

Effect of sulphite on spore germination and rhizoid development in the tropical fern *Lygodium japonicum* (Filicales: Lygodiaceae)

A.N. Sahi¹ and S.K. Singh²

¹ Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi - 221005, India.

² Environmental Engineering Section, MECON, Doranda, Ranchi (Bihar), India.

(Rec. 17-VIII-1992. Acep. 2-XI-1993)

Abstract: A 24 hr red light treatment followed by white light enhanced germination rate on spores of *Lygodium japonicum* Swartz. Sulphite adversely affected germination and caused abnormalities in rhizoidal growth. In sulphite-free media the apical growth left behind the swollen portion of the rhizoids. *L. japonicum* gametophytes are good bioindicators of SO₂ pollution.

Key words: *Lygodium japonicum*, pollution, sulphite, spore, germination, growth.

Sulphur dioxide is a major air pollutant in urban-industrial areas, leading in plants to growth retardation (Bell *et al.* 1979, Buckenham *et al.* 1982) and reduction of photosynthesis (Jones and Mansfield 1982) and carbohydrate contents (Heath 1980). Sulphur dioxide is reported to affect the germination of spores of some bryophytes, and of pollen in pine, lilly etc. (Keller and Beda 1984, Mitsugi *et al.* 1985, Nakada *et al.* 1970). Once inside the cells SO₂ dissolves in water to form H₂SO₃ which dissociates into SO₃²⁻, HSO₃⁻ and H⁺ depending upon the pH of the medium (Puckett *et al.* 1973). It has been suggested that an aqueous solution of sulphite be applied as an alternative to SO₂ fumigation to study the effect on plants (Miller and Xerikos 1979, Garsed 1981). Since the life cycle of ferns is short and the developmental process of fern gametophyte is light-dependent (Furuya 1983) and easily observable under the microscope, Wada *et al.* (1986) proposed an excellent model system to investigate the toxicity of SO₂ to fern cells. Moreover, the cultures grown under liquid medium can easily be manipulated to observe

the effects of exogenously added substances. In the present study, the effect of sulphite on the germinating process of *Lygodium japonicum* Sw. spores and further development are analysed.

MATERIAL AND METHODS

Spores of *Lygodium japonicum* Swartz. a tropical fern cultivated in the University Botanical Garden were collected and imbibed in water for 24 hr before sowing aseptically in AF Dyer's nutrient media after sterilization with 0.3% sodium hypochlorite solution (Dyer 1979). The petri dishes (5 cm) were kept separately at 24±2°C under white light (8.7 Wm⁻²) and red light (4.8 Wm⁻²) to see the effect of several light treatment periods on spore germination (Table 1). For each treatment 100 spores were picked on glass slides from the aseptic cultures, mounted in Acetocarmine-Chloral hydrate stain and examined under 100 magnifications for germination counts (Edwards and Miller 1972). Spore coats often dissociated from germinating spores and

TABLE 1

Effect of red light treatment of various duration followed by white light on spore germination of L. japonicum

White light initiation		Red light initiation					
Days	Germination (%)	8hr		16 hr		24 hr	
		Days	Germination (%)	Days	Germination (%)	Days	Germination (%)
5	93	5	92	5	90	3	90

TABLE 2

Effect of K-phosphate buffer, sulphite, sulphate on fern spore germination of L. japonicum (values are average \pm SE)

Concentration of K-phosphate buffer (M)	Control	Spore germination (per cent)			
		Na ₂ SO ₃ conc. (M)		Na ₂ SO ₄ conc. (M)	
		1 mM	10 mM	1 mM	10 mM
1 mM	93.66 \pm 1.52	75.66 \pm 3.21	19.66 \pm 2.08	89.66 \pm 4.04	41.33 \pm 1.53
10 mM	67.33 \pm 2.08	57.33 \pm 3.05	17.33 \pm 1.52	55.33 \pm 3.05	28.33 \pm 1.53
100 mM	27.00 \pm 2.00	27.33 \pm 4.04	12.66 \pm 1.53	25.33 \pm 2.08	13.33 \pm 1.52

aborted or empty spore coats were not counted (Table 1).

Some modifications were carried out in the rest of the experiments. Approximately 0.5 mg of spores were imbibed for one day in darkness and then suspended in 6 ml of culture medium (potassium phosphate buffer, 1 mM, 10mM and 100 mM; pH 6.0) to be cultured under continuous red light of 4.8 Wm⁻² for 3 days. The definition of spore germination was the emergence of rhizoid or protonemal cell out of a spore coat. All spores under one microscopic field was scored for per-cent germination (three replicate samples per culture and three replicate cultures per treatment). Sample size for each observation varied from 20 to 60 spores per microscopic field. Approximately 300 spores were scored per treatment to calculate standard errors of the average per-cent germination. Sulphite was applied as a solution of Na₂SO₃ (dissolved just before use in an autoclaved medium using sterilized glassware). Sulphite was not autoclaved to prevent destruction. Solutions of Na₂SO₄ were also prepared in the above manner (Table 2).

To see the effect of sulphite treatment on spore germination and rhizoidal growth the spores were cultured under continuous red light

(R) of 4.8 Wm⁻² for four days after imbibition in dark (D) for 48 hrs (Table 4). Sulphite treatment of 3 mM (Na₂SO₃) in K-phosphate buffer was given for 24 hrs on consecutive days to each set during spore germination. Data were taken on the 7th day of spore inoculation and are presented as mean \pm SE.

RESULTS

The treatment of 24 hr of red light followed by white light enhanced the rate of germination (Table 1). However, continuous exposure of red light could not bring any significant difference in the formation of antheridia, archegonia and sporophyte.

Metal ions of AF Dyer nutrient media promotes oxidation of sulphite; hence, for the growth of fern gametophytes a replacement with potassium-phosphate buffer was made in the present work (Fuzzi 1978, Beilke and Gravenhorst 1978, Hegg and Hobbs 1978). Beyond 10 mM concentration of K-phosphate buffer the per-cent spore germination decreased irrespective of the presence or absence of sulphite or sulphate in the medium. At 1 mM concentration of buffer, spore germination was

TABLE 3

pH changes of sulphite and sulphate on different concentrations of K-phosphate buffer and incubation times

Concentration (M)	Incubation time (days)	Concentration of K-phosphate buffer		
		1 mM	10 mM	100 mM
Control	0	6.01	6.00	6.01
	5	6.11	6.01	6.02
10 mM Na ₂ SO ₃	0	6.97	6.59	6.21
	5	6.38	6.23	6.20
1 mM Na ₂ SO ₃	0	6.71	6.04	6.01
	5	6.31	6.03	6.02
10 mM Na ₂ SO ₄	0	6.27	6.06	5.99
	5	6.39	6.08	6.02
1 mM Na ₂ SO ₄	0	5.87	6.12	5.99
	5	6.17	6.07	6.13

TABLE 4

Effect of sulphite treatment on spore germination and rhizoidal growth (D = Dark, R = Red light)

Duration of sulphite treatment (hr)	Light condition	Germination (%)	Rhizoidal abnormalities (%)	Rhizoid length (µm)
0-24	D	76.33 ± 2.51	5.71 ± 1.04	36.33 ± 3.05
24-48	D	70.66 ± 2.08	7.10 ± 1.61	33.66 ± 1.53
48-72	R	65.00 ± 3.60	10.32 ± 1.40	35.66 ± 2.08
72-96	R	71.33 ± 3.05	13.08 ± 0.52	24.66 ± 1.69
96-120	R	74.66 ± 3.57	68.87 ± 4.57	20.00 ± 2.64
120-144	R	81.33 ± 3.05	71.60 ± 2.73	16.33 ± 1.53

93.66% but a slight inhibition with respect to control was demonstrated on application of sulphite and sulphate (Table 2). Inhibition in spore germination varied significantly with increase in sulphite (ANOVA, $F = 289.42$; $p < 0.001$) and buffer concentrations ($F = 204.50$; $p < 0.001$). Their interaction was also significant ($F = 38.35$; $p < 0.001$). Similarly, spore germination was significantly inhibited by sulphate ($F = 289.42$; $p < 0.001$) and buffer concentrations ($F = 327.40$; $p < 0.001$). Interaction between sulphate treatment and buffer concentration was significant ($F = 23.52$; $p < 0.001$).

Sulphite and sulphate may change the pH of the K-phosphate buffer. Hence the buffering capacity of the medium was tested (Table 3). When using the 10 mM and 100 mM concentrations of K-phosphate buffer the dissolved sulphite showed very little variation in pH (with respect to the control). Nevertheless, after dissolving sulphite at 1 mM (buffer concentration) pH increased initially; a gradual decrease was observed by the 5th day of the experiment. The variation in the pH of buffer at all concentrations due to sulphite and sulphate did not differ from the control (Student's t-test, $p < 0.05$).

A sulphite treatment (Na_2SO_3) of 3 mM was given to fern spores for 24 hr on consecutive days from day one to day six (Table 4). Observations were made on the 7th day to ascertain sulphite sensitivity on the processes of spore germination, induced rhizoidal abnormalities and rhizoidal length. Germination declined sharply on the third day of sulphite application *i.e.* the first day of red light treatment. Rhizoidal abnormalities were high on the third and fourth day of red light irradiation when the rhizoid showed active apical growth. Swollen rhizoids appeared on the seventh day. The per-cent spore germination (ANOVA $F = 3.30$; $p < 0.005$), rhizoidal abnormalities ($F = 175.83$; $p < 0.001$) and rhizoid length ($F = 12.39$; $p < 0.001$) varied significantly with sulphite treatment.

After the 7th day, spores were washed with sterile distilled water and re-transferred to a buffer medium and AF Dyer's nutrient media separately for growth. Normal protonemal growth was observed in both conditions *i.e.* after removal of sulphite from the medium.

DISCUSSION

Light has no effect on dormant dry spores, but regarding imbibition the photo-induced germination is enhanced under red light for two days. Phytochrome is one of the photoreceptors controlling the light induced spore germination (Sugai *et al.* 1977) Initial increase in the pH followed by a gradual decrease after dissolving sulphite to 1 mM concentration of K-phosphate buffer varies the phytotoxicity of sulphite because of a change in the pH of the medium (Hocking and Hocking 1977, Garsed 1981). A slight shift of pH (Table 3) to a higher value results in rapid increase in the ratio of sulphite (Puckett *et al.* 1973) and then to less toxic sulphate (Neiboer *et al.* 1976) causing acute to chronic injury symptoms (Environmental protection Agency 1976).

Little information is available on the precise mechanism of the toxicity of SO_2 on fern spore germination. The sulphite-sensitivity of these spores during germination was detected by a decrease in the germination rates and by abnormal rhizoids. The sulphite sensitive timing on the germination rate was the first day of red light treatment (Table 4). A delay of two

days in germination was found in the spores kept in sulphite plus red light irradiated conditions. Sulphite affected early stages of germination much before the rupture of spore wall. Red light absorption by the phytochromes must have triggered a series of biochemical and physiological changes inside the spore for differentiation into protonema and rhizoids (Wada *et al.* 1987).

Apical growth of the rhizoids was retarded as soon as sulphite was applied and the growth in horizontal direction increased resulting a swollen shape of the apical tip. On the Other hand when sulphite was removed from the medium, the rhizoid resumed normal apical growth resulting in the swollen shape being left behind. This is possibly due to reversible changes in synthesis and/or arrangement of components such as microfibrils on the cell wall (Wada *et al.* 1987). Rhizoid growth is a very sensitive indicator of contamination of the medium (Dyer 1979)

The spore germination of *L. japonicum* is very sensitive to sulphur dioxide, therefore it can prove to be a good bioindicator of SO_2 pollution in the urban-industrial air sheds of India.

RESUMEN

Un tratamiento de 24 hr de luz roja seguida de luz blanca aumentó la tasa de germinación en esporas del helecho *Lygodium japonicum* Swartz. En cambio el sulfuro la disminuyó y causó un crecimiento anormal en los rizoides. En medios de cultivo sin sulfuro el crecimiento apical dejó atrás la porción engrosada de los rizoides. Los gametófitos de esta especie son buenos bioindicadores de contaminación con SO_2 .

REFERENCES

- Beilke, S. & G. Gravenhorst. 1978. Heterogenous SO_2 oxidation in droplet phase. *Atmosph. Environ.* 12: 231-239.
- Bell, J.N.B., A.J. Rutter & J. Relton. 1979. Studies on the effects of low levels of sulphur dioxide on the growth of *Lolium perenne* L. *New Phytol.* 83: 627-643.
- Buckenham, A.H., M.A.T. Parry & C. Whittingham. 1982. Effects of aerial pollutants on the growth and yield of

- spring barley *Hordeum vulgare* cv. magnum. Ann. Appl. Biol. 100: 179-188.
- Dyer, A.F. 1979. The culture of fern gametophytes for experimental investigation. In A.F. Dyer (ed.). The Experimental Biology of Ferns. Academic, London.
- Edwards, M.E. & J.H. Miller. 1972. Growth regulation by ethylene in fern gametophytes. III. Inhibition of spore germination. Am. J. Bot. 59: 458-465.
- Environmental Protection Agency (USA). 1976. Diagnosing vegetation injury caused by air pollution. EPA 68-02-1344, EPA Res., Triangle Park, North Carolina.
- Furuya, M. 1983. Photomorphogenesis in ferns, p. 569-600. In W. Shropshire Jr. & H. Mohr (eds.). Encyclopedia of Plant Physiology. Springer-Verlag, Berlin.
- Fuzzi, S. 1978. Study of iron (III) catalysed sulphur dioxide in aqueous solution over a wide range of pH. Atmosph. Environ. 12: 1439-1442.
- Garsed, S.G. 1981. The use of sulphite solutions for studying the effects of SO₂ on higher plants. Environ. Pollut. Ser. A 25: 303-311.
- Heath, R.L. 1980. Initial events in injury to plants by air pollutants. Ann. Rev. Plant Physiol. 31: 395-431.
- Hegg, D.A. & P.V. Hobbs. 1978. Oxidation of sulphur dioxide in aqueous systems with particular reference to the atmosphere. Atmosph. Environ. 12: 241-253.
- Hocking, D. & M.B. Hocking. 1977. Equilibrium solubility of trace atmospheric sulphur dioxide in water and its bearing on air pollution injury to plants. Environ. Pollut. 13: 57-64.
- Jones, T. & T.A. Mansfield. 1982. The effect of SO₂ on growth and development of seedlings of *Phleum pratense* under different light and temperature environments. Environ. Pollut. 27: 57-71.
- Keller, T. & H. Beda. 1984. Effects of SO₂ on the germination of conifer pollen. Environ. Pollut. 33: 237-243.
- Mitsugi, H., Y. Nakagawa & H. Watanabe. 1985. Effects of sulphur dioxide and nitrogen dioxide alone and in combinations on the germination and protonemal growth of the moss spores. J. Jap. Soc. Air Pollut. 20: 198-204.
- Miller, J.E. & P.B. Xerikos. 1979. Residence time of sulphite in SO₂ 'sensitive' and 'tolerant' soybean cultivars. Environ. Pollut. 18: 259-264.
- Nakada, M., S. Fukni & S. Kanno. 1976. Effects of exposure to various injurious gases on germination of lily pollen. Environ. Pollut. 11: 181-187.
- Neiboer, E., D.H.S. Richardson, K.J. Puckett & F.D. Tomassini. 1976. The phytotoxicity of sulphur dioxide in relation to measurable responses in lichens. In T.A. Mansfield (ed.) Effects of Air Pollutants on Plants. Cambridge University, Cambridge.
- Puckett, K.J., E. Nieboer, E.P. Flora & D.H.S. Richardson. 1973. Sulphur dioxide: Its effect on photosynthetic ¹⁴C fixation in lichens and suggested mechanisms of phytotoxicity. New Phytol. 72: 141-154.
- Sugai, M., K. Takeno & M. Furuya. 1977. Diverse responses of spores in light-dependent germination of *Lygodium japonicum*. Pl. Sci. Lett. 8: 333-338.
- Wada, M., H. Shimizu, A. Kadota & N. Kondo. 1986. A model system to study the effect of SO₂ on plant cells. I. Experimental conditions in the case of fern gametophytes. Environ. Control Biol. 24: 95-102.
- Wada, M., H. Shimizu & N. Kondo. 1987. A model system to study the effect of SO₂ on plant cells. II. Effect of sulfite on fern spore germination and Rhizoid development. Bot. Mag. Tokyo 100: 51-62.