characteristic appressorium from which infection hypha emerged (Figure 5).

The various characters revealed by the uredinial sorus of the rust under the present consideration is in conformity with the form-genus *Peridiopsora* Kamat & Sathe apud Sathe[9,10].

In Uredinales, by and large genera are identified primarily on the features of telial state. Many rusts where telial state has not been discovered, assigning them to any particular genus is rather difficult. Since rust fungi exhibit host specialization this attribute has been exploited by mycologists for making a detailed study of aecia and uredinia and assigning them to different form genera.

The entire structure of the uredinial sorus is also in conformity with the structure of *Caeoma mori* Barclay. It is, therefore, essential to transfer the species to the form genus *Peridiopsora* and this is effected here as *Peridiopsora mori* (Barclay) Prasad K. V. *et al.* comb. nov. (≡*Caeoma mori* Barclay in *J. Asiatic Soc. Bengal*, 1890, **59**, 97 Figs.).

In conclusion, the rust fungus infecting *Morus alba* L. in India is *Peridiopsora mori* (Barclay) Prasad *et al.* and the other names become synonyms of the same.

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Isolation of a chlorate-resistant line from protoplast cultures of *Hyoscyamus muticus* L.

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A chlorate-resistant line resistant to 40 mM potassium chlorate ($KClO_3$) has been isolated from suspension culture protoplast derived colonies (30–35 celled stage) of *Hyoscyamus muticus*. In comparison to the wild type, the selected line ClO^rI registered nearly 3-fold decrease in the nitrate reductase enzyme activity (157.25 units/g fresh wt.). This characterization of ClO^rI was reflected in their slower growth on medium having NO_3 as sole nitrogen source.

ISOLATION of conditional mutants such as nitrate reductase deficient, amino acid and vitamin requiring mutants *in vitro* and their use have led to the progress of developing a majority of somatic hybrids to-date[1,2]. The importance of such mutations is further enhanced in developing universal hybridizers[3,4]. Although several technical advancements such as fluorescent activated cell sorting (FACS) and microculture techniques have emerged for the efficient screening of somatic hybrids[5,6], the importance of inducing selectable markers to desirable genetic background still prevails. It has been studied earlier that the screening for chlorate resistance can indirectly result in recovery of nitrate reductase (NR) deficient mutants[7]. In this communication we report the isolation of a chlorate-resistant line from protoplast cultures of *Hyoscyamus muticus*. The importance of this mutant with respect to somatic hybridzation will be discussed.

Protoplasts from suspension culture cells were isolated according to the method reported earlier[8]. Dose response studies were conducted to determine the concentration of potassium chlorate ($KClO_3$) which completely inhibited the growth of cells. Protoplast-derived colonies (30–35 celled stage) were plated on the medium incorporated with 10, 20, 30 and 40 mM concentrations of $KClO_3$. 20 mM $KClO_3$ totally inhibited growth. Selection pressure was applied at a supralethal dose (40 mM). Resistant colonies were selected after 8–10 weeks of growth in the stress medium. Chlorate-resistant callus was subjected to characterization by testing its capacity to utilize nitrate as the sole nitrogen source in relation to ammonium and other reduced forms of nitrogen such as casein hydrolysate. Regeneration of plants from a chlorate-resistant line was obtained.

Assay for nitrate reductase activity was measured by the *in vitro* assay method[9]. Tissue samples included callus and leaves of wild type and chlorate-resistant lines and leaves of *in vitro* grown double mutant plants of *Nicotiana tabacum*[3]. Double mutant of *N. tabacum*, obtained through the courtesy of Dr D. Pental, Tata Energy Research Institute, New Delhi, India was used as standard check.

Chlorate-resistant lines were selected by incorporating 40 mM of $KClO_3$ as supralethal dose. The screening for chlorate resistance was carried out by plating $1 \times 10^3 - 5 \times 10^3$ protoplast-derived colonies (Table 1). After eight weeks of incubation on the stress medium, a total of six colonies survived in the first passage. Out of

Table 1. Chlorate-resistant colonies selected from protoplast cultures of *Hyoscyamus muticus**

No. of colonies ($\times 10^3$) plated per ml of the selective medium	No. of resistant colonies obtained	Designated as line no.
1.0	1	ClO^r 1
2.0	–	–
3.8	1	ClO^r 2
4.5	2	ClO^r 3, ClO^r 4
5.0	2	ClO^r 5, ClO^r 6

*Observations taken after 8–10 weeks of culture.

these one each (designated as ClO^r1 and ClO^r2) was recovered from cultures having plating density of 1 and 3×10^3 while two resistant lines were isolated each from cultures with 4×10^3 (ClO^r3 and ClO^r4) and 5×10^3 (ClO^r5 and ClO^r6) plating densities. In order to assess the stability of the resistance lines selected, they were transferred to medium devoid of $KClO_3$ and allowed to grow for 6–8 weeks. Thus, the lines growing on chlorate-free medium were transferred again to medium containing 40 mM $KClO_3$. It was observed that except for line ClO^r1 all other selected lines failed to survive the ClO_2 (chlorite) toxicity indicating, thereby, their leaky or carry-over nature. This line grew normally by repeated subculturing (at 4-week intervals) on the selection medium, both in presence and absence of 20 and 40 mM $KClO_3$. The selected line exhibited comparable growth in relation to the wild type callus on a full strength MS medium in the absence of chlorate (Figure 1). It has maintained the resistant trait for over three and half years now.

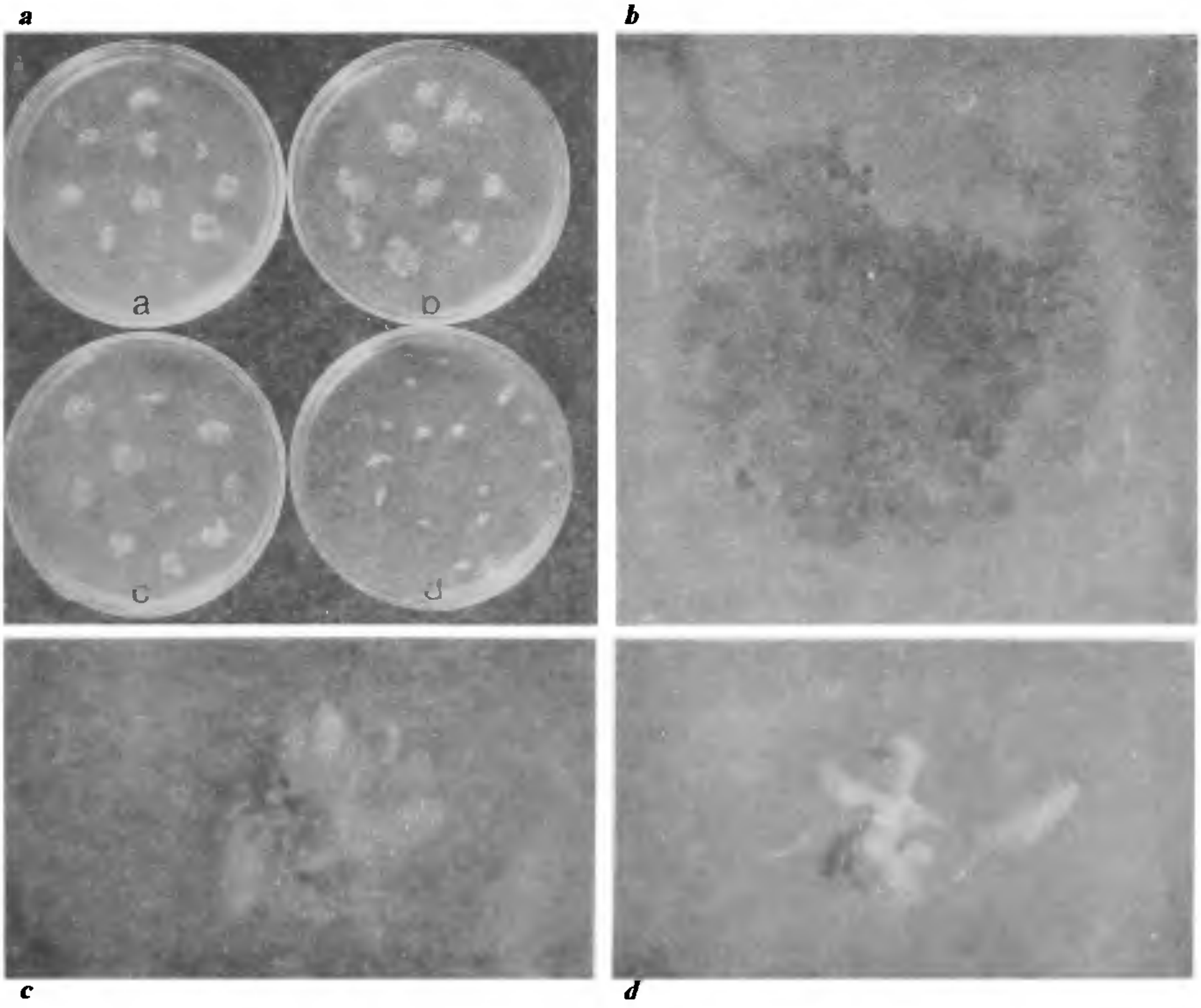

Figure 1. Selection and plantlet regeneration from a chlorate-resistant line in *H. muticus*. *a*, Comparative growth of chlorate-resistant (a, b) and the wild type (c, d) callus after 4 weeks in the absence (a, c) and presence (b, d) of 20 mM chlorate. *b*, Callus growth of chlorate-resistant line observed in a 4-week-old culture in the presence of 20 mM chlorate. *c* and *d*, Regeneration of shoots from callus of chlorate-resistant line in the presence of 20 mM chlorate.

In order to assess the efficacy of the different nitrogen sources on growth and expression of chlorate resistance of ClO^r 1 line, different nitrogen sources were tested both in presence and absence of 20 mM $KClO_3$ (Table 2). It was observed that the presence of NH_4^+ in the medium was essential for the optimum growth and expression of chlorate-resistant trait. The growth of resistant callus was good when the medium was supplemented with 20 mM NH_4NO_3 (Table 2). Best and profuse growth of resistant line was obtained when the medium was supplemented with NH_4NO_3 (20 mM) and CH (1 g l). Some response was also obtained with even an additional supplement of KNO_3 to the medium. The presence and absence of KNO_3 in the medium did not affect the growth of the callus. But on the contrary, the absence of NH_4NO_3 in the medium was critical. The additional supplement of CH in the media facilitated the best growth indicating that the resistant line alone cannot utilize nitrate in the medium for its reduced nitrate reductase activity. The presence of CH alone in the medium showed moderate growth of the resistant line, indicating the role of NH_4 ion in the expression of chlorate resistance. This type of finding has also been reported in *N. tabacum*[10]. Addition of 10 mM succinic acid in the medium supplemented with either NH_4Cl or NH_4NO_3 brought about marginal inhibition of proliferation of ClO^r 1 when compared with that in its absence. However, succinic acid improved the growth response when applied along with 20 mM KNO_3.

The nitrate reductase assay is summarized in Table 3. The wild type line exhibited NR activity equivalent to 443.56 and 427.33 units $g\,l^{-1}$ fresh wt in callus and leaf tissue respectively. In comparison, the selected line

Table 3. Relative *in vivo* activity of the nitrate reductase enzyme in leaf and callus tissues of *H. muticus* (WT and ClO^r 1) and *Nicotiana tabacum* (WT and NR^- SR^+)

Tissue sample	Nitrate reductase activity (units*, g^{-1} fresh wt)
N. tabacum leaves (NR^- SR^+)	0
N. tabacum leaves (WT)	661 66
H. muticus leaves (WT)	427.33
H. muticus callus (WT)	443.56
H. muticus callus (ClO^r 1)**	157.25

*1 unit = 1 n mole NO_2^- released/60 min; **In presence of 20 mM $KClO_3$ in the medium; NR^- = Nitrate reductase deficient; SR^+ = Streptomycin resistant; WT = Wild type.

ClO^r 1 registered nearly 3-fold decrease in enzyme activity (157.25 units $g\,l^{-1}$ fresh wt). Hence, it is apparent that ClO^r 1 line though not totally NR-deficient, had an impairment in the functioning of NR enzyme. The characteristic of ClO^r 1 was also reflected in slower tissue growth on medium having NO_3^- as sole nitrogen source (Table 2).

Shoots were regenerated from the ClO^r 1 callus line on MS medium supplemented with 0.1 mg l^{-1} NAA and 0.5 mg l^{-1} BAP. The regeneration frequency was 10–15% lower compared to 60 to 70% in control cultures. The leaves in the regenerated shoots were morphologically abnormal and thick. These shoots, when transferred to a rooting medium (half strength MS nutrient) produced profuse callusing at the base of the shoot instead of rooting response. So the plants could not be transferred to the glasshouse. Thus, the progeny analysis could not be carried out to study the inheritance of the altered trait. However, interestingly, calluses obtained from these chlorate-resistant shoots were tested for their resistance to chlorate. These were comfortably growing at 20 mM level of $KClO_3$. This

Table 2. Effect of different nitrogen sources on growth of the wild type (WT) and chlorate-resistant variant line (ClO^r 1) of *H. muticus*

Nitrogen source	WT		ClO^r 1	
	(−)ClO_3	(+)ClO_3	(−)ClO_3	(+)ClO_3
1. None	+	−	−	−
2. NH_4Cl (10 mM)	+ + +	−	+ + +	+ +
3. NH_4NO_3 (20 mM)	+ + +	−	+ + +	+ +
4. KNO_3 (20 mM)	+ + +	−	+ +	+ +
5. NH_4NO_3 (20 mM)	+ + +	−	+ + +	+ + +
6. KNO_3 (20 mM)	+ + +	−	+	+
7. CH (1 g/l)	+ + +	−	+ +	+ +
8. NH_4NO_3 (20 mM)+ KNO_3 (20 mM)+ CH (1 g/l)	+ + + +	−	+ + + +	+ + + +
9. KNO_3 (20 mM)+ CH (1 g/l)	+ + +	−	+ +	+ +
10. NH_4NO_3 (20 mM)+ CH (1 g/l)	+ + +	−	+ + + +	+ + + +
11. MS basal (full strength)	+ + + +	−	+ + +	+ + +

CH = Casein hydrolysate; − = no growth; + = poor, + + = moderate; + + + = good; + + + + = profuse; (−) ClO_3 = without chlorate; (+) ClO_3 = with 20 mM chlorate. Treatments 1–10 were in MS[13] medium devoid of NH_4NO_3 and KNO_3 salts. Treatments 2–4 were having 10 mM succinate in the medium.

indicates that the selected trait was of a stable nature and could be carried from cell to whole plant and back.

The selected line will be of use for the selection of interspecific somatic hybrids between *H. muticus* and non-regenerating related incompatible species such as *H. albus* and *H. niger*. The selection scheme in this case would be based on the facts that the ClO^r 1 line of *H. muticus* will grow at a lower rate on only nitrate-containing medium with chlorate and *H. albus*, *H. niger* will not grow and regenerate shoots. It is likely if inactivated (with iodoacetamide) chlorate-resistant line will be fused with either *H. albus* or *H. niger*, and the fusion product will not only grow in the selection medium but may also regenerate shoots.

Chlorate resistance leads to nitrate reductase deficiency[7]. The resistant line can be of fundamental use for understanding the mechanism of chlorate resistance in higher plants with impaired nitrate reductase activity or its absence. Further, the variant (mostly nia type) may be a starting material for the development of universal hydridizers. Availability of such types of mutants (alone or double) has led to the production of somatic hybrids[11,12].

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Brainstorming session in the area of 'Plant Senescence'

The Department of Science & Technology is planning to organize a brainstorming session in the area of 'Plant Senescence' in order to review the state-of-art and to generate research proposals for possible funding. The proposed discussion will be in the areas of;

- Flower senescence
- Fruit senescence
- Environmental aspects of senescence
- Hormonal aspects of senescence
- Molecular and photo-biology of senescence
- Senescence of organelles

Interested scientists, particularly in the age group of 30 to 40 years, are invited to apply for participation in the brainstorming session. A pre-proposal on prescribed format should reach this Department by September 30, 1993. Formats may be obtained from Dr Parveen Farooqui, Director & Head (Life Sciences), Department of Science & Technology, New Delhi 110 016.