HEALTH AND PHYSIOLOGICAL POTENTIAL OF SOYBEAN SEEDS SUBMITTED TO DIFFERENT STORAGE CONDITIONS

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SUMMARY

The objective of this study was to verify the health and physiological potential of soybean seeds submitted to different storage conditions. The harmful effect of fungi on seed quality before storage was observed. Generally, there was a reduction in fungal occurrence with storage, principally for the conditions of the cold chamber and the uncontrolled laboratory environment, resulting in increasing germination and seedling emergence.

Palavras-chave: Field fungi, *Glycine max*, Storage fungi, Seed quality

POTENCIAL SANITÁRIO E FISIOLÓGICO DE SEMENTES DE SOJA SUBMETIDAS A DIFERENTES CONDIÇÕES DE ARMAZENAMENTO

RESUMO

O presente trabalho teve como objetivo verificar o potencial sanitário e fisiológico de sementes de soja submetidas a diferentes condições de armazenamento. Antes do armazenamento constatou-se o efeito negativo dos fungos na qualidade fisiológica das sementes. De maneira geral, com o armazenamento houve queda na incidência dos fungos, principalmente nos ambientes de câmara fria e em condições não controladas, refletindo na melhora da qualidade fisiológica.

Keywords: Fungos de campo, Fungos de armazenamento, *Glycine max*, Qualidade de sementes

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the crops which has most expanded in Brazil, reaching 90,025,000 tons for the 2013/14 crop (CONAB, 2014). The use of high quality seed has been one of the factors which most contributed to the production increase.

After harvest, the seeds are dried, cleaned and stored until sowing. Storage of soybean seeds is practically obligatory (KROHN & MALAVASI, 2004) because the harvest period, which is between January and May in Brazil, does not coincide with sowing, which starts in mid-September.

Storage is a key factor for seed producers and should be done under conditions which permit the physiological potential to be maintained until seeds are used (GALLI et al., 2007). The seeds longevity is affected by the seed initial quality and the storage conditions (CATÃO et al., 2013). If storage conditions are unfavorable, seed germination and vigor will be reduced and adversely affect crop development (MARCOS FILHO, 2005).

From the moment of their formation until storage, seeds serve as an important vehicle (ITO et al., 2003) to saprotrophic and pathogenic microorganisms (DOMINJAN et al., 2005) and can be infected by various fungi. There are two groups of fungi linked to seeds, those called field fungi and those called storage fungi (TANAKA et al., 2001).

In soybean, the field fungi include the genera *Colletotrichum, Phomopsis, Cercospora* and *Fusarium*, among others (RATHOD & PAWAR, 2012; WAIN-TASSI et al., 2012). The association of these fungi with seeds occurs while these are still in the field, before harvest, and they can cause damage after sowing. These fungi do not affect seed physiological quality during storage because they only develop when seed moisture content is higher than 20% and relative humidity higher than 95% (DHINGRA, 1985; MARCOS FILHO, 2005). A reduction in seed moisture content halts development of the field fungi, and these may remain dormant or gradually die (DHINGRA, 1985).

On the other hand, the storage fungi, represented by the genera *Aspergillus* and *Penicillium*, can survive in low humidity environments and are able to proliferate in succession to the field fungi (TANAKA et al., 2001, RATHOD & PAWAR, 2012, ANWAR et al. 2013). These genera can be present in low percentages in recently-harvested seeds (TANAKA et al., 2001), but generally infect the seeds after harvest,

causing more or less damage according to the temperature, relative humidity (MARCOS FILHO, 2005), seed moisture content and storage time (DHINGRA, 1985). Storage fungi alter seed tissues, causing heat by respiration, consume of seed reserves, seed discoloration and mycotoxins production (MARCOS FILHO, 2005), resulting in seed deterioration (TANAKA et al., 2001).

A seed borne pathogen may be present externally, internally or associated with the seed as contaminant and may cause seed abortion, seed rot, seed necrosis, reduction of the germination capacity as well as seedling damage resulting in development of disease at large stages of plant growth by systemic or local infection (PATEL et al., 2014). Seeds are regarded as highly effective means for transporting plant pathogens over long distances (PATEL et al., 2014).

During the seed storage, many factors can affect the seed quality and the fungi viability, including temperature and relative humidity. Thus, correct storage conditions are fundamental for maintaining seed batch quality and studies on this topic should be made. The objective of the present study was to verify the health and physiological potential of soybean seeds submitted to different storage conditions.

MATERIALS AND METHODS

The experiments were done in the laboratories and greenhouse of the Departments of Phytopathology and Nematology and Crop Science of Escola Superior de Agricultura Luiz de Queiroz - ESALQ/USP. Three batches of the Coodetec 206 soybean cultivar, with different vigor levels, were first evaluated for health and physiological quality and then stored for six months under the following conditions: cold chamber (temperature of 10 ºC and Relative humidity (RH) around 85%), dry chamber (20 ºC and RH 50%) and laboratory environment (no temperature or relative humidity control). Samples were analyzed every three months with the following tests:

Determination of moisture content

The oven method was used at 105 ^{o}C, with two repetitions for each treatment as described in the Seed Analysis Rules (BRASIL, 2009).

Health Test

The blotter method was used to detect the fungi present on seeds (MATHUR & KONGSDAL, 2003). Three wetted sheets of filter paper were placed in Petri plastic dishes (9 cm in diameter) and 10 soybean seeds were sowed, separated from each other. The Petri dishes were kept in incubation chamber at a temperature of 20 ±

2 ºC and alternating light (12 hours of white fluorescent light and 12 hours of dark) for seven days. The funghi evaluations were made with a stereoscopic microscope and also with an optical microscope when necessary.

Germination Test

Seeds were distributed on germination paper towel rolls, moistened with an amount of water equivalent to 2.5 times the weight of the dry substrate and placed to germinate at 25°C (BRASIL, 2009). Evaluations were made four and eight days after the beginning of the test, observing vigor at the first count and germination at the second count.

Seedling emergence test in a greenhouse

The seeds were sown in plastic boxes (43x30x11 cm), containing sterilized soil. The boxes were kept in a greenhouse and after seedling emergence, the number of normal seedlings emerging was noted. The emergence speed index and the total emerged seedlings were determined 14 days after the test began.

Accelerated aging test

The accelerated aging test was done according to a method described by Marcos Filho (1999). A single, uniform layer of seeds was placed on mesh inside plastic boxes (11x11x3 cm), containing 40 mL of water. The closed boxes were kept in aging chambers at 41 ^{o}C for 48 h. After this period, the aged seeds were submitted to germinate according to germination test and evaluated after the fifth day (ISTA, 1985).

Experimental design

The experimental design was completely randomized for the laboratory tests and in completely randomized blocks for the greenhouse tests, with four repetitions of 50 seeds for each test. The results were analyzed using a factorial 3x3 design (storage time x storage conditions).

RESULTS AND DISCUSSION

The moisture contents of the three seed batches submitted to the three storage conditions were registered (Table 1). The seeds had a moisture content around 10% at the beginning of storage, which is considered ideal for maintaining the physiological potential of soybean seeds (MARCOS FILHO, 2005). After storing under laboratory conditions (uncontrolled environment), the moisture content remained around 9% for the three seed batches. Under the dry chamber conditions (T = 20 ºC and RH = 50%), the moisture contents fell to 5-6% and for the cold chamber (T = 10 ºC and RH = 85%) were above 15%.

The incidence of *Phomopsis* spp. in batches 1 and 2 showed a treatment variation

due to the interaction between the environmental factors and storage time (Table 2). There was a reduction in *Phomopsis* occurrence after the third month, which became observable after 6 months under the laboratory and cold chamber conditions. *Phomopsis* spp. incidence in batch 3 was very low and did not show any variations which could be attributed to the interaction of the environmental and storage time factors, but a drop in incidence was observed after the third month. There was no variation in the occurrence of *Cercospora kikuchii* on soybean seeds due to the interaction of the storage time and environmental conditions but a reduction in fungal incidence was observed after the third month for batches 2 and 3 and at 6 months for batch 1 (Table 2).

Isolation frequences of *Aspergillus* spp. and *Penicillium* spp. frequently appear on soybean seeds (ANWAR et al. 2013). The behaviors of these storage fungi were similar to the others (Table 3). For *Aspergillus* spp., only batch 2 showed an interaction between time and storage environment factors, with a reduction in incidence after the third month in the cold chamber environment, which only differed significantly from the laboratory treatment. There was an interaction between these factors for *Penicillium* sp. for batches 1 and 3, with a smaller incidence in the dry chamber and laboratory treatments for batch 1 and only for the dry chamber for batch 3. About the storage time, both fungi generally showed, the same reduction in the sixth month of storage.

Table 1. Moisture content of three soybean seed batches stored in three environments conditions for six months (Piracicaba, 2011).

		Storage period		
Batch	Environment	Start	3 months	6 months
1	U.E.	10.05	8.98	9.47
	D.C.	10.05	7.27	5.63
	C.C.	10.05	15.56	16.27
2	U.E.	10.40	8.81	8.61
	D.C.	10.40	6.91	5.14
	C.C.	10.40	17.16	14.91
3	U.E.	10.18	9.46	9.24
	D.C.	10.18	6.75	5.65
	C.C.	10.18	15.52	16.48

*U.E. = uncontrolled environment (laboratory); D.C. = dry chamber; C.C. = cold chamber

Table 2. Incidence (%) of field fungi in three soybean seed batches stored in three environments conditions for six months (Piracicaba, 2011).

Batches	Environment*	*Phomopsis* spp.				*Cercospora kikuchii*			
		Storage period (months)				Storage period (months)			
		Start	3	6	Mean	Start	3	6	Mean
1	U.E.	31	13	1	15.0b**	5	1	0	2.0 ^{ns}
	D.C.	31	18	20	23.3a	5	3	1	2.7
	C.C.	31	10	6	15.7b	5	3	0	3.0
	Mean	31.0A	13.7B	9.0C		5A	2A	0.3B	
CV			16.7%				58.1%		
2	U.E.	24	15	0	13.0b	4	0	0	1.3b
	D.C.	24	20	15	19.7a	4	2	1	2.3^{a}
	C.C.	24	11	6	13.7b	4	2	1	2.3^{a}
	Mean	24A	15.3B	7.0C		4A	1.3B	0.7B	
CV			22.0%				44.6%		
3	U.E.	8	5	0	4.3^{ns}	7	0	0	2.3b
	D.C.	8	5	4	5.6	7	4	2	4.3^{a}
	C.C.	8	4	2	4.6	7	0	0	2.3ab
	Mean	8.0A	4.6B	2.0B		7.0A	1.0B	0.7B	
CV			47.0%				84.4%		

*U.E. = uncontrolled environment (laboratory); D.C. = dry chamber; C.C. = cold chamber

**Means followed by small letters in the columns and capital letters in the rows differ among themselves according to the Tukey test at the 5% probability level; ns = not significant

Table 3. Incidence (%) of storage fungi in three soybean seed batches stored in three environments conditions for six months (Piracicaba, 2011).

Batches	Environment*	*Aspergillus* spp.				*Penicillium* sp.			
		Storage period (months)				Storage period (months)			
		Start	3	6	Mean	Start	3	6	Mean
1	U.E.	72	57	69	66.0^{ns**}	63	27	17	35.7b
	D.C.	72	69	54	65.0	63	56	21	46.7b
	C.C.	72	73	31	58.7	63	91	88	80.7a
	Mean	72A	66.3A	48.6B		63A	58.0A	42.0B	
CV			10.1%				16.0%		
2	U.E.	63	62	63	62.7a	68	45	44	52.3^{ns}
	D.C.	63	59	47	56.3ab	68	37	36	47.0
	C.C.	63	44	39	48.7b	68	51	61	60.0
	Mean	63A	55AB	49.7B		68A	44.3B	35.2B	
CV			15.1%				20.6%		
3	U.E.	3	10	4	5.7^{ns}	55	66	13	44.7a
	D.C.	3	5	4	4.0	55	27	10	30.7b
	C.C.	3	4	1	2.7	55	55	52	54.0a
	Mean	3.0^{NS}	6.3	3.0		55.0A	49.3^{a}	25.0B	
CV			62.5%				156.2%		

*U.E. = uncontrolled environment (laboratory); D.C. = dry chamber; C.C. = cold chamber

**Means followed by small letters in the columns and capital letters in the rows differ among themselves according to the Tukey test at the 5% probability level; ns=not significant in the column and NS= not significant in the row

About the seed physiological potential, the values for the first germination count, germination and accelerated aging are shown in Table 4. In all the batches, the germination after three months storage increased, which was maintained until six months. Batch 1 showed higher percentage seed germination under laboratory conditions when compared to the cold chamber.

As observed for germination, tests based on seedling growth, first germination count (Table 4) and seedling emergence speed (Table 5), showed increases in vigor after three months storage, which were maintained until the sixth month. However, there were no variations due an interaction between environmental factors and storage time and no reduction in seed vigor was observed based on seedling percentage emergence after storage.

For the accelerated aging test (Table 4), an interaction between the time and storage environment factors was only observed for batches 2 and 3, with a reduction in vigor after three months storage. Batch 1 seeds stored under laboratory and dry chamber conditions, showed a higher vigor compared to those stored in the cold chamber. For batch 2, the highest vigor was observed for those seeds stored in the dry chamber whereas for batch 3, the conditions in the dry and cold chambers favored the maintenance of seed vigor.

The high relative humidity of the cold chamber caused an increase in seed moisture, which adversely affected the conservation of the physiological potential, even at low temperatures. When the seed moisture content, relative humidity and temperatures are maintained in low levels, physiological potential will be better conserved (MINOR & PASCHAL, 1982), with the relative humidity being more critical than the temperature (MARCOS FILHO, 2005).

The results obtained for the field fungi, *Phomopsis* spp. and *C. kikuchii* were expected, because these fungi require a moisture content around 25% for their survival (AGARWAL & SINCLAIR, 1996) and as the moisture content falls, these pathogens lose their viability.

The most important factors influencing infection by storage fungi are the seed moisture content, environmental relative humidity, temperature and storage time (DHINGRA, 1985). For these fungi, there is a tendency for an increase in the incidence with increasing storage time since these fungi are adapted to storage conditions (CARVALHO & NAKAGAWA, 2000).

Table 4. Germination and accelerated aging of three soybean seed batches stored in three environments conditions for six months (Piracicaba, 2011).

Batch	Environment*	First germination count				Percentage germination				Accelerated aging			
		Storage period (months)				Storage period (months)				Storage period (months)			
		Start	3	6	Mean	Start	3	6	Mean	Start	3	6	Mean
1	U.E.	63	79	81	74^{ns**}	79	88	92	86a	67	72	60	66a
	D.C.	63	86	74	74	79	90	84	84ab	67	54	82	68a
	C.C.	63	81	66	70	79	86	80	82b	67	53	49	56b
	Mean	63C	82A	74B		79B	88A	85A		67^{NS}	60	64	
CV			6.8%				5.6%				11.1%		
2	U.E.	80	82	79	80^{ns}	80	88	86	85^{ns}	67	67	46	60b
	D.C.	80	82	84	82	80	84	90	85	67	61	80	69a
	C.C.	80	84	85	83	80	88	89	86	67	39	64	57b
	Mean	80B	83A	83A		80B	87A	88A		67A	56B	63AB	
CV			8.2%				10.1%				10.4%		
3	U.E.	78	90	87	83^{ns}	88	95	91	91^{ns}	67	49	33	50b
	D.C.	78	91	89	86	88	95	93	92	67	56	69	64a
	C.C.	78	92	92	87	88	94	95	92	67	48	60	58a
	Mean	78B	91A	89A		88B	95A	93A		67A	51B	54B	
CV			4.6%				6.0%				9.5%		

*U.E. = uncontrolled environment (laboratory); D.C. = dry chamber; C.C. = cold chamber

**Means followed by small letters in the columns and capital letters in the rows differ among themselves according to the Tukey test at the 5% probability level; ns=not significant in the column and NS= not significant in the row

Table 5. Speed and percentage emergence of three batches of soybean seeds stored in three environments conditions for six months (Piracicaba, 2011).

Batches	Environment*	Emergence speed				Percentage emergence			
		Storage period (months)				Storage period (months)			
		Start	3	6	Mean	Start	3	6	Mean
1	U.E.	6.5	7.4	8.0	6.8 ns**	88	80	88	85 ns
	D.C.	6.5	8.2	7.3	7.0	88	87	88	88
	C.C.	6,5	7.8	7.1	6.6	88	84	88	87
	Mean	6.5 NS	7.8	7.5		88 NS	84	88	
CV			12.7%				31.3%		
2	U.E.	4.8	7.7	4.4	5.6 ns	87	79	47	71 ns
	D.C.	4.8	7.0	8.7	6.8	87	82	90	86
	C.C.	4.8	7.4	8.6	6.9	87	89	88	88
	Mean	4.8B	7.4A	7.2[a]		87 NS	83	75	
CV			32.5%				24.9%		
3	U.E.	5.1	8.1	8.8	7.3 ns	86	96	90	91 ns
	D.C.	5.1	7.1	8.2	6.6	86	84	91	87
	C.C.	5.1	7.9	7.6	6.9	86	88	80	85
	Mean	5.1B	7.7A	8.2A		86 NS	89	87	
CV			16.3%				11.6%		

*U.E. = uncontrolled environment (laboratory); D.C. = dry chamber; C.C. = cold chamber

**Means followed by small letters in the columns and capital letters in the rows differ among themselves according to the Tukey test at the 5% probability level; ns=not significant in the column and NS= not significant in the row.

However, the results obtained in this experiment showed a reduction in the level of these fungi incidence, probably as a result of the variation in the seed moisture content. Tanaka et al. (2001) obtained the same result for *Aspergillus* spp. in some corn batches, observing a tendency for reduction in seeds stored in a cold chamber. For *Penicillium* spp., these authors observed an increase, which was smaller in the cold chamber.

Under the laboratory and dry chamber conditions there was a reduction in seed moisture content, which was greater for the dry chamber, reaching around 5.5% after six months storage, which is below the ideal for the development of these fungi. According to Agarwal & Sinclair (1996), the moisture content necessary for the growth of storage fungi varies from 12-18% for soybeans depending on the fungus species.

Marcos Filho (2005) has showed that seed germinative potential decreases with time as a part of the natural deterioration process of seeds. However, the opposite was observed in this experiment, possibly due to the significant reduction in the incidence of *Phomopsis* spp., *Aspergillus* spp. and *Penicillium* sp., considered as seed rotting agents.

Fungi growing on the stored grains reduce the germination rate, carbohydrate, protein, total oil content, increase moisture content and also enhancing other biochemical changes of seeds (BHATTACHARYA & RAHA, 2002). Seeds can be deteriorated by the action of the storage fungi *Aspergillus* spp. and *Penicillium* spp. Tanaka & Corrêa (1981) proved this by inoculating bean seeds with these fungi and storing them for 16 months; after this period, the seeds, which initially had a germinative potential of 81%, showed 60% germination for non-inoculated seeds and 40% and 32% for those inoculated with *Aspergillus* sp. and *Penicillium* sp., respectively. Most of the species of *Aspergillus* are dominant and play vital role in the seed biodeterioration, reducing seed germination and vigor (CHAVAN, 2011; ROCHA et al., 2014).

Waint-Tassi et al. (2012) observed reduction in the physiological potential of seed with a high incidence of *Phomopsis* sp. However, in seeds stored under favorable conditions, the fungi rapidly lost its viability and this improved laboratory germination results. Other authors also have verified an increase in seed vigor and germination after 60 days storage together with a reduction in the incidence of *Phomopsis* sp. (MARTINS FILHO et al., 2001).

The increase in seed vigor observed in the tests based on seedling growth (first germination count and emergence speed), may also be due to a reduction in the occurrence of the main pathogens associated with the seeds until the sixth month of storage. The seedling emergence test did not indicate any increase in vigor after storage, agreeing with those results obtained by Henning (1987). This author found that the adverse effect of *Phomopsis* spp. on germination was not repeated for seedlings emerging from these seeds. This may be explained by the pathogens being present principally on the seed coat, which remains in the soil after seedling emergence.

The accelerated aging test confirms that after storage, seed physiological potential is reduced and seeds cannot withstand the stress conditions they are submitted to during accelerated aging test It shows that, even in the seed coat, the fungi probably affect the biochemical protection mechanism in seeds. The fungi biochemical action is related to enzyme involved in the plant cell wall degradation and in the toxins production which will cause decomposition of viable tissues, thereby causing an increase in respiratory rate of the seed host, resulting in seed deterioration (HENNING et al., 2009).

Thus, the results of lower germination and vigor obtained from the remaining tests at the beginning of storage are associated with the high incidence of pathogens such as *Phomopsis* spp., *Aspergillus* spp. and *Penicillium* sp.

CONCLUSION

There is a reduction in field and storage fungi occurrence in soybean seeds with storage, especially for the conditions of the cold chamber and the uncontrolled laboratory environment, resulting in the increase of germination and seedling emergence.

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