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Viability of Coffee Leaf Rust (*Hemileia vastatrix*) Urediniospores Stored at Different Temperatures

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Viability of spores samples of *Hemileia vastatrixf*, the fungal pathogen causing leaf rust disease in coffee was checked after storing at room temperature, -20°C and -80°C for one year. While the spore samples lost their viability as early as 45 days at room temperature and thus retained viability for about 120 days at -20°C. Spores stored at -80°C retained about 52% viability even at the end of one year. The results clearly indicated that urediniospores of *Hemileia vastatrix* can be preserved at -80°C for one year and more.

Coffee is the most important agricultural product in the world, being second only to the petroleum in the global trade. India is among the top six largest coffee producers in the world. India produced 43,72,000 bags and exported 31,45,000 bags in the year of 2008/09 (ICO, http://www.ico.org/). Globally 65% of total coffee production comprises of the arabica coffee. *C. canephora* accounts for the remaining 35%. *Coffea arabica* varieties produce good coffee quality, but the genetic diversity in the species is limited, rendering the plants highly susceptible to pests and diseases. Coffee leaf rust (CLR) (orange rust) is the most destructive disease of coffee caused by the fungus *Hemileia vastatrix*. Even though *H. vastatrix* can attack several species of the genus *Coffea*, it is most devastating on *C. arabica*. It was first of all discovered in Lake Victoria in Africa in 1861 and subsequently observed in Srilanka in 1868 and in Brazil in 1970, reported from India in 1869. The pathogen has spread to all the coffee producing countries in the world. The infection is manifested as yellow-orange powdery lesions on the abaxial surface of leaves, and leads to impaired photosynthesis, premature defoliation and reduced floral initiation resulting in tremendous yield loss that may reach as much as 70%. The urediniospores produced in these lesions are responsible for the dispersal, survival and infection structure of the pathogen. Teliospores are occasionally produced, germinate to produce basidiospores. However, infection of basidiospores to coffee has not been demonstrated and so far, no alternate host has been found. The pathogen exists in several physiological forms or races and breaks down the resistance in the host plant in due course by evolving new races. While 45 *H. vastatrix* races have been identified globally, as many as 37 races (26 designated + 11 new races) have been isolated from India [1]. At present, the isolated and identified rust races are maintained on susceptible plants under green house conditions. It requires frequent re-inoculation from old leaves to new leaves in isolated chambers to avoid cross contamination. Maintenance of large number of races requires larger space and manpower. The infected leaves defoliated after few days of infection which may cause loss of known races and to re-isolate the same race requires couple of years. Hence, the study for preservation of known rust races with high viability was optimized.

The degree of resistance to rust by coffee lines depends on the type of rust race [2]. Many studies are presently being carried out to understand the molecular basis of the interaction between coffee and rust [3]. Parallel to this, plant breeders are putting in tremendous efforts to generate introgressed lines that exhibit disease resistance to different races of rust.

One major requirement for studies like screening for natural resistance in the host germplasm and screening of fungicides to control rust outbreaks is the continuous availability of viable spores. Since, *H. vastatrix* is a biotropic fungus and culture of the fungus on an artificial medium is not possible. *H. vastatrix* cultures are usually maintained by repeated infection on susceptible plants under controlled conditions, which is a tedious and laborious process. An alternative to overcome this problem is to preserve spores under appropriate conditions. Though there is a considerable literature available on the germination conditions of coffee rust [4], there are only limited studies on preservation of CLR spores [5,6]. The present study was conducted to identify the condition for preserving the CLR spores at least for a year.

Urediniospores samples of race I and race VIII of *H. vastatrix* were harvested from the mature pustules on leaves of susceptible germ types of arabica coffee plants maintained in green house at Regional Coffee Research Station, Thandigudi, Tamil Nadu, India. Harvested spore samples were put in pharmaceutical grade gelatin capsules and transported to Plant Biotechnology Division, Mysore under ambient conditions for the study. These spore samples were used in the present study. Viability of the spores was calculated at different intervals upto one year after storing at dark room temperatures, -20°C and -80°C.

Storage and testing the viability of spores

Spore samples in 10 mg aliquots in 0.5 ml micro tubes (Tarson) were stored at room temperature, -20°C in a deep freezer (Forma Scientific) and -80°C in an ultra-deep freezer (Forma Scientific) in dark. Viability of spore samples was checked before storage and at different intervals of storage in different treatments up to one year. For checking the viability, small aliquots of spore samples were germinated on water agar. Spore samples stored at -20°C and -80°C were incubated in a water bath (Grant) at 40°C for 10 minutes as suggested by Kushalappa (1989) before plating on water agar. The spore aliquots (about 10 mg) were suspended in 5 ml sterile reverse-osmosis grade water by vortexing for 10 seconds. 1 ml spore suspension was spread uniformly on 2% water agar in petri plates under sterile conditions. The agar plates were sealed

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with parafilm (Sigma) and incubated overnight for 16 h at 23°C in dark in an incubator (Sanyo). After incubation, the spores were observed under a light microscope (Nikon) at 10X magnification and stored for germination. Five replicated plates of each sample were used to assess the germination frequency. On each plate, 100 fields of view of around 0.6 cm2 were observed randomly and the germinated/ungerminated spores were counted (Figure 1). Germination percentage was calculated for each replicate. Arithmetic mean was calculated from the percentage germination value of the five replicates of each set of experiment and the standard deviation was calculated. Viability of spores stored at different temperatures is given in Table 1.

Room temperature 25 ± 5°C

The germination of fresh spores was 30% in both the races race I and race VIII. By 15 days of storage at room temperature (25 ± 5°C), germination percentage was greatly reduced to 94% in race I and 77% in race VIII. By 60 days onwards the germination was negligible, although the aliquots of the same stock preserved under cold storage were still viable.

Deep freezer (-20°C)

Rust race stored at -20°C showed some improvement in germination for race VIII with about 55% spores being germinated. However, no significant change was observed in case of race I stored at -20°C until 60 days of storage where the mean germination percentage recorded was around 45%. By 60 days race VIII spores preserved at -20°C had showed reduction in revival. Race I spores stored on -20°C germination percentage was reduced completely on 210 days of preservation whereas viability was retained in race VIII spores for one year of preservation.

Ultra-deep freezer (-80°C)

After the storage at -80°C for 15 days, there was some increase in germination percentage in both the races. Germination percentage of spores stored at -80°C showed fluctuation over the sampled period from 21.16 to 51.98%. Although the higher viability was noticed after 15 days for both the races at -80°C, it disappeared by 30 days in case of race I with germination percentage falling to around 30%. A second peak of higher viability was noticed for race-I after 60 days of storage when the germination percentage was recorded almost 55%. Preservation beyond 120 days showed a uniform increase in germination percentage and was last recorded to be about 52% at the end of one year. Race VIII spores preserved at -80°C differed from race I in their state of higher

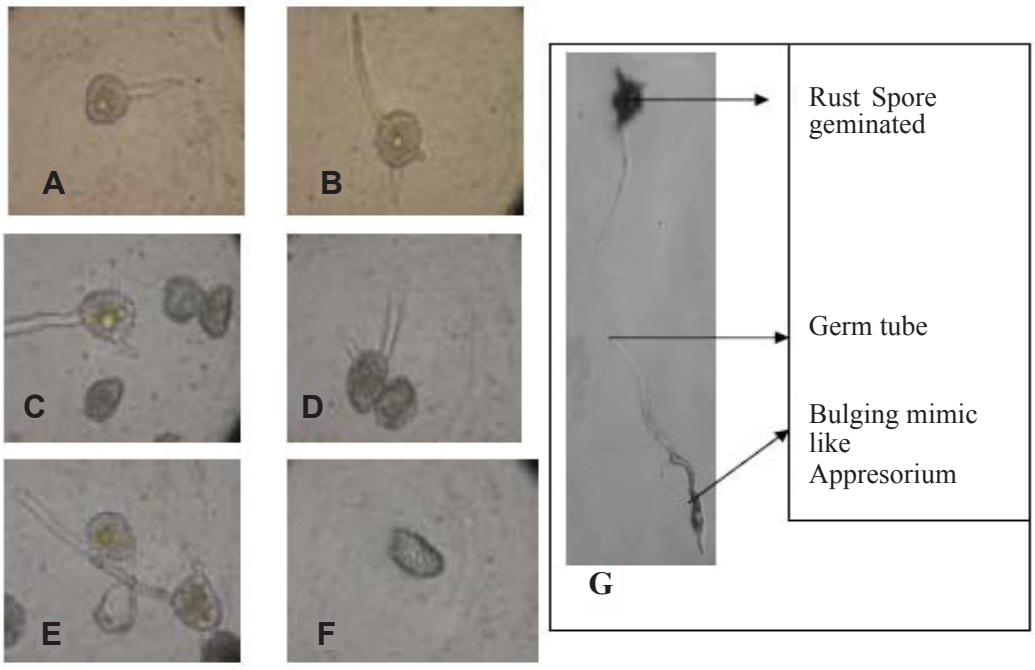

Figure 1: Germination of *Hemileia vastatrix* urediniospore after storing at different temperatures a, b, c: Germination of fresh urediniospores cultured for 8h,[(a)] 12h,[(b)] and 16h[(c)] on water agar. Swollen appresorium like structure is seen at the end of the germ tube. (D) Several viable spores have germinated after storing at -80°C for one year indicating good viability. (E) Non-viable spore.

Storage temperature	Race	Period of storage in days						
		0	15	30	60	120	210	360
Room temperature	I	30.06 ± 4.15*	1.2 ± 1.2*	4.32 ± 5.35*	0	0.2±0.45*	0.2±0.44*	0
	VIII	29.14 ± 5.35*	6.68 ± 3.40*	14.62 ±8.16*	0.4 ± 0.55*	0	0	0
-20°C(Deep freezer)	I	21.68 ± 4.59*	25.06 ± 5.98*	28.48±15.35*	44.72±7.04*	13.74±5.06*	0.8±0.73*	0.46 ± 0.27*
	VIII	40.72 ± 10.9*	55.58 ± 4.86*	48.1±11.68*	15.36±5.90*	17.4±6.53*	7.1±3.39*	1.36 ± 0.34*
-80°C(Ultra deep freezer)	I	30.78 ± 5.99*	40.74 ± 8.70*	29.56±3.30*	54.92±3.12*	25.94±10.71*	30.66±8.98*	43.58 ± 4.44*
	VIII	41.48 ± 10.80*	49.02 ± 3.39*	46.48±8.51*	50.46±16.54*	40.86±13.57*	21.16±11.1*	51.98±3.13*

(* Mean percentage of germination with standard deviation).

Table 1

viability, which was maintained almost constant from 15 days to 60 days but fell steeply after 210 days. A second state of higher viability was observed after 360 days when the spores recorded viability of 52%.

Cold storage at both -20°C and -80°C seems to enhance germination in both the races at least up to 15 days. This easy preservation condition would eliminate the need of cumbersome processes like maintenance culture on susceptible plants or constant supply of liquid nitrogen for storage at -196°C. The results obtained herein would benefit researchers of various fields, providing them easy preservation of rust considering the fact that viability of coffee rust is more sensitive to temperature and light conditions [4]. Coffee rust spores were maintained up to 150 days with high viability in liquid nitrogen at -196°C [5].

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Reference

1. Prakesh NS, Ganesh D, Bhat SS (2005) Population dynamics of coffee leaf rust and recent advances in rust research in India 411- 442.
2. Rodrigues CJ, Bettencourt AJ, Rijo L (1975) Races of the pathogen and resistance to coffee rust. Annu Rev Phytopathol 13: 49-70.
3. Fernandez D, Santos P, Agostini C, Bon MC, Petitot AS, et al. (2004) Coffee (*Coffea Arabica* L.) genes early expressed during infection by the rust fungus (*Hemileia vastatrix*). Mol Plant Pathol 5: 527-536.
4. De Jong EJ, Eskes AB, Hoogstraten JGJ, Zadoks JC (1987) Temperature requirements for germination, germ tube growth and appressorium formation of urediospores of *Hemileia vastatrix*. European Journal of Plant Pathology 93: 61-71.
5. Kushalappa AC, Eskes AB (1989) Biology and Epidemology. Coffee rust: Epidemiology, resistance and management: CRC Press.
6. Escobaro C, Cristancho AM (2007) Estudio de metodolongias para la conservacion de urediniosporas de la roya del cofeto. Cenicafe 58: 324-332.