SEED HEALTH OF COMMON BEAN STORED AT CONSTANT MOISTURE AND TEMPERATURE

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ABSTRACT: Fungal incidence in stored common bean *(Phaseolus vulgaris* L.*)* is the main concern in order to preserve seed health and viability. The main aim of this study was to analyse these quality parameters in hermetically stored seeds at 10.2, 13.1, 16.2, 18.5% moisture content (MC) and 25, 30, 35, 40°C, through seed germination and health tests. Water activity recorded at 10.2 and 18.5% MC were 0.448 and 0.700, respectively. Low seed moisture content reduced *Alternaria* spp. incidence at 25-30°C. Highest incidence of *Fusarium* spp. (7.5%) occurred at 16.2% MC and 35-40°C. Highest incidences of *Rhizoctonia* spp. (8-10%) were recorded at 16.2-18.5% MC and 30-40°C. *Penicillium* spp. and *Aspergillus* spp. were predominant throughout the experiment and the highest incidences (80-100%; 20-30%, respectively) were scored at 18.5% MC and 30-35°C and 13.1-18.5% MC at 35°C, respectively. The higher the seed MC the higher the fungi incidence while lower seed MC decreased the incidences by 25%. Storage conditions below 30°C and 13.0% MC appear suitable to preserve common bean seed in relation to viability and health, up to a 8-month period.
Key words: seed healthiness, hermetic storage, fungal incidences

SANIDADE DE SEMENTES DE FEIJÃO ARMAZENADAS A UMIDADE E TEMPERATURA CONSTANTES

RESUMO: A incidência de fungos em sementes de feijão (*Phaseolus vulgaris* L.) é a preocupação principal de cientistas e tecnologistas de sementes visando preservar a sua sanidade e viabilidade. O objetivo deste estudo foi analisar esses parâmetros de qualidade em sementes hermeticamente armazenadas com graus de umidade de 10,2, 13,1, 16,2 e 18,5% a 25, 30, 35, 40°C, através de testes de germinação e de sanidade. Os valores de atividade de água obtidos para graus de umidade de 10,2 e 18,5% foram de 0,448 e 0,700. Baixos graus de umidade reduziram a incidência de *Alternaria* spp. a 25-30°C. A maior incidência de *Fusarium* spp. (7,5%) ocorreu com grau de umidade de 16,2% a 35-40°C. As maiores incidências de *Rhizoctonia* spp. (8-10%) foram registradas para graus de umidade de 16,2 e 18,5% a 30-40°C. Os fungos *Penicillium* spp. e *Aspergillus* spp. foram predominantes durante todo o experimento, sendo que as maiores incidências (80-100%; 20-30%) foram registradas para 18,5% de umidade a 30-35°C e 13,1-18,5% de umidade a 35°C, respectivamente. Quanto maior o grau de umidade da semente maior foi a incidência de fungos, enquanto que os valores mais baixos de umidade reduziram essas incidências a 25%. Temperaturas de armazenamento abaixo de 30°C e graus de umidades inferiores a 13,0% parecem ser as condições adequadas para preservar a viabilidade e a sanidade de sementes de feijão por até 8 meses.
Palavras-chave: patologia de sementes, armazenamento hermético, incidência de fungos

INTRODUCTION

Brazil is one of the largest common bean producers in the world, therefore requiring high technology for the maintenance of seed quality parameters as to physical purity, germination and health percentages. Several factors may affect common bean seed conservation, mainly including seed health, moisture content (MC), temperature (T), relative humidity (RH) and the action of fungi and insects. High T and MC accelerate degenerative processes in biological systems, causing gradual, irreversible and accumulative losses in vigour and viability (Delouche & Baskin, 1973). Seeds present a lower respiration rate during storage than external and internal fungi (Lazzari, 1993).

Seeds are the vehicles for transmission of several fungi and frequently introduce new pathogens in exempt areas, so that the integration between seed health and germination tests is recommended to control seed transmitted diseases (Zaumeyer & Thomas, 1957; Singh & Mathur, 1974; Bolkan et al., 1976.; Neergaard, 1977). However, most of the research has

emphasised seed health in open-stored common bean, not taking into account controlled environments.

Field fungi activity is delayed during storage at low seed MC since they require ≥ 90% air RH for growth (Lazzari, 1993). Fast development and high aggressiveness of these pathogens could kill the seed after sowing due to the action of powerful enzymes and toxins. On the other hand, storage fungi usually develop in seeds in equilibrium at 65-90% air RH (around 12-13% MC) (Loewer et al., 1994). A decrease in field fungi and an increase in storage fungi populations occur after harvest, in an ecological succession. The main objective of this research was to analyse seed germination and fungi incidences in common bean under controlled storage conditions to define an optimum MC, storage period and T required to maintain high seed health and viability.

MATERIAL AND METHODS

Seven kg of common bean seeds cv. IAC-Carioca ETE, were harvested in the 1998-99 season, in Campinas, São Paulo State, Brazil. Seed MC was adjusted at 25°C to 10.2, 13.1, 16.2 and 18.5% MC, from an initial value of 15.1%, either by rehydration over water in a closed plastic box or by dehydration over silica gel, aiming to avoid possible damage to the seeds caused by fast dehydration / rehydration. Seeds were sealed in laminated aluminium-foil packets (polyester structure / aluminium / low-density polyethylene, with a total thickness of 120 mm) and stored in incubators maintained at 25, 30, 35 and 40°C (± 0.5°C).

Seed MC (fresh weight basis) was determined in three 5 g ground seed samples at 130-133°C for 2 h (ISTA, 2004). Water activity (A_w) was determined using three seed samples for each MC in a hygrometer using the dew point technique, at 25°C ± 0.3 (± 0.01A_w). Germination tests were performed at 25°C using 4 × 50 seed replicates for each MC / temperature / sampling date combination, placed in rolled paper towels moistened with deionised water, with initial and final seedling counts recorded at the 5th and 9th days, respectively (ISTA, 2004).

Fungi incidences were determined by the blotter test (Neergaard, 1977), using 200 seeds (20 × 10 replicates) for each MC / temperature / sampling date combination, incubated at 20°C during seven days under 12-h alternating cycles of NUV-light (320-400 nm) and darkness, followed by evaluation under a stereoscopic microscope. Seeds were placed in plastic Petri dishes (9 cm diameter), with three filter papers moistened with sterilised water and previously decontaminated in a 1% sodium hypochlorite solution for 5 minutes (Berjark, 1984; Usberti & Amaral, 1999). Seed fungi incidences were estimated through observations of their structures (Barnett & Hunter, 1972).

Sampling intervals for seed health tests were quite variable due to the different levels of deterioration in relation to MC and storage T (Table 1). Preliminary results revealed no significant differences among initial and sampling time MCs. Fungi incidences for each combination among storage period, T and MC were compared using Fisher's LSD test ($p < 0.05$). Prior to statistical analyses, germination percentages and fungi incidences were transformed into arcsine $\sqrt{}$%/100 and $\sqrt{}$(x+1), respectively.

RESULTS AND DISCUSSION

Water activity

A_w is the quotient of seed vapour pressure over pure water vapour pressure at the same temperature and is an important parameter in storage studies since it is closely related to rate and intensity of common bean seed deterioration (Sartori, 1996). The A_w values recorded on each seed MC (45-70% RH) were 0.448,

Table 1 - Sampling intervals (days) for seed health tests on common bean seeds stored at 25, 30, 35, 40°C and 10.2, 13.1, 16.2, 18.5% moisture content (MC).

10.2% MC				13.1% MC				16.2% MC				18.5% MC			
25°C	30°C	35°C	40°C	25°C	30°C	35°C	40°C	25°C	30°C	35°C	40°C	25°C	30°C	35°C	40°C
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
189	189	118	118	150	150	189	109	130	15	4	3	24	15	4	2
242	242	200	200	242	200	214	116	200	24	7	7	104	23	7	4
		207	205		242		123	214	60	11	11	109	49	11	7
		210	210				130	259	100	23	18	116	60	21	10
		214	214				132		109	56	22	123	63	29	18
		230	216				137		130	60	25	130	70	39	22
							140			63	29	140		43	29
											32				

0.571, 0.674 and 0.700 for 10.2, 13.1, 16.2 and 18.5% MC, respectively.

Statistical analyses

Statistical analyses of fungi incidences on common bean seeds are presented in Table 2, for each combination of storage period, T and MC. No statistical interaction were recorded among fungi incidences, storage T and seed MC. *Penicillium* spp. and *Aspergillus* spp. revealed the highest incidences among fungi throughout the experiment (Figures 1 and 2). Regression lines of fungi incidences were observed in shorter

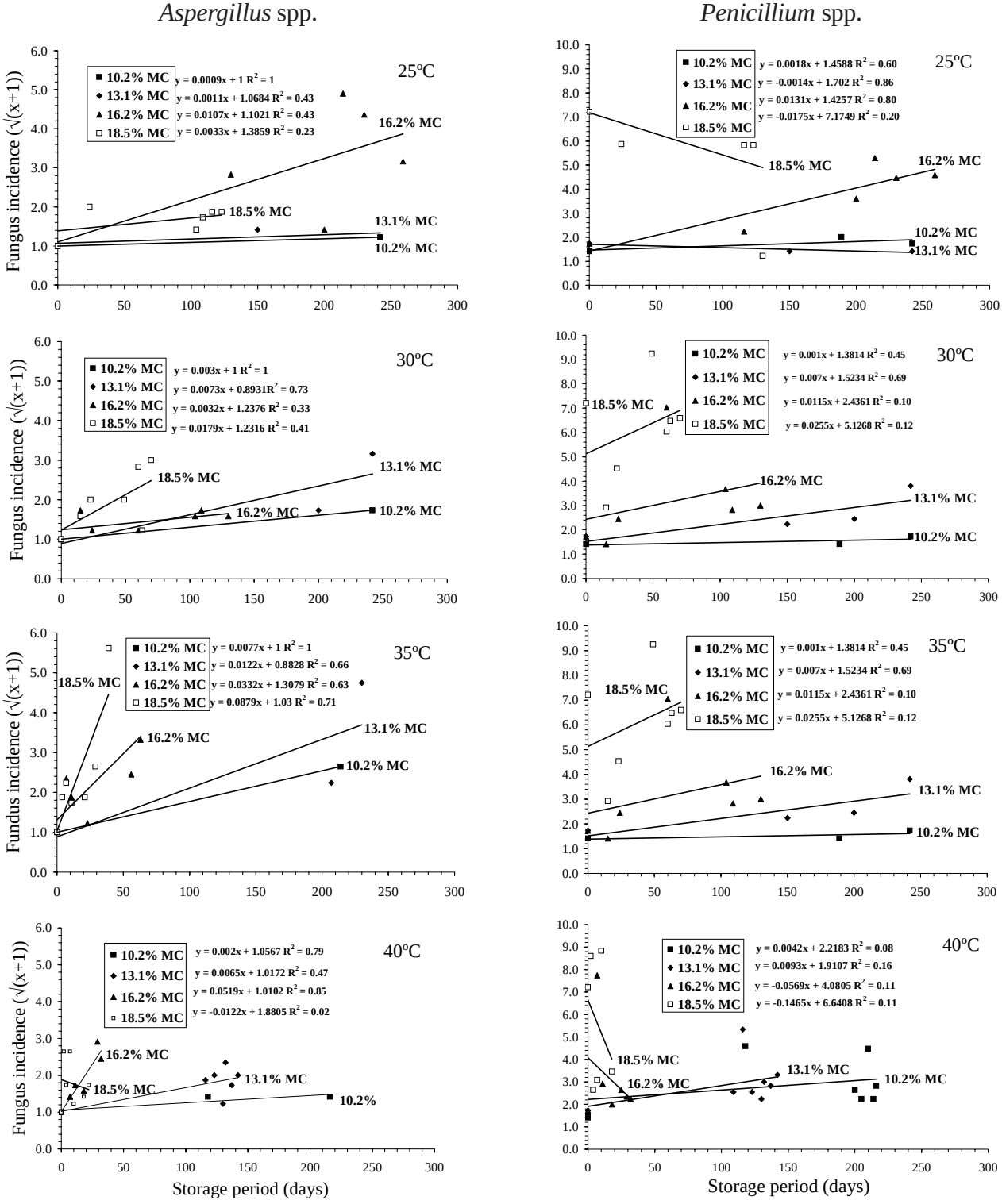

Figure 1 - Regression lines of storage fungi (*Aspergillus* spp. and *Penicillium* spp.) incidences (√(x+1)) in common bean seeds stored at 25, 30, 35, 40ºC and 10.2, 13.1, 16.2, 18.5% moisture content (MC).

storage periods according to increases on seed MC and storage T.

The determination coefficients estimated for storage fungi incidences (Figure 1), ranging from 0.08 to 1, revealed a decreasing tendency, according to increases on MC and storage T. However, the values recorded for field fungi incidences (Figure 2) were quite variable, ranging from zero to 1, without showing a specific tendency.

Storage fungi incidences

Penicillium spp. and *Aspergillus* spp. are the main storage fungi in common bean and usually invade the seeds during and after maturation, causing damage as soon as they find appropriate conditions. The primary coloniser is *Aspergillus* spp., which subsequently allows the development of *Penicillium* spp. (Faiad et al., 1996). *Penicillium* spp. and *Aspergillus* spp. incidences scored in common bean seeds stored at 25, 30, 35, 40°C and 10.2, 13.1, 16.2, 18.5% MC are presented in Figure 1.

***Aspergillus* spp.** - Fungal incidences higher than 20% were recorded at different MC / temperature combinations, mainly in early storage periods and 16.2-18.5% MC, except at 10.2-13.1% MC / 35-40°C, when these values were scored until 200-230-day storage periods. Regardless of T, 10.2-13.1% seed MC reduced fungal invasion until the 214-day storage when values around 6-8% were registered at 35°C; however

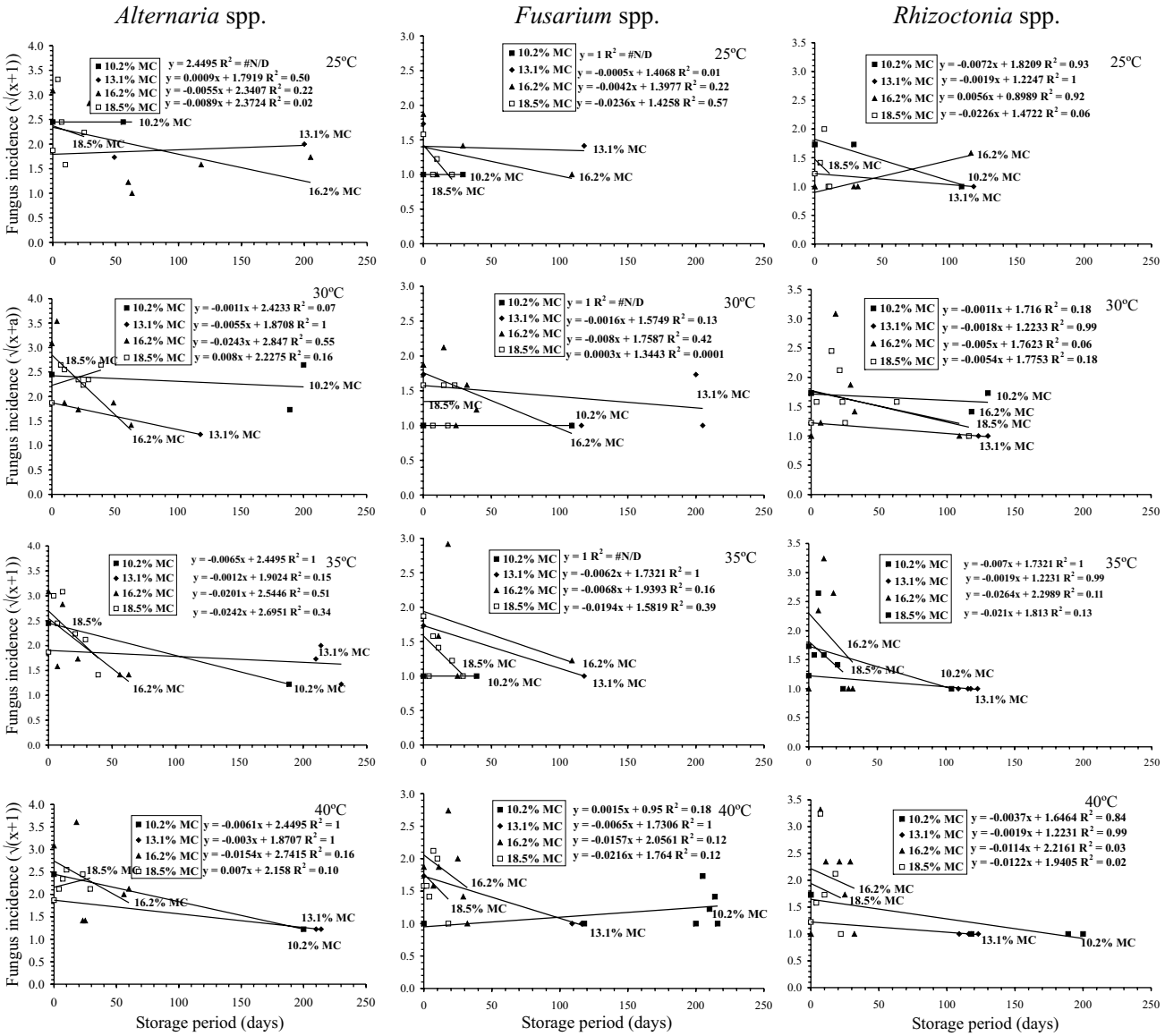

Figure 2 - Regression lines of field fungi (*Alternaria* spp., *Fusarium* spp. and *Rhizoctonia* spp.) incidences (√(X+1)) in common bean seeds stored at 25, 30, 35, 40ºC and 10.2, 13.1, 16.2, 18.5% moisture content (MC).

Table 2 - Statistical analyses of fungi incidences on common bean seeds stored at 25, 30, 35, 40°C with 10.2, 13.1, 16.2, 18.5% moisture content (MC). Same letters in the column mean no differences among incidences (Fisher LSD's test, $p < 0.05$).

they increased by about 20% at 40°C. Highest and earliest values were recorded at 16.2-18.5% MC / 35-40°C, respectively.

The presence of *Aspergillus* spp. in bean seeds was reported by Christensen (1972); Faiad et al. (1996) and Sinha et al. (1999); however, its occurrence was always associated with the presence of *Penicillium* spp. (Bolkan et al., 1976; Dhingra & Sinclair, 1978). A high incidence of *Aspergillus* spp. (77%) was detected in bean seeds during 24-month open storage conditions at 85-95% RH (Hernandez et al., 1994).

***Penicillium* spp. -** *Penicillium* spp. had the highest incidences in common bean seeds, mainly at 18.5% MC and 30-40°C (80-100%) while still higher values (around 60%) were recorded at 25°C and 16.2-18.5% MC. The higher the storage T, the higher the fungus invasion during early storage periods. Lowest seed MC (10.2-13.1%) reduced fungus incidences; however, at 30-35°C, the values reached 10%, while at 40°C some values were higher than 20%. The best T range for fungus invasion was 30-35°C and MC higher than 16%.

Highest incidences of *Penicillium* spp. were observed at 18.5% MC at all T, while *Aspergillus* spp. contamination was only pronounced at 35°C; so, the higher the seed MC, the higher the fungal incidences at early storage periods. Additional high T effects on fungi incidences could also be noted. *Penicillium* spp. and *Aspergillus* spp. presented the highest incidences in common bean seeds during hermetic storage. These results agree with Terveit (1945); Wilcox et al. (1974); Bolkan et al. (1976); Dhingra & Sinclair (1978) and Hernandez et al. (1994).

Field fungi incidences - The most common field fungi detected in fresh bean seeds were *Alternaria* spp. and *Fusarium* spp. (Figure 2), which require seed MC in equilibrium to 90% RH for growing and cause great impacts in crop yields (Abawi et al., 1977; Pieczarka & Abawi, 1978; Sinha et al., 1999). Field fungi incidences remained below 12% throughout the experiment and lowest MC / T combinations (10.2-13.1% and 25-30°C) reduced the values below 6% (Figure 2). Highest incidences of *Alternaria* spp. (8-12%) were observed at 16.2-18.5% MC, however low MC were effective in restraining them. The higher T, the greater the fungus incidence. On the other hand, *Fusarium* spp. occurred mainly at 16.2% MC and 35-40°C and remained below than 3.5% at 25-30°C. *Rhizoctonia* spp. highest incidence (8-10%) was detected at 18.5% MC and 30-40°C, however at 25°C the fungus occurrence was reduced. Field and storage fungi incidences were clearly reduced for the lowest MC (10.2%, 13.1%) at 25-30°C, with values remaining below 5 and 10%, respectively (Figures 1 and 2).

Seed germination

Increases in MC reduced seed viability and this effect was more pronounced for highest seed MC (16.2, 18.5%), unrelated to storage T (Figure 3). The

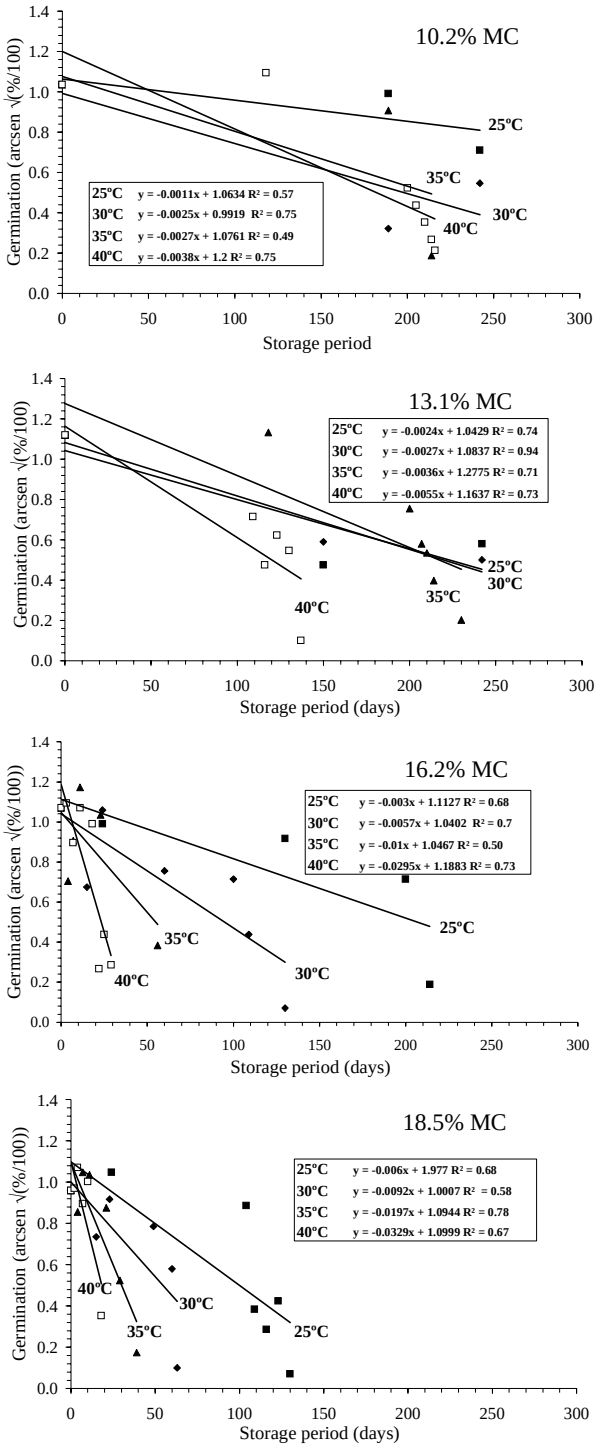

Figure 3 - Regression lines of standard germination (arcsine √(%/100)) of common bean seeds stored at 25, 30, 35, 40ºC and 10.2, 13.1, 16.2, 18.5% moisture content.

determination coefficients calculated for germination percentages were quite high, ranging from 0.49 to 0.94, without showing a specific tendency according to MC and storage T.

Some germination percentage reductions observed throughout the storage period might be influenced by previous high incidences of *Penicillium* spp. (60.5% at 130-day; 36.5% at 132-day) (Figure 1). Moreover, some early high incidences of *Aspergillus* spp. at 40 to 60-day storage could play an additional role in the deterioration process.

Similar results were reported by Chisholm & Coates (1997), evaluating germination percentages and fungi incidences in three leguminous seeds during storage with subsequent germination reduction and increase in fungi incidences mainly at 28°C. Stored beans (10.3-14.2% MC at 30°C) with high initial seed germination percentage and MC around 11.5% might maintain viability for eight months (Aguirre & Peske, 1991). Sanhewe & Ellis (1996) have also reported that bean seed quality was higher at cold temperatures during development and maturation.

CONCLUSIONS

Storage conditions with moisture content and temperature lower than 13.1% and 30°C, respectively, appear to be adequate for maintenance of seed viability and healthiness up to 8-months storage. Such storage conditions, which might be easily reached by seed sun drying and open storage, suggest a closing remark as to potential benefits and offer seed producers with a strategy for maintaining seed viability.

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