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Survival of *Rhizoctonia bataticola* in groundnut seed under different storage conditions

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Summary

Rhizoctonia bataticola (sclerotial stage of *Macrophomina phaseolina*), which causes charcoal rot disease of groundnut, is an important pathogen of this crop. Under warm and wet conditions in the field, the pathogen enters the seed and remains inside without any visible symptoms. Groundnut germplasm stored for various durations in the medium term storage (4°C) commonly shows 10-29% infection of this pathogen. To determine the longevity and survival of this pathogen in groundnut seed, a study began on 15 May 1995 with healthy and *R. bataticola* infected groundnut seed stored at 20, 4 and -18°C. Initial levels of infection and moisture were determined in both the seed lots. Independent samples from the two bulks were drawn every year for determining seed germination and the level of pathogen infection. Agar plate method was used for pathogen detection and paper towel method for seed germination. After five years of storage, the germination of healthy seed was not affected at any of the temperatures. However, a drastic reduction in the germination of infected seed occurred at all temperatures even within three years of storage. This reduction in germination was associated with a significant increase in the incidence of *R. bataticola* in infected seed stored at all temperatures after 5 years of storage. In the case of healthy seed, there was a little increase in the incidence of infection at 20 and 4°C, but no increase occurred at -18°C. The results show that *R. bataticola* can survive and its incidence can substantially increase in infected groundnut seed even at -18°C, a temperature recommended for long term storage. The implication of these results is that for maintaining a high level of germination in groundnut seed, only disease-free, or seed with very low infection should be used for long term storage.

Introduction

Groundnut (*Arachis hypogaea* L.), a native of America, is an important crop in Asia, Africa, Europe and Oceania. The crop is an indispensable source of edible oil and livestock feed in Asia and Africa. Groundnut is cultivated on an estimated area worldwide of about 19.3 million hectares although, the largest area (14.5 million hectares) and about 70% of the world's production of this crop (15.4 million tons) are in Asia (Fletcher *et al.*, 1992). ICRISAT is the world's repository of groundnut and holds 15342 accessions in its gene bank. A part of these accessions (appr. 5000) is now under long term storage conditions (-18°C with no RH control). Efforts are under way to keep all the remaining germplasm accessions in this storage facility in the next five years.

The long term storage conditions, not only enhance the longevity of the germplasm, but also help in the survival of seed-associated pathogens. Thus the gene banks may also serve as reservoirs of the seed-borne pathogens (Diekmann, 1988; Plucknett *et al.*, 1987).

Keeping in view the enormous increase in the international movement of germplasm during the last two decades (Plucknett and Smith, 1989), seed-borne pathogens are cause of serious concern if they can survive even under these storage conditions. A large number of seed-associated fungi (about 60) have been reported on groundnut and many of these are seed-borne (Subrahmanyam *et al.*, 1990; Girish *et al.*, 2001).

R. bataticola causes diseases such as charcoal rot and pod rot of groundnut is an important seed-borne pathogen of this crop (Garren and Wilson). Pod rot is a quite widespread disease in the SAT region causing considerable losses in several countries (Abdou and Khadr, 1974; Mercer, 1977). During the 1978-79 growing season, up to 72% pod rot infection was observed at ICRISAT farm (Proceedings of the International Workshop on groundnuts 1980). In routine health tests of groundnut seed in 2000, 29% of the 673 accessions yielded 1–35% *R. bataticola* infection. In fact, 10-29% *R. bataticola* infection has been constantly observed in groundnut accessions kept in medium term storage that were tested for seed health for quarantine clearance.

There are many reports on storability and methods of maintaining the viability of groundnut seed under different storage conditions (Bewley, 1986; Kurdikeri *et al.*, 1996; Nautiyal and Zala, 1993; Nautiyal *et al.*, 1996; Rao, *et al.*, 1996 Tripathi *et al.*, 1996). However, no effort appears to have been made to study the survival of seed-borne pathogens under these storage conditions for any pathogen. Keeping in view the economic importance of *R. bataticola*, a 10-year study was initiated to determine the survival of this pathogen in apparently healthy and infected seed of groundnut at three storage temperatures, 20°C, 4°C and -18°C. In this paper, the results are reported after five years of storage.

Materials and methods

Collection of seed

Apparently disease-free and *R. bataticola* infected pods of cultivar TMV 2 were collected from Adoni area in Kurnool district of Andhra Pradesh state, India. The pods were dried under room temperature in the laboratory at 25-27°C and then threshed separately. Thirty-three packets each containing 350 seed (200 seed for germination, 100 seed for pathological study and 50 seed for moisture detection) were prepared from both apparently healthy and infected seed lots. The packets were grouped into three sets of 11 packets each for both seed lots. A set of 22 packets made up of 11 packets from each seed lot, was kept at 20°C, the second at 4°C and the third at -18°C.

Testing procedure

Seed from one packet of each seed lot from each of the three temperatures of the two seed lots were tested on the first day of the experiment for moisture content, germination and pathogen infection. Thereafter, one packet from each treatment was withdrawn from storage every year for five years.

Moisture detection

The moisture content was determined using the low constant temperature oven method

(ISTA, 1976). The seeds were ground and two replicates of 5-10g per sample were dried in ventilated oven at 105°C for 16 h. The moisture content was expressed on a wet weight basis.

Germination test
From each packet, two hundred seed (four replications, each with 50 seeds) were tested for germination at each sampling time. The seed were sown on three-layered, moistened paper towels which were rolled and incubated at 22±2°C with 12h/12h Near Ultraviolet light/dark for 10 days. Records were taken for seed germination at 10 days and percent germination was calculated. When radicle and plumule growth were very poor, these were counted as ungerminated.

Pathogen detection test
One hundred seed from each of the six packets, representing two seed lots and three storage conditions, were tested each testing time. The Standard agar plate method was used. Before plating on agar, seed were surface sterilized with 0.1% $HgCl_2$ for 2 min followed by several changes in distilled water. Five seeds were placed on each agar plate and there were 20 replications for each treatment. The plates were incubated at 22±2°C under a 12 h light cycle for 7 days. At the end of the incubation period, each seed was examined microscopically for the presence of the pathogen. Infection/contamination with other microorganisms, if any, was also recorded. A record of seed germination was also taken.

Statistical analysis
Data on infection, germination and moisture for each year was separately analyzed using a one-way analysis of variance (Sendecor and Cochran, 1980). The six combinations of the two seed types and three storage temperatures were taken as treatments. Pair wise differences among treatments were tested using least significant difference at 5% level of significance. Since infection and germination data were analyzed with as well as without arsine transformation. Since the inferences were similar in the two cases results are presented for untransformed data

Results

Effect of storage duration and temperature on germination
Two seed types (apparently healthy and infected) differed in their germination response to storage temperature. The germination of healthy seed did not differ at three storage temperatures for five years. However, the germination of infected seed was decreased with the increase in storage duration at all the storage temperatures (table 1). Some of the small differences in germination of healthy seeds at 4°C and the differences between the germination of infected seeds at one storage time were significant, although they were not consistently so. Similar pattern in germination of the two seed types was observed on potato dextrose agar (table 2).

Table 1. Germination in paper towels of apparently healthy and *Rhizoctonia bataticola* infected groundnut seed stored at 20, 4 and -18°C for 5 years, ICRISATCenter, Patancheru.

Treatment	Storage Temperature (°C)	Germination (%)*					
		1 day	1 year	2 year	3 year	4 year	5 year
Healthy seed	20	98	98	100	98	97	92
	4	98	90	94	92	95	91
	-18	100	99	99	100	97	98
Infected seed	20	84	82	71	72	74	63
	4	85	83	80	70	73	62
	-18	81	84	77	74	75	66
SED		*0.62*	*1.33*	*0.92*	*0.79*	*1.45*	*0.67*

*Data are means of four replicates each with 50 seed.

Table 2. Germination on PDA of healthy and *Rhizoctonia bataticola* infected groundnut seed stored at 20, 4 and -18°C for 5 years, ICRISAT Center, Patancheru.

Treatment	Storage Temperature (°C)	Germination (%)*					
		1 day	1 year	2 year	3 year	4 year	5 year
Healthy seed	20	95	95	97	96	92	90
	4	97	92	94	95	93	87
	-18	93	92	94	92	93	92
Infected seed	20	81	82	76	77	69	59
	4	81	81	83	79	70	62
	-18	80	81	79	81	76	64
SED		*0.14*	*0.15*	*0.16*	*0.16*	*0.17*	*0.21*

* Data are means of four replicates each with 50 seed.

Effect of storage duration and temperature on infection

Incidence of *R. bataticola* in healthy seeds remained unchanged at -18°C. At other temperatures, there was a small increase from 0.2% to 4-5% after 5 years. However, the incidence in the infected seeds increased after four years of storage and was double of the initial infection at 20 and -18°C after 5-year of storage. Statistically, *R. bataticola* infection in infected seeds differed at each temperature and each storage duration but these differences were small (table 3). No other fungi were detected on healthy or diseased seed.

Table 3. Incidence of *Rhizoctonia bataticola* in infected and healthy groundnut seed stored at 20, 4 and -18°C for 5 years, ICRISAT Center, Patancheru.

Treatment	Storage Temperature (°C)	Incidence (%)*					
		1 day	1 year	2 year	3 year	4 year	5 year
Healthy seed	20	0	1	2	1	3	5
	4	2	3	2	3	3	4
	-18	1	1	0	1	1	1
Infected seed	20	17	16	20	18	24	34
	4	19	18	18	23	26	33
	18	16	15	19	18	22	32
SED		*0.13*	*0.16*	*0.16*	*0.17*	*0.19*	*0.22*

*Data are the mean of 20 replicates each with 5 seeds.

The pattern of correlation of storage duration and temperature with moisture content was inconsistent. Also, there was no correlation between the moisture content and germination and also between moisture content and infection of healthy seed. There was, however, significant negative correlation between germination and infection of apparently healthy seed at 20°C and between germination and infection in infected seed at all the temperatures (table 4).

Table 4. Correlation among germination, infection and moisture under different temperatures and seed types (n=6).

Seed type	Temp (°C)	$\Upsilon(M_1G)$	$\Upsilon(M_1I)$	$\Upsilon(G_1I)$
Healthy	20	0.04	0.32	-0.82 *
	4	-0.3	0.50	-0.71
	-18	-0.52	-0.17	0.09
Infected	20	-0.77	0.65	-0.82 *
	4	0.56	-0.39	-0.93 **
	-18	-0.35	0.31	-0.93 **

G = Germination, I = Infection, M = Moisture.; * $P<0.05$, ** $P<0.001$

Discussion

There was no reduction in germination of healthy seed at -18°C but germination of infected seed was reduced even at this temperature, which is being used for long term conservation of seed (Cromarty *et al.*, 1982). Similar results have been reported in case of lentil seed infected with *Ascochyta fabae* f.sp. *lentis* (Kaiser *et al.*, 1989).

Incidence of *R. bataticola* substantially increased in infected seeds at all temperatures. This clearly demonstrates that the pathogen can survive and multiply even at -18°C. It is likely that the pathogen may multiply even at temperature lower than -18°C. A classical example of this is the survival of *Ascochyta rabiei* f.spp. *lentis* after four years of storage at -196°C (Kaiser *et al.*, 1989). However, increase in the level of infection at a given temperature will depend on the initial level of infection provided the genetic constitution of the infected seeds is same. This is supported by the small increase in infection in healthy seeds, with low level of initial infection, compared with the substantial increase and doubling of infection in the infected seed lot. The implication of this result is that only disease-free seed or seed with a very low level of infection should go for long term conservation at –18°C. This necessitates conducting seed health test prior to storage for conservation, which will help in discarding heavily infected seed lots and will ensure high viability of seed for longer period of time.

References

Abdou, Y.A. and Khadr, A.S. (1974). Systemic control of seedling and pod rot diseases of peanuts. *Plant Disease Reporter,* **58**, 176–179.

Bewley, J.D. (1986). Membrane changes in seeds as related to germination and the perturbations resulting from deterioration in storage. CSSA, **I,** 27–48.

Cromarty, A.S., Ellis, R.S. and Robert, E.H. (1982). The design of seed storage facilities for genetic conservation. *International Board of plant Genetic Resources (IBPGRI),* 96pp.

Diekmann, M. (1988). Seed health testing and treatment of germplasm at the International Center for Agricultural Research in the Dry Areas (ICARDA). *Seed Science and Technology,* **16**, 405–417.

Fletcher, S.M. and Ping Zhang Carley, D.H. (1992). Groundnut production, utilization and trade in the 1980's. In; Nigam, S.N. ed. Groundnut: A global perspective. *Proceedings of an International Workshop, 25–29 November 1991,* ICRISAT Center, India, 17–32, International Crops Research Institute for the Semi-Arid Tropics, Patancheru andhra Pradesh, India.

Garren, K.H. and Wilson, C. (1951). Peanut diseases. Pages 262–324 *In*: 'The Peanut', the unpredictable legume. Washington, D.C., USA; National Fertilizer Association.

Sendecor, G.W., Cochran, W.G. (1980). *Statistical methods.* Seventh edition. The Iowa State University press. Ames, USA. 1-458.

Girish, A.G., Singh, S.D., Chakrabarty, S.K., Rao, P., R.D.V.J., Surender, A., Varaprasad, K.S. and Bramel, P.J. (2001). Seed microflora of five ICRISAT mandate crops. *Seed Science and Technology,* **29,** 429–443.

Hewitt, P.D. (1987). Pathogen variability in seed in deep freeze storage. *Seed Science and Technology,* **15**, 73–77.

International Seed Testing Association (1976). International rules for seed testing. *Seed Science and Technology,* **4,** 3–49.

Kaiser, W.I., Stanwood, P.C. and Hannan, R.M. (1989). Survival and pathogenicity of *Ascoshyta fabae* f. spp. *lentis* in lentil seeds after storage for four years at 20 to –196°C. *Plant Disease,* **73**, 762–764.

Kurdikeri, M.B., Giriraj, K., Darkpur, H.W., Hiremath, N.V. and Hanumaratti, N.G. (1996). Groundnut seed viability under ambient storage conditions of Dharwad. *Karnataka Journal of Agricultural Sciences,* **9**, 363–365.

Mercer, P.C. (1977). A pod rot of peanut in Malawi. *Plant Disease Reporter,* **61**, 51–55.

Nautiyal, P.C. and Zala, P.V. (1993). Groundnut genotypes with longer seed viability. *Groundnut news,* **5**, 2–3.

Nautiyal, P.C., Joshi, Y.C. and Zala, P.V. (1996). A storage method to prolong seed viability in groundnut. *Agriculture and Equipment International,* **48,** 43–44.

Plucknett, D.L. and Smith, N.J.H. (1989). Quarantine and Exchange of crop genetic resources. *Bioscience,* **39,** 16–23.

Plucknett, D.L., Smith, N.J.H., Williams, J.T. and Murthi Anishetty, N. (1987). Gene Banks and the world's food Princeton University press, Princeton, N.J. 247 pp.

Porter, D.M., Powell, N.L. and Cobb, P.R. (1977). Severity of *Sclerotinia minor*, blight of peanuts as detected by infrared aerial photography. *Peanut Science,* **4,** 75–77.

Proceeding of the International Workshop on Groundnut. International Crops Research Institute for the Semi-Arid tropics (ICRISAT), Patancheru P.O. 502 324, Hyderabad andhra Pradesh, India. 13-17 Oct. 1980. Page 197.

Rao, V.V.R., Reddy, M.S. and Rao, K.C. (1996). Effect of fungicidal treatment on the viability of groundnut. *Seed Research,* **29,** 66–69.

Subrahmanyam, P., Reddy, D.V.R., Sharma, S.B., Mehan, V.K. and McDonald, D. (1990). A world list of groundnut diseases. *Legumes Pathology Progress Report* **12**, 14. Legumes Program, International Crops Research Institute for the Semi-Arid Tropics, Patancheru andhra Pradesh, India.

Tripathi, S.K., Patra, A.K., Samui, R.C., Nanda, M.K. (1996). *Indian Journal of Plant Pathology* **1**, 180–184.