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Influence of fungi associated with watermelon seeds on physiological and health quality

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Abstract

Crop cultivation begins with direct sowing or seedlings production; it is important to use high quality seeds, highlighting physiological and health attributes. This study aimed to evaluate the physiological and health quality of watermelon seeds using different methodologies. We used four lots of untreated watermelon seeds of Crimson Sweet plant variety produced in the same year. Physiological performance was evaluated by a standard germination test, first count of germination, accelerated aging, electrical conductivity of seeds (Ec) and seedling emergence (S). Health quality was evaluated by a blotter method test where seeds were planted on well water-soaked blotters (filter papers), and incubated for seven days at 22ºC under 12h alternating cycles of light and darkness; seedling symptom in paper roll test where seeds were planted such as a standard germination test, and incubated for seven days at 22ºC under 12h alternating cycles of light and darkness; seedling symptom in autoclaved sand carried out with the seedlings' emergence. First count of germination and electrical conductivity of seeds showed the lower quality of lots 1 and 2 in relation to the other lots, which were evaluated as similar in seedlings' emergence. Identification of seedlings' symptoms in paper roll and sand was not sensitive enough to quantify *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. presence in watermelon seeds. Electrical conductivity and first count of germination tests allow the evaluation of physiological performance of watermelon seeds properly, based on the correlation value (+/- 1). The blotter test is the most efficient test to evaluate health quality of watermelon seeds. *Aspergillus* sp. incidence is correlated with low physiological performance of watermelon seeds.

Keywords: *Aspergillus* sp.; *Citrullus lanatus*; physiological potential; seed pathology; seed viability.
Abbreviations: AA_accelerated aging; AS_abnormal seedlings; ASAA_abnormal seedlings in the accelerated aging test; ASG_abnormal seedlings in the standard germination test; DS_dead seeds; DSAA_dead seeds in the accelerated aging test; DSG_dead seeds in the standard germination test; DSSE_dead seeds on seedlings' emergence; EC_electrical conductivity; ESI_emergence speed index; FAO_Organization Agricultural foundation; FCG_first count of germination; G_standard germination; MC_moisture content; MCAA_water content after AA; NS_normal seedlings; NSAA_normal seedlings in the accelerated aging test; SE_Seedlings' emergence; SSPR_symptoms in seedlings in paper roll; SSS_symptoms in seedlings in sand.

Introduction

Watermelon (*Citrullus lanatus* Thumb) is a member of the cucurbit family (*Cucurbitaceae)*, one of the main vegetable fruits propagated by seeds. According to the Fao data, in 2013 the watermelon area harvested was 3,490,000 hectares, a yield of about 31 tonnes per hectare and a seed production of about 28,700 tonnes, representing approximately 40% of cucurbit crop production worldwide (Fao, 2015). Watermelon growing starts with direct sowing in the field or by seedlings' production. In both cases, propagation by seeds or seedlings, the use of high quality seeds is essential, highlighting physiological and health attributes. In Brazil, most of the watermelon growing is done by small farmers (Ibge, 2012), the importance of commercialization of healthy seeds that will provide a suitable stand in the field is emphasized. Seeds with high physiological potential are necessary to obtain a fast and uniform germination because the seeds' vigor influences the plants' initial development (Casaroli et al., 2009). Information on seed vigor is very important, particularly for expensive seeds, such as vegetables (Abdo et al., 2005). However, to ensure a suitable initial plant stand, seeds with high health quality are needed in addition to high physiological potential. A direct relationship between the incidence of pathogens on seeds and loss of germination capacity and seed vigor, may exist, affecting plant establishment in the field. Vegetable crops are very vulnerable to the occurrence of diseases. Therefore, workable production of these species depends on high physiological and health seed quality. The diseases initially present in the seed may give rise to progressive disease development in the field and reduce the commercial value of the crop; because of this, ensuring seed health is important (Fao, 2010). Consequently, the aim of this study was to evaluate the physiological and health quality of watermelon seeds, using different methodologies and to evaluate the influence of fungi associated with seeds in germination and early seedlings' performance of watermelon.

Results and Discussion

Physiological quality of four untreated watermelon seed lots

In the germination test, it was possible to identify lot 1 as the least vigorous of the evaluated lots. The percentages of normal seedlings were 97, 95 and 93%, respectively, for lots

3, 2 and 4 (Table 1). Lot 1 had the lowest germination percentage of 84%, and differed from the other lots. The percentage of dead seeds in the germination test was the highest in lot 1 with 13% different from the other lots, which did not differ from one another (Table 1). There were no differences in abnormal seedlings' percentages for germination and AA tests, and the percentages of dead seeds in the AA test (Table 1).

In the AA test, lot 2 had a higher percentage of germination after the stress period, with 77% germination, followed by lots 3, 1 and 2 with 72, 69 and 62% of normal seedlings, respectively (Table 1). These results disagree with Bhering et al. (2003), who concluded that the AA test, conducted at 41 °C for 48 h and 100% RH, was effective for the ranking of watermelon seed lots. However, in this study, the ranking was not according to the results of seedling emergence. The initial moisture content of the seeds and its water content after the AA test showed little variation, less than 2% between lots, within the limits suggested by Tekrony (2003) and Marcos Filho (2005) for performing the test (Table 1).

The FCG test made it possible to detect vigor differences among the evaluated lots (Table 2). Lot 3 with 80% germination had the best result, followed by lot 4 with 77% germination, lot 2 with 74% germination and lot 1 with 59% germination (Table 2). According to Almeida et al. (2010), FCG could be used to obtain preliminary information about seed lots' vigor by the ease of implementation. By SE, it was not possible to detect differences between lots 3 and 4, high vigor lots that showed 76 and 82% SE, respectively (Table 2); however, these lots differed from lot 2, of medium vigor, with 67% SE. Lot 1, classified as low vigor, had 47% SE, very different from the other evaluated lots (Table 2).

The ESI of lot 1 was lower than for the other lots analyzed (Table 2), an expected result due to the low vigor lot verified for germination, AA, FCG and SE. According to the EC test result, it was possible to separate the lots into four levels of vigor, considering lot 4 as the most vigorous and lot 1 the least vigorous (Table 2). After reaching the point of physiological maturity, the seed goes to a low moisture content condition, which may vary according to environmental conditions, especially relative humidity. During the drying process, seeds may undergo a structural disruption and thereby form in lower water content with higher cell disruption, which may result in loss of integrity of cell membranes.

According to Panobianco et al. (2007), the integrity of cell membranes varies according to the degree of deteriorative biochemical or physical damage that may be considered as a fundamental cause of changes in the level of seed vigor, which may be directly evaluated considering the EC measurements in the soaked seed solution. Similar results were obtained by Vieira and Dutra (2006) for squash, Almeida et al. (2010) for watermelon and Abdo et al. (2005) for cucumber. In agreement with these authors, the EC test has potential to be used as a vigor indicator for the above-mentioned species. According to Table 3, SE was positively correlated with the germination test, FCG and ESI. SE had a high and negative correlation with the EC test, it means the greater the SE, the smaller were the read values of leachate (Table 3). The EC test showed negative significant correlations also with germination, FCG and ESI (Table 3).

These results confirm the potential of the EC test to evaluate watermelon seed vigor. Only the AA test was not correlated with the other assessment tests of physiological watermelon seed quality.

Health quality of four untreated watermelon seed lots

Health analysis showed a high incidence of different fungi associated with the seeds: *Aspergillus* sp. and *Penicillium* sp. (Table 4). These genera are known as storage fungi, causing rot and reduction in the seeds' viability and longevity (Farias and Lucca-Filho, 2012). The incidence of *Aspergillus* sp. was 99% in lot 1 and 81% in lot 4, and incidence of *Penicillium* sp. was 83% in lot 3 (Table 4). It is important to note that in the standard germination and AA test, lot 1 had lower results than the other lots evaluated (Table 1). However, fungi known to be seed contaminants, such as *Cladosporium* sp. and *Rhizopus* sp., showed 10% or lower incidence in the evaluated lots (Table 4).

In the health quality of seeds, analysis by the blotter test detected the following fungi associated with the seeds: *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp. and *Cladosporium* sp. In the other methods used, identification of symptoms in seedlings in paper roll and sand, only *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. were detected. Among the seed tests to evaluate health quality, the blotter test was the most efficient, allowing verification of a greater number of contaminated seeds (Table 5).

It was found that the incidence of *Aspergillus* sp. presented a negative correlation with SE (r = –0.6950), germination (–0.8540), FCG (–0.7838), normal seedlings after AA test (–0.5396) and ESI (–0.7085) of watermelon seed lots (Table 6). The same fungus had a significant positive correlation with the percentage of dead seeds in the germination test (r = 0.8111), showing the influence of the fungus in physiological performance in the evaluated watermelon seed lots (Table 6). Likewise, Muniz et al. (2004) found a correlation between the incidence of *Aspergillus* sp. and number of dead seeds in different vigor tests for melon seeds, attributing these results to the fact that the fungus is a major cause of rot in seed and may be associated with inappropriate seed storage.

There was no significant correlation between physiological performance variables and the incidence of *Cladosporium* sp., *Rhizopus* sp. and *Penicillium* sp. (Table 6) corroborating Casaroli et al. (2006), who found no negative interference from the fungi *Cladosporium* sp., *Rhizopus* sp. and *Penicillium* sp. on the physiological quality of pumpkin seeds.

According to the Pearson correlation analysis, the physiological performance of watermelon seeds was influenced by the presence of *Aspergillus* sp. (Table 6).

A correlation between the physiological performance of the seeds and the presence of *Aspergillus* sp. is explained by the fact that the genus is known as a storage fungus associated with the seed deterioration process in inappropriate storage conditions, resulting mainly in rotted seeds. For the other fungi detected in the evaluated watermelon seed lots, there was no correlation with the variables of physiological quality evaluated.

An overview analysis of the results allows verifying the efficiency of EC (50 seeds/75 mL of water at 25 °C for 24 h) and FCG in the separation of watermelon seed lots into vigor levels. Both tests, EC and FCG, allowed verification of the inferior quality of lots 1 and 2 in relation to the other evaluated lots, similarly to the SE. Regarding the methodology for analysis of health quality, the blotter test methodology was the most efficient in detecting fungi present in watermelon samples evaluated in this research. Symptoms' identification tests on seedlings' sand and paper roll do not show enough sensitivity to quantify the presence of *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. in watermelon seeds. Furthermore, the influence of the

Table 1. Initial moisture content (MC), normal seedlings (NS), abnormal seedlings (AS) and dead seeds (DS) for germination, AA and water content after AA (MC AA) of watermelon seeds.

Lot	MC	Germination			AA			
		NS	AS	DS	NS	AS	DS	MC AA
		%						
1	7.6	84 ± 0.87a	3 ± 0.20a	13 ± 0.81b	69 ± 0.97a	11 ± 0.55a	20 ± 1.11a	18.6
2	7.6	95 ± 0.68a	2 ± 0.58a	3 ± 0.37a	77 ± 0.55a	11 ± 0.92a	12 ± 0.92a	19.3
3	7.7	97 ± 0.40a	1 ± 0.20a	2 ± 0.24a	72 ± 0.49a	13 ± 0.32a	15 ± 0.6a	18.7
4	7.3	93 ± 0.75a	3 ± 0.40a	4 ± 0.63a	62 ± 0.63a	14 ± 0.68a	24 ± 0.93a	18.4

Means±SE followed by the same letter in a column do not differ according to the Tukey's test at 5% probability.

Table 2. First count of germination (FCG), seedlings' emergence (SE), emergence speed index (ESI) and electrical conductivity (EC) of watermelon seeds.

Lot	FCG (%)	SE (%)	ESI	EC (μS cm^{-1} g^{-1})
1 (30529)	59 ± 0.75 d	47 ± 0.98 c	5.83 ± 0.53 b	74.54 ± 0.41 d
2 (30538)	74 ± 0.97 c	67 ± 0.37 b	10.41 ± 0.48 a	49.19 ± 0.68 c
3 (30615)	80 ± 0.97 a	76 ± 0.97 a	10.07 ± 0.31 a	23.97 ± 0.25 b
4 (30634)	77 ± 0.20 b	82 ± 1.31 a	11.7 ± 0.42 a	16.94 ± 0.30 a

Means±SE followed by the same letter in the column do not differ according to the Tukey's test at 5% probability.

Table 3. Pearson linear correlation coefficients (*r*) in the evaluation tests of physiological quality of watermelon seed lots.

Variable	SE	G	FCG	AA	ESI	EC
SE	1.00	0.7726*	0.9434*	-0.2177^{ns}	0.9021*	–0.9708*
G		1.00	0.8863*	0.3026^{ns}	0.6272*	–0.7274*
FCG			1.00	-0.0148^{ns}	0.8166*	–0.9409*
AA				1.00	-0.1112^{ns}	0.3431^{ns}
ESI					1.00	–0.8264*
EC						1.00

*significant at 1% probability. ns = not significant. Seedlings emergence (SE), germination (G), first count of germination (FCG), accelerated aging (AA), emergence speed index (ESI) and electrical conductivity (EC).

Table 4. Fungi incidence (%) in watermelon seed lots.

Fungi				
Lot	*Aspergillus* sp.	*Cladosporium* sp.	*Penicillium* sp.	*Rhizopus* sp.
1 (30529)	99.80 ± 0.20a	0 ± 0.00c	27.80 ± 7.26b	2.40 ± 1.47a
2 (30528)	56.60 ± 3.46a	10.00 ± 2.19a	44.60 ± 14.58ab	6.40 ± 1.78a
3 (30615)	62.40 ± 2.09a	7.40 ± 2.54ab	82.60 ± 8.99a	2.60 ± 1.12a
4 (30634)	80.60 ± 2.42a	2.00 ± 0.71bc	33.20 ± 3.10b	2.60 ± 0.81a

Means±SE within a column followed by the same letter do not differ according to the Tukey's test at a level of 5% probability.

Table 5. Incidence (%) of fungi *Aspergillus* sp., *Penicillium* sp., and *Rhizopus* sp. in watermelon seed lots by symptoms of seedlings in paper roll (SSPR) test, symptoms in seedlings in sand (SSS) and dead seeds on seedling emergence (DSSE).

Fungi	Lot 1 (30529)		
	SSPR (%)	SSS (%)	DSSE (%)
Aspergillus sp.	18.00	0.00	23.00
Penicillium sp.	0.00	0.00	0.00
Rhizopus sp.	3.00	1.00	0.00
Lot 2 (30538)			
Aspergillus sp.	3.00	1.00	13.00
Penicillium sp.	1.00	0.00	0.00
Rhizopus sp.	2.00	2.00	0.00
Lot 3 (30615)			
Aspergillus sp.	4.00	0.00	11.00
Penicillium sp.	2.00	0.00	0.00
Rhizopus sp.	0.00	0.00	0.00
Lot 4 (30634)			
Aspergillus sp.	4.00	0.00	5.00
Penicillium sp.	1.00	0.00	0.00
Rhizopus sp.	1.00	1.00	0.00

Table 6. Pearson correlation coefficient (*r*) between incidence of *Aspergillus* sp. and seed physiological quality variables in watermelon seed lots.

Variable	Fungi
	Aspergillus sp.
SE	–0.6950*
G	–0.8540*
FCG	–0.7838*
ASG	0.5254**
DSG	0.8111*
NSAA	–0.5396**
ASAA	-0.1587^{ns}
DSAA	0.6401*
ESI	–0.7085*
EC	0.5712*

ns = not significant; * significant at 1% level of probability; ** significant at the 5% level of probability. Seedlings' emergence (SE), standard germination (G), first count of germination (FCG), abnormal seedlings in the standard germination test (ASG), dead seeds in the standard germination test (DSG), normal seedlings in the accelerated aging test (NSAA), abnormal seedlings in the accelerated aging test (ASAA), dead seeds in the accelerated aging test (DSAA), emergence speed index (ESI) and electrical conductivity (EC).

incidence of *Aspergillus* sp. on the physiological performance of watermelon seed lots should be emphasized, especially on the incidence of dead seeds for standard germination and AA tests.

Materials and Methods

Plant materials

This study was carried out at the Laboratory of Seed Analysis and in the greenhouse of Universidade Federal de Pelotas. Four untreated watermelon (*C. lanatus*) seed lots cultivar Crimson Sweet were used and submitted to the following tests: Moisture content: conducted in an oven with forced air circulation at 105 ± 3 ºC for 24 h, in accordance with the Rules for seed analysis (Brasil, 2009), using two samples of 4.5 g seed for each lot. Results were expressed as mean percentage weight loss per lot.

The watermelon seed lots were produced according to the same production standard of the seed company. However, they may have different physical, physiological and, health seed quality. The seed lots commercial numbers are lot 1 (30529), lot 2 (30528), lot 3 (30615) and, lot 4 (30634).

Physiological performance evaluation

Standard germination test: four subsamples of 50 seeds were used, and the test was performed on paper towel. Evaluations were conducted at five and fourteen days after sowing and the results expressed in percentage of normal seedlings, according to Ista (2012).

First count of germination (FCG): performed under the same conditions as the germination test and assessed at five days after sowing, following the recommendations of Ista (2012).

Accelerated aging (AA) test: a total of 200 seeds (four replicates of 50 seeds) per seed lot were distributed over an aluminum screen placed inside a plastic box (Gerbox®) containing 40 mL of distilled water, maintained at 41 °C for 48 h. After this period, the seeds were allowed to germinate following the methodology used in the standard germination test described above. The percentage of normal seedlings was assessed on the fifth day after sowing, as described by Bhering et al. (2003). The moisture content of the seed was assessed before and after the AA test.

Electrical conductivity (EC): assessed by the mass method with four replications of 50 seeds of pure seed portions per seed lot. The seeds were weighed and soaked in 200 mL plastic cups containing 75 mL of deionized water. The readings were taken 24 h after soaking at 25 °C. The conductivity value provided by the device Digimed®, model 32 was expressed in mS cm^{-1} g^{-1} per seed. Seedlings' emergence (SE): watermelon seeds were spread at a depth of 2 cm in trays containing washed and screened sand. Evaluation was taken at 14 days after sowing, counting the number of seedlings and determining the SE rate. Emergence speed index (ESI): performed under the same conditions as the SE test, the number of seedlings emerged in each tray was counted daily. Data from the assessments were applied to the formula proposed by Maguire (1962) to get ESI: ESI = E1/N1 + E2/N2 + ... En/Nn, where E1, E2, …, En = number of normal seedlings counted at the first count, second count and last count; N1, N2, …, Nn = the number of days from sowing to first, second and last counts.

Seed health quality evaluation

Blotter test: 400 seeds were plated in Gerbox® boxes containing well water-soaked blotters (filter papers), and incubated for seven days at 22ºC under 12h alternating cycles of light and darkness according to the blotter test method (Brasil, 2009). The identification of the fungi based on the way they grow on seeds "habit characters", and on the morphological chaeacters of fruiting bodies, spores/conidia observed under a compound microscope.

Identification of seedling symptoms in paper towel: 200 seeds were plated on well-soaked towel paper, and incubated for seven days at 22ºC under 12h alternating cycles of light and darkness for seven days. Isolations were performed in Petri dishes containing PDA culture medium for correct identification of the pathogen from tissue presenting symptoms. Five replications of each lot were used. All seeds were inspected and separated them into two groups: 1) healthy-looking seedling, 2) seedlings showing symptoms.

The identification of the fungi based on the way they grow on seeds "habit characters", and on the morphological characters of fruiting bodies, spores/conidia observed under a compound microscope.

Identification of seedling symptoms in sand: performed under the same conditions as for SE. After assessment of SE, those that displayed a range of observed symptoms were removed from the soil with a spade. Then, soil was carefully removed and the seedlings gently washed, blotted dry, wrapped in paper towels, and placed in a plastic bag. Samples were transported immediately to the university diagnostic laboratory for analysis. Plant tissues that displayed a range of symptoms were isolated in Petri dishes containing PDA culture medium for correct identification of the pathogen.

The identification of the fungi based on the way they grow on seeds "habit characters", and on the morphological characters of fruiting bodies, spores/conidia observed under a compound microscope.

Experimental design and statistical analysis

The experimental design was a completely randomized layout with five replications. Data expressed as percentage were subject to the following expression: arcsin ($\sqrt{x \div 100}$), except for seed health data, which were subject to the expression ($\sqrt{x+0.5}$), and an analysis of variance (Anova) was performed for all data. In the statistical procedure, Anova was performed separately for each test and the means of the lots were compared using the Tukey test at 5% probability and a Pearson correlation coefficient analysis was done using the Winstat 1.0 statistical package (Machado and Conceição, 2003). It used four lots and five replications for each test.

Conclusions

The EC and FCG tests allow the evaluation of the physiological performance of watermelon seed lots properly, based on the correlation value between these test and SE. The blotter test is the most efficient test to evaluate the health quality of watermelon seeds allowing the correct identification and quantification of different genera of fungi that other health quality tests did not detect. *Aspergillus* sp. incidence is correlated with the low physiological performance of watermelon seeds evaluation by SE, G, FCG, DSG and ESI.

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