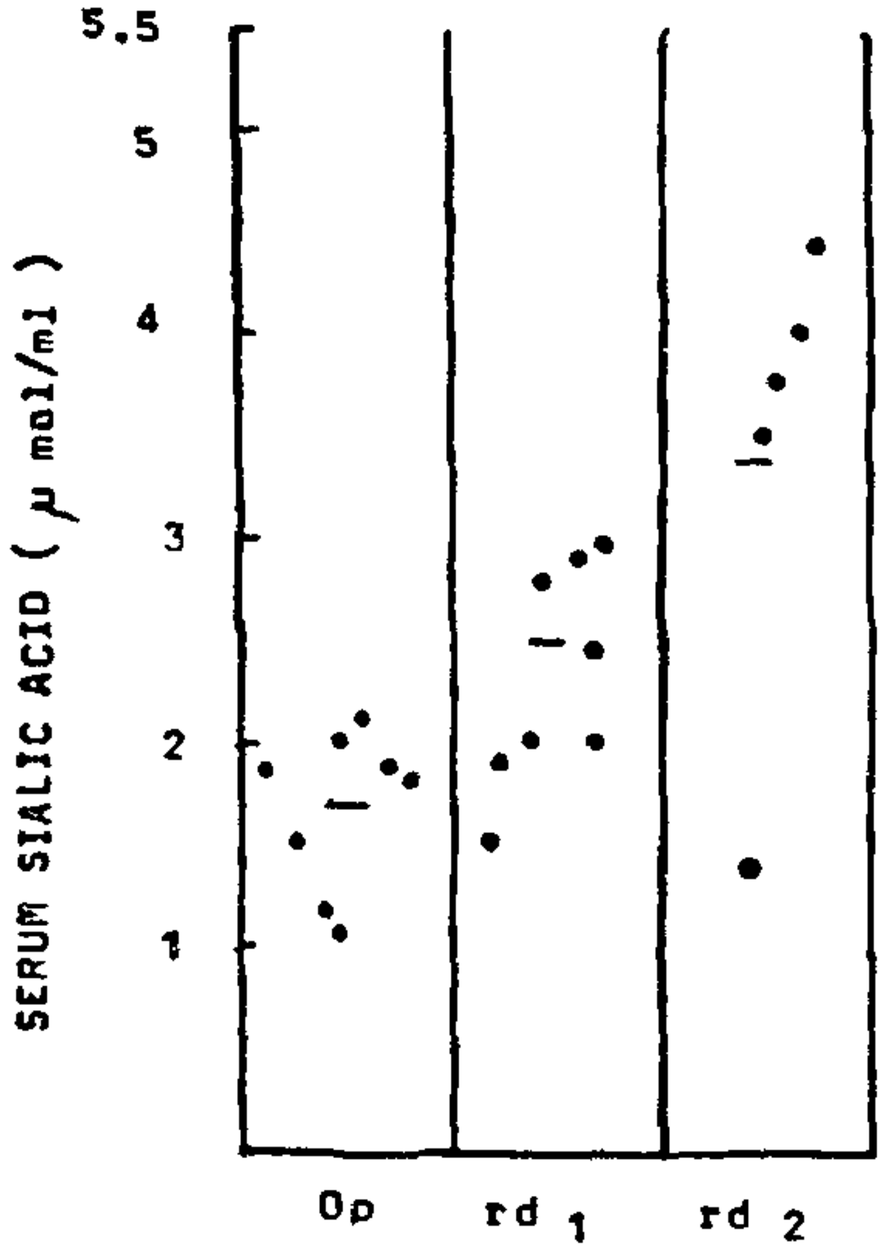

Figure 2. Serum Sialic acid in cancinoma of cervix patients after operation (op) and radiotherapy (rd_1 & rd_2). (rd_1) patient responding to therapy. (rd_2) patient not responding to therapy. (Bar indicates mean in each case).

the patients with cancer was varying magnitude. Eight patients showed satisfactory responses to the therapy by way of tumor regression and their serum level of sialic acid were much below than the comparable untreated control; out of five patients who did not respond to radio therapy, however four patients revealed high values of serum sialic acid.

Raised serum level of sialic acid have been demonstrated in association with the human ovarian carcinoma, melonama and breast cancer[5,10,11]. The present study reveals the relationship between the clinical stages of carcinoma of cervix and the level of serum sialic acid as well as monitor responses of the same towards radical surgery and radiotherapy. As tumor markers are assuming an increasingly important role in clinical oncology and markers may be helpful in diagnosis, they have far greater merit as monitors of tumor burden in following disease advancement or response to treatment. The results of the present study assumes significance in the light of monitoring the tumor burden and consequently may help in the evaluation of the management of cervical carcinoma patients.

10 January 1983.

1. Faques W. L., Brown B. E., Barrentt M. J., Brey S., Wallace Jr. and Waltner W. Jr., *J. Biol Chem.*, 1977, **252**, 4533.
2. Deman J. J. and Bruyneel A. E. *Biochem. Biophys. Res. Commun.*, 1975, **62**, 895.
3. Emmelot P., *Eur J. Cancer.*, 1973, **9**, 1319.
4. Buck, A. C., Glick, C. M. and Warren L. *Biochemistry.*, 1970, **9**, 4567.
5. Silver, H. K. B., Rangel, D. M. and Morton, D. L., *Cancer (Phila).*, 1978 **41**, 1947.
6. Warren, L., Fuhrer, J. P. and Buck, C. A., *Proc. Natl. Acad. Sci.*, U.S.A., 1972, **69**, 1938.
7. Van Beek, W. P., Smets, L. A. and Emmelot, P. *Cancer Res.*, 1973, **33**, 2913.
8. Silver, H. K. B., Karim K. A. and Archibald, E. L. *Am. Assoc. Cancer Res.*, 1978, **19**, 403.
9. Warren, L. *J. Biol Chem.*, (1959) **234**, 1941.
10. MacBeth R. A. L. and Bekesi G. *Cancer. Res.*, 1962, **22**, 1170.
11. Hogan - Ryan A., Fennelly J. J., Jones M., Cantwell B. and Duffy, M. J. *The Brit. J. Can.*, 1980, **41**, 587.

SYNCHRONY IN *DRECHSLERA SOROKINIANA* POPULATION DURING PRIMARY INFECTION OF BARLEY AND WHEAT LEAVES

B. S. YADAV
Department of Botany, Sanatan Dharm College, Muzaffarnagar 251 001, India.

PRIMARY infection is a multicomponent process including spore germination, formation of appressoria, penetration, and colonization. Each phase is distinguishable on the basis of morphology or differential sensitivity to environmental factors or both[1,2]. Study of biochemical changes which occur in the microclimate of host surface during different phases of infection is necessary for clear understanding of the host-parasite interaction[3-5]. Such studies can only be meaningful if the factors essential for synchronous development of the parasite population are known. Synchronous germination of *Drechslera sorokiniana* (Sacc.) Subram. and Jain spores occurs *in vivo*[6] if they are collected from 20 day-old cultures and incubated at $28 \pm 1^\circ$ C after making a suspension (10^5 spores ml) in phosphate buffer solution (0.01 M, pH = 6). The present investigation was aimed at look-

ing for synchrony in other component phases in the parasite population's infection of its host.

Seeds of barley cultivar (DL 70) and wheat cultivar JANAK (HD-1982) were disinfected with 0.1% (w/v) mercuric chloride solution, washed thoroughly with distilled water, and sown in earthenware pots (8–10 seeds/pot). *Drechslera sorokiniana* was isolated from infected barley leaves by single spore culture technique, tested for pathogenicity, and grown for spore production on PDA slants. Spores from twenty day-old cultures were suspended in phosphate buffer solution[7] (0.01 M, pH = 6). The inoculum was adjusted to 10^5 spores/ml by replicated haemocytometer counts and serial dilutions. Drops of uniform size from spore suspension (infection drops) were placed on cleared leaf pieces (5–10 mm long) or uncleared excised host leaves. Spores, on leaf surfaces were then incubated in petriplate moist chambers at 28 ± 1° C. Glucose solution (2%, w/v) was added separately to the infection drops before incubation over cleared leaves to observe its effect upon primary infection.

Cleared leaves were obtained by keeping the young fully expanded leaves in 3:1 (v/v) alcohol-acetic acid mixture for 24–36 hr to remove opacity[8]. Leaves were then washed thoroughly 6–8 times with sterile distilled water and used for placing the infection drops. During incubation of spores over uncleared leaves the young, fully expanded, uninjured, excised leaves from barley and wheat plants at about booting stage were placed over wet Whatman No. 1 filter paper in petriplate moist chambers with both ends between the folds of wet cotton pads. Leaves were then inoculated with infection drops. After specified incubation periods the inoculated leaves were cut into pieces (5–10 mm) and placed in small petri dishes containing alcohol-acetic acid mixture (3:1, v/v) to clear them. They were then rehydrated through an alcohol series. The spores were stained with 0.02% (w/v) trypan blue in lactophenol and mounted in lactophenol. Development stages of the parasite, including germination (200 spores were counted), formation of appressoria, penetration pegs, and invasion hyphae were examined directly under the microscope. Criteria used for germination were the same as suggested by Manners[9].

Appressoria developed between 10th and 14th hr after incubation on cleared leaves and within 12th and 15th hr after inoculation on excised leaves. Maximum percentage of appressoria formed over cleared and uncleared leaves was almost identical (table 1). Majority of appressoria produced on cleared leaf surface inoculated with unenriched infection drops failed to penetrate the host epidermis becoming malformed or branched. Only few of them produced infection pegs between 12th and 18th hr after inoculation. In enriched infection drops, cleared leaves were penetrated within 12th and 15th hr after inoculation. On excised leaves of barley as well as wheat, most penetrations were observed 14–18 hr after inoculation. Colonization hyphae developed in cleared leaves by 14–19 hr (unenriched infection drops) or 14–17 hr (enriched infection drops) after incubation began. Colonization in excised leaves occurred at 16–19 hr after inoculation. The frequency of penetration, of cleared leaves with enriched infection drops and of excised leaves (barley as well as wheat), was approximately thrice as large as cleared leaves with unenriched infection drops while colonization increased almost six times under these conditions (table 1).

When *D. sorokiniana* conidia were inoculated on uncleared leaves the curves representing per cent appressoria formation, per cent penetration and per cent colonization were almost identical to the curve representing per cent conidial germination (figure 1). On cleared leaves (unenriched), however, only the curves representing per cent appressoria formation and per cent germination were identical.

Primary infection of barley and wheat leaves by *D. sorokiniana* is made up of several component phases[10] which are distinguished on a morphological basis. Most appressoria penetrated uncleared leaves within 14 and 18 hr after inoculation as observed[11,12] of the penetration of *Poa pratensis* by this fungus. When optimum conditions for spore germination of *D. sorokiniana*[6] were provided the parasite population on

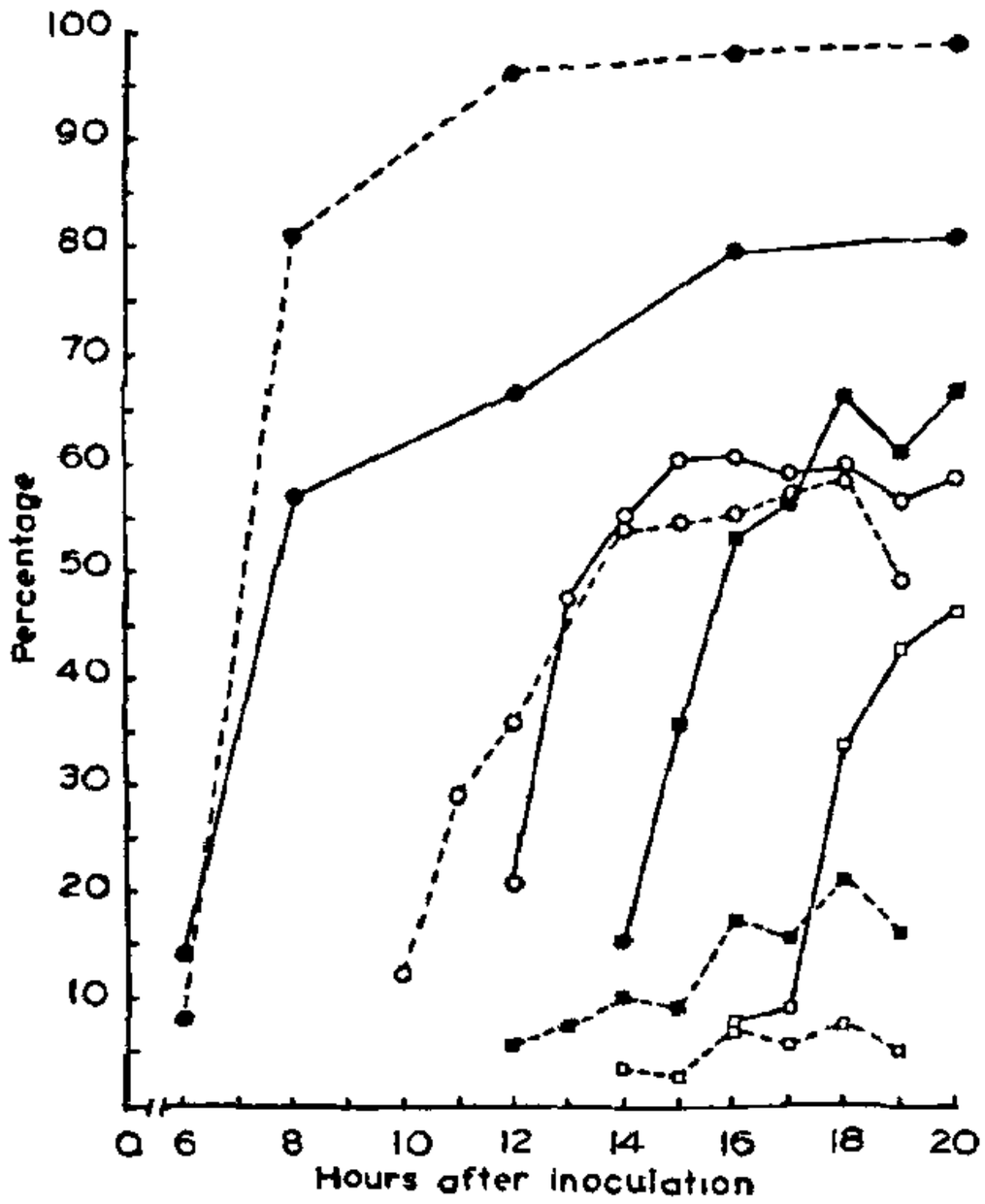

TABLE 1

Percentage of appressoria formation, penetration and colonization by Drechslera sorokiniana on unenriched and enriched cleared leaves and on uncleared leaves of barley and wheat.

Incubation period (hr)	Per cent appressoria formation				Per cent penetration				Per cent.colonization			
	Cleared leaves		Uncleared leaves		Cleared leaves		Uncleared leaves		Cleared leaves		Uncleared leaves	
	Unenriched	Enriched	Barley	Wheat	Unenriched	Enriched	Barley	Wheat	Unenriched	Enriched	Barley	Wheat
10	12.25	7.92	–	–	–	–	–	–	–	–	–	–
11	29.09	31.86	–	–	–	–	–	–	–	–	–	–
12	35.64	40.39	20.57	16.50	5.55	12.19	–	–	–	–	–	–
13	45.09	56.37	47.52	33.33	7.60	18.26	–	–	–	–	–	–
14	53.69	56.52	55.22	55.55	10.09	71.81	15.31	6.95	3.67	10.26	–	–
15	54.22	59.00	62.00	60.57	9.17	73.72	35.48	13.49	2.75	29.66	–	–
16	54.94	38.41	61.50	60.38	17.11	73.72	52.84	45.60	6.30	38.13	6.5	8.80
17	57.00	59.52	62.43	59.00	15.79	75.00	57.81	57.54	5.26	49.17	8.95	31.35
18	58.82	61.57	61.57	59.50	20.83	76.00	65.65	63.86	7.50	48.80	33.60	42.01
19	49.20	58.54	55.55	56.32	16.16	66.66	60.86	62.60	5.05	45.83	42.61	49.56
20	–	63.50	55.98	58.50	–	71.67	66.66	68.90	–	47.24	46.15	44.44

excised leaves was reasonably synchronous since most units of the population attained optimum of each phase within 4 hr period of its beginning (table 1). Also, penetration and colonization processes on excised leaves were shortened appreciably compared with that of cleared leaves (figure 1). This was apparently because (i) the clearing process results in loss of nutrients[13], (ii) *D. sorokiniana* requires exogenous nutrients for penetration and colonization[10] of host leaves and (iii) nutrients leach out of excised barley and wheat leaves into the infection drops[14] and are reabsorbed by *D. sorokiniana* spores[15]. This viewpoint gets further support by the shortening of penetration and colonization phases on cleared leaves when the infection drops were enriched (table 1). Because of its fairly well-defined kinetics on excised host leaves, this host-parasite combination lends itself to qualitative and quantitative biochemical studies of early host-parasite interactions. It is possible to predict what portion of the parasite population is in a particular stage of development at a particular time.

The author is grateful to Dr. C. L. Mandahar, Professors K. S. Thind, K. K. Nanda and S C. Verma of Panjab University, Chandigarh for encouragement, Dr. Paul E. Waggoner of TCAES, USA, for invaluable suggestions and UGC, New Delhi for award of fellowship.

25 November 1982

1. Masri, S. S. and Ellingboe, A. H., *Phytopathology*, 1966, **56**, 304.
2. Nair, K. R. S. and Ellingboe, A. H., *Phytopathology*, 1965, **55**, 365.
3. Inman, R. E., *Phytopathology*, 1965, **55**, 341.
4. Robinson, W. P. and Hodges, C. F., *Phytopathology*, 1977, **67**, 1239.
5. Sziraki, I., Balaze, E. and Kiraly, Z., *Physiol. Plant Pathol.*, 1975, **5**, 45.
6. Yadav, B. S., *J. Indian Bot. Soc.*, 1980, **59**, 9.
7. Deverall, B. J. and Wood, R. K. S., *Ann. Appl. Biol.*, 1961, **49**, 461.
8. Sass, J. E., *Botanical microtechnique*, Iowa State College, Press, Iowa, 1958.
9. Manners, J. G., In *The fungus spore*, (ed.) M. F. Madelin, Butterworths, London, 1966.
10. Yadav, B. S., *Aust. J. Bot.*, 1981, **29**, 71.
11. Mower, R. G., *Histological studies of susceptpathogen relationships of Helminthosporium sativum* P. K. and B. *and Helminthosporium vagans* Drechs. and *Curvularia lunata* (Wakk.) Boed. *on leaves of merion and common Kentucky bluegrass*. Ph.D. Thesis, Cornell University, 1961.
12. Weihing, J. L., Jensen, S. G. and Hamilton, R. I., *Phytopathology*, 1957, **47**, 744.
13. Mandahar, C. L. and Arora, R. K., *Proc. All India Symp. on Physiol. Host-pathogen Interact., India*, 1977.
14. Tukey, H. B. Jr., *In Ecology of leaf surface microorganisms*, (eds), T. F. Preece and C. H. Dickinson, Academic Press, London, 1971, 67.
15. Yadav, B. S. and Mandahar, C. L., *Indian Phytopathol.*, 1980, **33**, 87.

IN VITRO HYBRIDIZATION IN AN INCOMPATIBLE CROSS — BLACKGRAM × GREENGRAM

S. S. GOSAL AND Y. P. S. BAJAJ
Tissue Culture Laboratory, Punjab Agricultural University, Ludhiana 141 004, India.

DURING the last decade although considerable progress has been made in the improvement of grain-legumes (pulses), yet their yield has remained almost static. This is attributed to the lack of sufficient genetic diversity in the base populations of these crops. Thus need is felt to increase the genetic variability by resorting to means other than conventional[1]. The present communication is, therefore, a part of the project undertaken to achieve wide hybridization in pulses using *in vitro* techniques. By combining the application of growth regulators and embryo culture, interspecific hybrids have been obtained in an otherwise incompatible cross[2,3], involving two important Indian pulses, blackgram (mash) × greengram (mung). The ultimate objective is to combine the desirable traits of these two parents. Whereas blackgram is resistant to the yellow-mosaic virus and seed shattering, greengram possesses larger number of seeds per pod and contains more easily digestible and relatively high protein[4].

The emasculated flowers of blackgram (*Vigna mungo* cv. Mash 1-1) pollinated with pollen of greengram (*V. radiata* cv. Russian Mung) were treated with a distilled-water solution containing naphthalene acetic acid (25 mg/l + gibberellic acid (100 mg/l) and kinetin (5 mg/l) twice a day, for a week. The hybrid embryos were excised from the developing pods (14 days after pollination), and cultured on Murashige and Skoog's medium[5] (MS) supplemented with indoleacetic acid (1 mg/l) + kinetin (0.2 mg/l) + coconut water (70 ml/l). All manipulations were conducted aseptically in a Laminar Flow Cabinet (Klenzaids, Bombay), and the cultures were maintained at 25 ± 2° C.