect on germination speed of both disinfected and non-disinfected seeds, and uniformity of germination of disinfected seeds. Nevertheless, both total and inner infection (particularly infection of the embryo) of zinnia seeds increased as a result of priming. Re-

Table 4

Recovery of fungi from different seed parts before and after osmopriming (%)

Sample	Unprimed seeds			Primed seeds		
	pericarp	integument + endosperm	embryo	pericarp	integument + endosperm	embryo
Alternaria alternata						
I	11.0	26.0	1.0	64.0	55.0	28.0
II	36.0	33.0	5.0	64.0	17.0	10.0
III	28.0	24.0	2.0	39.0	28.0	28.0
IV	31.0	29.0	1.0	18.0	22.0	8.0
V	13.0	24.0	4.0	70.0	61.0	23.0
VI	20.0	17.0	2.0	38.0	34.0	20.0
VII	27.0	20.0	1.0	43.0	49.0	30.0
Alternaria zinniae						
I	29.0	31.0	2.0	41.0	27.0	16.0
II	36.0	19.0	0	33.0	21.0	4.0
III	23.0	20.0	4.0	32.0	24.0	23.0
IV	54.0	37.0	5.0	43.0	24.0	11.0
V	0	0	0	1.0	1.0	1.0
VI	43.0	34.0	8.0	17.0	24.0	13.0
VII	9.0	7.0	1.0	6.0	7.0	1.0
Cladosporium spp.						
I	0	0	0	1.0	2.0	0
II	9.0	4.0	2.0	25.0	22.0	11.0
III	0	1.0	0	15.0	13.0	33.0
IV	4.0	4.0	1.0	11.0	7.0	2.0
V	0	0	0	1.0	1.0	2.0
VI	4.0	1.0	0	8.0	3.0	7.0
VII	8.0	14.0	3.0	33.0	24.0	29.0
Fusarium spp.						
I	1.0	1.0	0	15.0	16.0	5.0
II	24.0	18.0	11.0	37.0	27.0	12.0
III	10.0	5.0	3.0	27.0	22.0	21.0
IV	7.0	8.0	2.0	35.0	33.0	14.0
V	3.0	3.0	0	3.0	27.0	9.0
VI	7.0	4.0	0	36.0	25.0	9.0
VII	12.0	16.0	4.0	40.0	50.0	23.0

Table 5

Total number of seeds infested with fungi before and after priming

Sample	Unprimed seeds	Primed seeds
I	71.0	100.0
II	96.0	99.0
III	70.0	99.0
IV	87.0	100.0
V	81.0	100.0
VI	90.0	100.0
VII	95.0	100.0

sults indicating possibilities of the increased seed infestation after priming were obtained by Tylkowska and Biniek (1996) and Tylkowska and van den Bulk (2001). These observations do not correspond with those obtained by Szopińska and Tylkowska (2003), which noted that priming did not affect total lettuce seed infestation, although the inner layers of seeds were infested to a higher degree after treatment. The authors observed that *A. alternata*, *B. cinerea* and *Cladosporium* spp. showed tendency to grow inside lettuce seed after priming. Tylkowska and van den Bulk (2001) obtained similar results for carrot seeds. They found that after osmopriming inner infection of the seed with *Alternaria* spp. increased.

Mycological analysis of disinfected and non-disinfected seeds gave only general information about inner seed infection, assuming that fungi present in non-disinfected seeds and absent in disinfected seeds, contaminated their surface and did not penetrate inner tissues. This information, although not very precise, can be a starting point to work out the proper strategies of seed treatment.

It seems crucial for minimising process of seed infestation by fungi during priming, that the high quality, i.e. healthy seeds should be primed first of all. Since, healthiness of seeds destined to priming is not tested commonly, additional – chemical or biological – seed treatment should be recommended to prevent development and spread of seed infection. The effects of chlorine pre-treatment, which partially removed the contaminating fungi, suggest that an adequate seed treatment may improve the quality of seeds, especially characterised with high superficial infestation. However, on the basis of obtained results, it is necessary to take into consideration the increased sensitivity of primed seeds to chemicals, to avoid a phytotoxic effect.

Streszczenie

WPŁYW OSMOKONDYCJONOWANIA NA KIEŁKOWANIE I WIGOR NASION *ZINNIA ELEGANS* ORAZ UMIEJSCOWIENIE W NICH GRZYBÓW

Nasiona (niełupki) siedmiu prób cynii (*Zinnia elegans*) poddano kondycjonowaniu w roztworze glikolu polietylenowego (PEG 8000) o potencjale osmotycznym

–1,0 MPa przez pięć dni w ciemności w temperaturze 20°C. Określano kiełkowanie i wigor nasion zarówno poddanych, jak i nie poddanych kondycjonowaniu. Testy wykonano na nasionach odkażanych w 1-procentowym wodnym roztworze podchlorynu sodowego i na nasionach nieodkażanych. Lokalizację inokulum w nasionach określano za pomocą dwóch metod histopatologicznych: analizy mikologicznej nasion nieodkażanych i odkażanych oraz analizy grzybów wyrosłych na poszczególnych częściach nasion wyszczepionych na pożywkę dekstrozowo-ziemniaczaną (PDA). Kondycjonowanie poprawiało zdolność kiełkowania nasion i zmniejszało liczbę siewek chorych, zwłaszcza w próbach nieodkażanych. Traktowanie znacząco poprawiało szybkość kiełkowania nasion nieodkażanych i odkażanych oraz zwiększało równomierność kiełkowania nasion odkażanych. Ogólne zasiedlenie nasion przez grzyby zwiększało się po kondycjonowaniu, zarówno nasion nieodkażanych, jak i odkażanych. Bez względu na traktowanie, grzyby głównie zasiedlały zewnętrzne warstwy nasion, tj. owocnię, osłonkę i bielmo, ale zasiedlenie wewnętrzne (zarodka) było większe po kondycjonowaniu.

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