Proc. Indian Acad. Sci., Vol. 88 B, Part II, Number 6, November 1979, pp. 479-486.

Stimulation of teliospore germination in smut fungi*

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MS received 19 April 1979; revised 14 July 1979

Abstract. Preheating and heated teliospore extracts have been found to be stimulatory to germination of the teliospores of ten different smut fungi used in this study. The effect is mostly on the percentage of germination.

Maximum per cent of spore germination was observed at higher concentrations of glucose and sucrose solutions. The percentage of germination of spores of the species included was more in sucrose than in glucose. Most of the vitamins of B-complex groups stimulated the initiation of germ tube.

Many of the growth regulators tried, except 2,4-dichlorophenoxy acetic acid have a stimulatory effect on teliospore germination. Higher percentage of teliospore germination was noticed in the case of gibberellic acid, followed by indole 3-acetic acid, beta-indole butyric acid and alphanaphthalene acetic acid.

Ethylenediamine tetraacetic acid, furfural, fumaric acid, oxalic acid and citric acid were also stimulatory, in that order.

Keywords. Stimulants; teliospore germination; smut fungi.

1. Introduction

The effects of germination stimulants have recently been extensively studied with the uredospores of the rust fungi which are particularly sensitive to higher straight chain aldehydes and alcohols (French 1961, 1962; French *et al* 1977, 1978). Germination stimulants have also been isolated from the fungal spores and include among others, coumarins and phenols (Allen 1957; Sussman 1966). In nature, the balance between inhibitors and stimulants probably serves as a regulatory factor in germination. Metabolic transformations of stimulatory aldehydes are carried out by the uredospores of stem rust (Scarles and French 1964).

Various stimulants or treatments have been devised from time to time to induce germination in smut teliospores (Fischer and Holton 1957; Schauz 1968; Saxena and Khan 1971). Noble (1924) made an extensive study on the effects of many different substances in attempting to stimulate germination of the spores of *Urocystis tritici*.

* The work reported herein is a part of the thesis submitted by the senior author for Ph.D. degree at the University of Mysore (1974).

Our studies have shown that the teliospores of some of smut fungi exhibited poor germination under 18–24° C and relative humidity 100 per cent. In an attempt to enhance the germination of teliospores of some of the smut fungi under investigation, the effect of preheating, heated teliospore extract, sucrose, glucose, vitamins, growth regulators and a few other chemicals have been studied, and the results are reported in this paper.

2. Materials and methods

Teliospores of the following species of the smut fungi were subjected to the effect of high temperature treatment:

(*a*) *Cintractia axicola* (Berk.) Cornu.
(*b*) *Sphacelotheca andropogonis-annulati* (Bref.) Zundel.
(*c*) *Sphacelotheca andropogonis* (Opiz) Bubak.
(*d*) *Sphacelotheca iseilematis* (Syd. & Butler) Mundk. & Thirum.
(*e*) *Sphacelotheca reiliana* (Kuhn) Clint.
(*f*) *Sphacelotheca rottboelliae* (Syd. & Butler) Mundk.
(*g*) *Ustilago utriculosa* (Nees) Tul.
(*h*) *Sorosporium indicum* Mundk.
(*i*) *Liroa emodensis* (Berk.) Ciferri.
(*j*) *Tilletia vittata* (Berk.) Mundk.

Teliospore suspensions of the above 10 species were prepared separately in distilled water and each sample was further divided into 7 equal parts. Six of each were subjected to 40°, 45°, 50°, 55°, 60° and 70° C for 5 min. The supernatant was decanted off and standard suspensions of the treated teliospores were prepared in distilled water and their germination was studied as described by Thirumalachar (1940). The seventh portion of the suspension (unheated) served as a control. The percentage of teliospore germination was calculated 24 hr after the initiation of spore germination.

2.1. *Effect of heated teliospore extract on spore germination*

The teliospore extract was prepared by taking concentrated spore suspensions of the above 10 species separately in 20 ml distilled water and heated to 80° C for 10 min on a water bath. The supernatant liquid was decanted off and filtered through Whatman filter paper No. 1. The filtrate was then used for making the standard suspensions of fresh teliospores in such a way that each sample was incubated for germination in its extract. Observations were made both for per cent germination and length of germ tube.

2.2. *Chemical treatment and teliospore germination*

The following chemicals were screened for their effects on the percentage of teliospore germination and germ tube length, by incubating spores in them.

(i) Glucose and sucrose solutions in distilled water at 1, 2, 3 and 5% concentrations.

(ii) The vitamins, thiamine, biotin, nicotinic acid, pantothenic acid and para-aminobenzoic acid (PABA) at 1 ppm concentration.

(iii) Alpha-naphthalene acetic acid (NAA), beta-indol butyric acid (IBA), indol-3-acetic acid (IAA), parachlorophenoxyacetic acid (PCPA), gibberellic acid (GA), and 2,4-dichlorophenoxy acetic acid (2,4-D) which are plant growth regulators, were used at 5 ppm concentration.

(iv) Ethylenediaminetetraacetic acid (EDTA), fumaric acid, oxalic acid and citric acid were also used at 10 ppm concentration. Furfural was tried at 1 ppm dilution. Germination in distilled water at room temperature (22° C) served as control in all the cases.

Number of teliospores counted per treatment were 400 in three replicates for all the experiments.

3. Observations

Preheating of teliospores (table 1) to 40°, 45° and 50° C had a stimulatory effect. Maximum percentage germination was at 45° C. At 60° C and above, the spores were killed. Teliospores of *C. axicola, S. andropogonis, S. rottboelliae, U. utriculosa, L. emodensis* could not withstand preheating even up to 55° C. In other species, however, the germination was reduced considerably at this temperature.

Heated teliospore extract has also shown the stimulatory effect on the percentage of teliospore germination (table 2). The effect was more pronounced in the case of *C. axicola, S. iseilematis, S. rottboelliae* and *S. indicum*.

Experiments on the effect of two sugars on teliospore germination of different species of smuts showed that sucrose (table 3) was the best carbon source for *S. andropogonis-annulati, S. andropogonis, U. utriculosa, S. indicum, L. emodensis* and *T. vittata* followed by glucose (table 3).

Table 1. Effect of preheating of teliospore on their subsequent germination.

Species	Percentage germination				
	Treatment at				Control
	40° C	45° C	50° C	55° C	22° C
Cintractia axicola	76	82	66	0	61
Sphacelotheca andropogonis-annulati	62	75	58	2	48
Sphacelotheca andropogonis	68	80	58	0	56
Sphacelotheca iseilematis	71	86	69	11	59
Sphacelotheca reiliana	65	68	45	28	12
Sphacelotheca rottboelliae	58	82	61	0	54
Ustilago utriculosa	70	78	66	0	60
Sorosporium indicum	81	86	72	5	54
Liroa emodensis	83	79	64	0	51
Tilletia vittata	75	81	72	7	60

Table 2. Effect of spore extracts on the teliospore germination.

Species	Percentage germination	
	Treated	Control (distilled water)
Cintractia axicola	75	56
Sphacelotheca andropogonis-annulati	66	52
Sphacelotheca andropogonis	72	60
Sphacelotheca iseilematis	81	58
Sphacelotheca reiliana	33	8
Sphacelotheca rottboelliae	85	61
Ustilago utriculosa	70	52
Sorosporium indicum	75	55
Liroa emodensis	66	57
Tilletia vittata	70	50

Table 3. Effect of glucose and sucrose on teliospore germination.

Species	Percentage germination								
	1%		2%		3%		5%		Control (distilled water)
	glucose	sucrose	glucose	sucrose	glucose	sucrose	glucose	sucrose	
Cintractia axicola	56	64	63	69	69	73	72	78	52
Sphacelotheca andropogonis-annulati	61	67	66	76	71	81	83	89	56
Sphacelotheca andropogonis	58	62	61	71	70	80	75	86	54
Sphacelotheca rottboelliae	66	59	66	68	68	71	72	76	50
Sphacelotheca reiliana	20	54	32	62	55	66	62	70	10
Sphacelotheca iseilematis	61	63	62	68	66	71	73	78	60
Ustilago utriculosa	60	62	65	66	70	73	82	89	54
Sorosporium indicum	62	65	68	70	75	81	80	92	58
Liroa emodensis	68	71	71	76	76	82	83	91	60
Tilletia vittata	60	65	65	70	70	76	78	82	53

3.1. *Effect of vitamins*

Most of the vitamins screened were stimulatory in that they increased the percentage of germination but not the germ tube length. Thiamine induced higher percentage of germination in the teliospores of *S. iseilematis*, *U. utriculosa* and *S. indicum*, followed by biotin, nicotinic acid, pantothenic acid and PABA.

Pantothenic acid and biotin exhibited no effect on the teliospore germination of *C. axicola*, *S. andropogonis*, *S. rottboelliae*, *L. emodensis* and *T. vittata* while thiamine, nicotinic acid and PABA induced higher percentage of germination. On the other hand, thiamine and nicotinic acid had the stimulatory effect on *S. andropogonis-annulati* and *S. reiliana* followed by biotin. Pantothenic acid had no effect while PABA was inhibitory. Sporidial formation was abundant in all cases.

Most of the growth regulators tried stimulated teliospore germination (table 4). However, except *S. reiliana* and *S. rottboelliae*, all were inhibited by 2,4-D. Slight stimulatory effect was noticed with PCPA. Higher percentage of teliospore germination was observed with GA, IAA, NAA and IBA.

Of the five miscellaneous chemicals tried, EDTA triggered maximum teliospore stimulation, followed by furfural, fumaric acid, oxalic acid and citric acid (table 5).

With regard to the effect of the chemicals in table 5 on the length of the germ tube, it was observed that the results did not agree with those obtained with the percentage of germination. The germ tube length showed very little increase in furfural and fumaric acid. However, there was great retardation in the growth of the germ tubes in EDTA, citric acid and oxalic acid.

4. Discussion

Teliospores appear to be stimulated to germinate after a few minutes exposure to temperatures of 40°, 45° and 50° C, particularly to 45° C (table 1). Noble (1924),

Table 4. Effect of plant growth regulators on teliospore germination.

Species	Percentage germination						
	NAA	IAA	IBA	GA	2,4-D	PCPA	Control (distilled water)
Cintractia axicola	62	66	64	68	45	55	51
Sphacelotheca andropogonis-annulati	66	68	63	72	50	56	54
Sphacelotheca andropogonis	70	75	71	78	52	65	60
Sphacelotheca iseilematis	68	76	72	80	50	68	56
Sphacelotheca reiliana	31	29	33	36	18	21	12
Sphacelotheca rottboelliae	75	73	62	75	58	63	50
Ustilago utriculosa	76	78	71	83	52	65	61
Sorosporium indicum	73	75	75	78	54	67	56
Liroa emodensis	74	71	73	82	46	65	52
Tilletia vittata	64	66	68	69	56	63	58

Table 5. Effect of an aldehyde and four carboxylic acids on teliospore germination.

Species	Percentage germination					
	Furfural	EDTA	Fumaric acid	Oxalic acid	Citric acid	Control (distilled water)
Cintractia axicola	74	78	71	63	67	63
Sphacelotheca andropogonis-annulati	80	87	69	66	61	52
Sphacelotheca andropogonis	75	80	68	64	58	50
Sphacelotheca iseilematis	78	83	71	66	61	58
Sphacelotheca reiliana	30	34	17	14	12	8
Sphacelotheca rottboelliae	81	84	76	74	60	61
Ustilago utriculosa	74	78	73	66	61	56
Sorosporium indicum	79	81	70	67	66	60
Liroa emodensis	77	84	71	68	61	54
Tilletia vittata	70	72	66	61	58	51

Smart (1936) and Lilly and Barnett (1951) believed that in general high temperature hasten the maturity, besides causing an increase in permeability of the spore wall as well as of the spore contents resulting in quicker ingress of water and in better germination. Shear and Dodge (1927), Goddard and Smith (1938) and Evans and Curran (1943) reported that preheating conidia of *Neurospora tetrasperma* to 30° C or more for a few minutes broke the dormancy and induced 100% germination, within 2 or 3 hr. The results from the present investigation seem to suggest high temperature as a teliospore stimulant in all the species studied. In view of this, it can be expected that if the teliospores of smut fungi are exposed to high summer temperatures in nature, severe smut infection could be expected in the following season.

The results in table 2 suggest that some water soluble substances present in the spore extract may have stimulated the teliospore germination, especially in *C. axicola*, *S. iseilematis*, *S. rottboelliae* and *S. indicum*.

Sucrose stimulated the germination of teliospores of all species included more than glucose. This was probably due to the fact that 1% sucrose solution contains more carbon (0·42) while that of 1% glucose solution contains less (0·4) carbon.

Considerable work has been done on the vitamin requirements on growth of smut fungi (Fischer and Holton 1957). However, nothing seems to be on record regarding the role of these factors in the germination of teliospores. Most of the vitamins of the B-complex group are stimulatory for the initiation of the germ tube. Our findings agree with those of Cooper (1939) and Singh Pritam (1966), who reported that thiamine and a few other vitamins induced high spore germination percentage in *Colletotrichum gloeosporides* Pens and *C. falcatum* Went.

The effects of growth regulators tried, on smut fungi were similar to observations made by Lin (1945), Wei and Ling (1948), Naito and Tani (1951), Peterson (1951) and Singh Pritam (1965) in several fungi.

EDTA was highly stimulatory to teliospore germination but was inhibitory to the further growth of the germ tube. A possible mode of action of EDTA in spore germination was proposed by Sussman (1953, 1954) who reported EDTA as a chelating agent, which acts first on the spore wall and then on the spore contents. It removes minerals lying on the former and those present endogenously within the latter, th reby increasing the permeability. As a result, there is more ingress of water or nutrients from the outside, conversion of insoluble food to soluble form, activation of the enzyme systems resulting in increased respiration and more germination.

Acknowledgements

The authors wish to record their thanks to Agricultural Research Service, USA, for the financial help under the PL–480 grants. Thanks are due to the authorities of the University of Mysore for laboratory facilities.

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