subglobose sori at the base of peduncle were also found (Fig. 1C).

FIG. 1A. Smut sori on flag leaf and the leaf below it in different stages of development with partially emerged smutted earhead of barley. 1B. Congregated barley leaves enclosing smutted earhead in the sheath whorl and their laminae torn away along the smut sori. 1C. Smut sori at the base of peduncle.

The morphological characters and size of the chlamydospores, and their mode of germination agreed closely with *U. hordei* causing covered smut of barley.

This is the first report of the development of the symptoms of covered smut of barley on leaf and peduncle. These symptoms can be utilized in the diagnosis of the disease even before the emergence of smutted ears in the barley variety DL 281, and in early roguing out of the affected plants.

Department of Plant Pathology,
Chandra Shekar Azad University
of Agriculture and
Technology, Kanpur,
April 24, 1978.

D. V. SINGH.
L. S. CHAUHAN.
H. K. SAKSENA.

PHYSIOLOGICAL EVIDENCES FOR QUICK REGENERATION OF WALL ON ISOLATED PLANT PROTOPLASTS

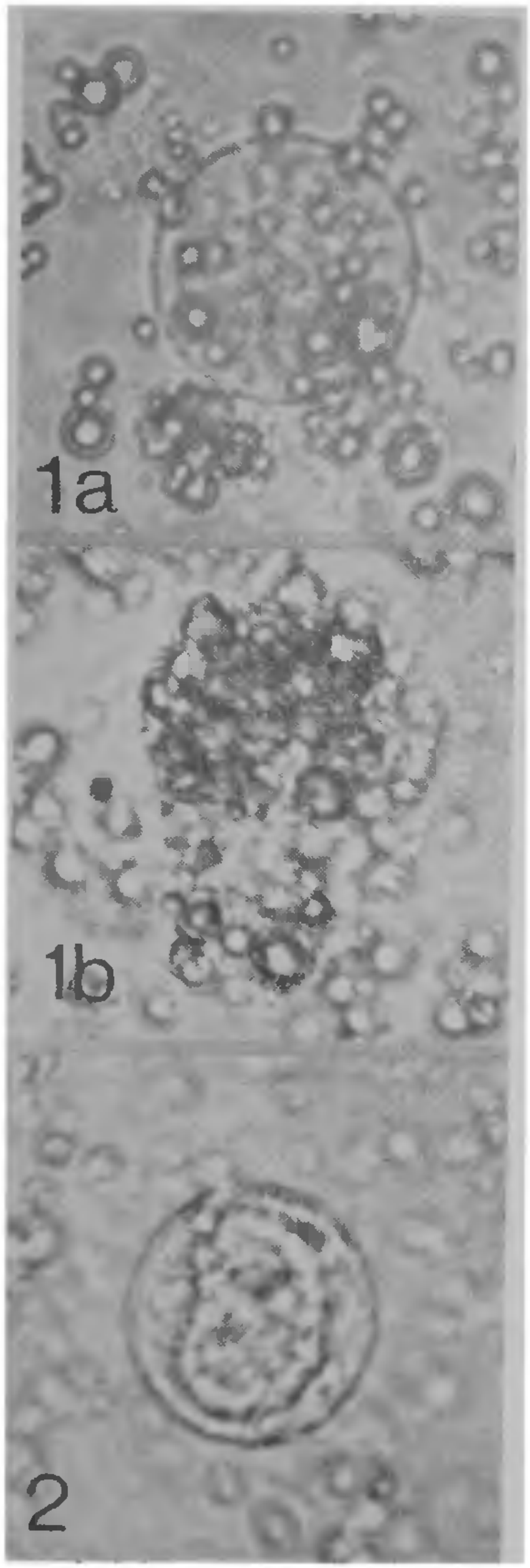
WALL regeneration around isolated protoplasts of green plants was first reported by Pojnar *et al.*[1] and later supported by Mishra and Colvin[2]. A number of reports that have appeared in the last decade or so have also provided evidences for wall regeneration[3–12]. A careful examination of the time course of wall regeneration as reported in these studies reveal that generally the time scale corresponding to this process is in terms of days since isolation[1,3–12]. However, there have been a few reports where this process has been described to be much quicker, of the order of hours since isolation. While Mishra and Colvin[2] reported a complete wall regeneration between 4–6 hours of isolation in protoplasts from green tomato fruits, Williamson *et al.*[14] found that in *Vicia hajastana* suspension cultures, the microfibril deposition was initiated after 10–25 minutes and within 20 hours, the protoplasts were observed to be densely covered with microfibrils. However, both the reports of quick regeneration of wall drew their evidences from ultrastructural studies under the electron microscope, calcofluor staining or X-ray diffraction studies. Since the cell wall is a physical functional barrier around a plant cell, it was presumed that it should be possible to demonstrate the development of the same around the protoplasts through simple plasmolysis and osmolysis experiments.

The wall regeneration studies were carried out on protoplasts isolated from the following plant materials :

1. Placenta cells and the cells of inner wall of Simla pepper (*Capsicum annum*).
2. Placenta cells and the cells of inner wall of green tomato fruits (*Lycopersicon esculentum*).
3. Young leaves around the growing point in cabbage head.

The methods for isolation of protoplasts have been previously described[13]. In brief, it involved (i) incubation of tissue sections in 5–10% pectinase dissolved in 0·25 M sodium nitrate for 1½ hours, (ii) decanting the enzyme mixture and macerating the softened tissue with a glass rod, (iii) filtering the macerate through a thick layer of cotton wool and (iv) observing the isolated protoplasts in the incubation medium in a fixative coated cavity slide under an optical microscope.

For osmolytic studies of wall regeneration, the medium with the protoplast was diluted with drops of distilled water and the microscopic field was so adjusted as to observe the same protoplasts upon dilution. The maximum dilution was to the extent of 5 drops of distilled water to one drop of the incubation medium. Such dilutions were performed at frequent intervals on samples kept on different slides.



FIGS. 1–2. Fig. 1 *a*. Freshly isolated protoplast from the placental tissue of young green tomato fruit (*Lycopersicon esculentum*) (Magnified 3500 times). Fig. 1 *b*. Same protoplast after bursting (Magnified 3375 times). Fig. 2. Plasmolysed cell developed from a protoplast after 3 hours of isolation showing the presence of wall (Magnified 3250 times).

Plasmolytic experiments were also conducted in the same manner on those protoplasts which showed resistance to bursting after incubation. To plasmolyse the protoplasts drops of saturated solution of sucrose were added.

When the dilution of the medium with distilled water was performed immediately after (Fig. 1 *a*, *b*) isolation, they were seen to burst open instantaneously. However, if the protoplasts of the same lot were left in the incubation medium for 2–3 hours after isolation and then the dilution of the medium was performed, it was observed that most of these did not burst even after five times the dilution. If the dilution was done after one to two hours of isolation, a few protoplasts bursted whereas others remained intact. It was possible to plasmolyse the newly regenerated cells from the naked protoplasts (Fig. 2) shoowing the presence of a wall.

The resistance to bursting can only be explained on the basis of the assumption that after 4–5 hours since the beginning of the plasmolysis and concurrently to the pectinase digestion (*i.e.*, 2–3 hours after isolation) the protoplasts are able to regenerate an envelope which is already strong enough to resist osmolysis effected through excessive dilution of the medium. It was also observed that during dilution, the spherical shape of the protoplasts changed to oblong or oval, but quickly reverted back to its original shape within 1–2 minutes.

The shape alteration suggests that the wall at this stage is fairly elastic and flexible and that these newly regenerated cells are actually those developed from naked protoplasts instead of being cells with undigested walls.

These observations of resistance to bursting and shape adjustments were repeated in all the plant materials used in a large number of trials. The regeneration of wall was reproducible in all the cases over almost identical span of time.

These observations provide physiological evidence for quick wall regeneration on isolated protoplasts and support other ultrastructural observation made earlier[2,14].

Dept. of Education in Science and Maths.,
National Council of Educational Research and Training,
Sri Aurobindo Marg,
New Delhi, *April* 26, 1978.

ARUN K. MISHRA.
MISS DAVINDER KAUR ARORA.

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NITROGEN FIXATION BY *AZOSPIRILLUM* SP. ISOLATED FROM BENOMYL-AMENDED RICE SOIL

THE participation of nitrogen-fixing *Azospirillum* in several grass-bacteria associations and its high nitrogen-fixing potential in various ecosystems have been recognised recently[1, 3]. The occurrence of nitrogen-fixing *Azospirillum* in rice roots and soils has been reported recently[2,4,6]. Although the intensive use of pesticides in agriculture can lead to ecological disturbances in the environment, the effect of pesticides on *Azospirillum* population and nitrogen fixation has not been investigated. The present report deals with the effect of benomyl, a systemic wide-spectrum fungicide, on the population of *Azospirillum* and nitrogen-fixing efficiency of cultures isolated from benomyl-amended soils.

Technical grade benomyl [methyl-1-(butyl-carbamoyl)-2-benzimidazole carbamate] was applied at *0, 10, 20 and 100 ppm concentrations to a flooded paddy soil. The population of Azospirillum sp. in* unamended and benomyl-amended soils was estimated as follows: Tenfold dilution of the soil samples were *transferred to 10 ml of nitrogen-free semi-solid malate* medium[3]. The inoculated culture tubes, replicated five times, were then incubated for 48 hr. at 30° C. Positive identification of *Azospirillum* sp. was recorded when there was the formation of very characteristic white, dense undulating fine pellicle a few mm below *the surface of the semi-solid medium and microscopic* observations for the confirmation of the presence of the typical *Azospirillum*. Population density of *Azospirillum* in soils was determined by most-probable numbers on 10, 20 and 30-day incubation under flooded conditions. The nitrogen-fixing efficiency of *Azospirillum* cultures obtained from benomyl-amended and unamended soils incubated for 10, 20 and 30 days was also determined.

Striking stimulation of *Azospirillum* population occurred at all concentrations (10, 20 and 100 ppm) of benomyl used, but this stimulation was less marked at the highest concentration of 100 ppm (Table I). *Azospirillum* cultures isolated from benomyl-amended soil, in general, exhibited greater nitrogen-fixing efficiency than the cultures from unamended soils irrespective of the duration of the soil incubation (Table II). Although *Azospirillum* cultures from 100 ppm benomyl-amended soils exhibited greater nitrogen fixation than the cultures from 10 and 20 ppm levels, nitrogen fixation was low in cultures isolated from 30-day incubated samples with 100 ppm benomyl.

TABLE I

Effect of benomyl on the population of Azospirillum *sp. in a flooded rice soil*

Benomyl concentration (ppm)	MPN of *Azospirillum* × 10^5/g dry soil		
	Incubation (days)		
	10	20	30
0	20	14	5
10	20	200	44
20	20	200	200
100	3	115	44

TABLE II

Nitrogen-fixing efficiency of Azospirillum *cultures isolated from benomyl-amended flooded soil*

Benomyl concentration (ppm)	mg N fixed/g. malate by *Azospirillum* sp.		
	Soil incubation (days)		
	10	20	30
0	2·97	5·07	6·10
10	5·20	5·87	10·23
20	7·17	11·80	10·10
100	9·33	12·00	7·13

Stimulation of nitrogen fixation in soil following pesticidal application has been reported[7]. It has