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An albino strain of *Ustilago nuda* from Canada

P.L. Thomas

Research Station, Agriculture Canada, 195 Dafoe Road, Winnipeg, Manitoba R3T 2M9. Contribution No. 1120.

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Offspring of a Canadian collection of *Ustilago nuda* from barley segregated for olive-brown and albino teliospores. Progeny of hybrids between smut strains showed that the albino spore color was determined by the same recessive gene as that in an albino strain from Bulgaria. This is the first report of an albino strain of *U. nuda* from North America.

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Les descendants d'une collection canadienne d'*Ustilago nuda* de l'orge se sont séparés selon leurs téliospores brun olive et albinos. Les nouvelles colonies issues de lignées d'hybrides de rouille ont prouvé que l'albinisme provenait du même gène récessif qui était apparu dans une colonie bulgare. C'est la première fois que l'on signale la présence d'une lignée albinos d'*U. nuda* en Amérique du Nord.

Albino strains of *Ustilago nuda* (Jens.) Rostr. have been reported only twice: by Mastenbroek (1949) in Holland and by Dimitrov (1967) in Bulgaria. This rarity can be attributed partially to the indurate nature of the spore mass and the resulting absence of inoculum in the air around receptive florets of barley (*Hordeum vulgare* L.).

The strain found by Mastenbroek no longer exists. Fullerton and Nielsen (1974) showed that the albino character in the Bulgarian strain is controlled by a recessive gene. This gene has been very useful as a marker in subsequent genetic studies (Thomas 1976, 1978, 1983). Given the rarity and usefulness of albinos, information on their occurence is of interest. The following is a report of the finding and verification of albino segregants among progeny of teliospores from a collection of *U. nuda* made in eastern Canada. Also investigated was the relationship of the genetic control of albinism in this strain to that in the Bulgarian strain.

Materials and methods

Florets of barley were inoculated with teliospores of the fungus at or 1-2 days after anthesis. The inoculum of both albino and brown strains was a suspension of 1 g of diploid teliospores per litre of water and was injected with a 22 gauge needle on a 5 mL syringe. Samples of brown spores were tested for heterozygosity for spore color by inoculating approximately 100 florets of barley. If we assume that one dikaryon produces all of the spores in each plant, this population would be large enough to allow detection of segregants, e.g. if 50 of the 100 florets develop into seeds that produce plants with sori, approximately 38 plants should have brown sori and 12 albino sori.

Two haploid cultures of opposite mating types were isolated from a germinating spore from one of the two plants that first produced albino spores. Haploid lines were isolated and cultured according to Nielsen (1968) and pairs with compatible mating types were inoculated into florets according to Thomas (1983).

The susceptible cultivar Regal was used as a host. Plants to be inoculated were grown in pots in growth cabinets and the seed from the inoculated florets was sown subsequently in beds of soil in greenhouses.

Results and discussion

Teliospores from strain #56-25, found in an infected spike of barley that had been in storage for 14 years, were subjected to routine increase in 1982. Inoculated florets of cv. Regal produced 60 viable seeds which in turn produced 26 infected plants. The spores in two of the infected plants were albino while those in the remainder were wild type in color (olive-brown). The albino spores were smooth walled in contrast to the echinulate wild-type. The albino sori produced by #56-25 are shown in Fig. 1 and have the same appearance and indurate nature as sori from the Bulgarian strain. It is extremely unlikely that the albino spores in the two plants resulted from contamination because several months had elapsed since any strains containing the albino gene from the Bulgarian strain were used in the laboratory, greenhouses or growth cabinets. The albino spores in the two plants were therefore unexpected, especially since a search of records at the research station showed no indication that strain #56-25 had segregated for albinism at any time in the past. Strain #56-25 was originally collected at Woodstock, New Brunswick, prior to 1949, and was screened on differential cultivars in 1949, 1951, 1953, and 1956. Sori on a single plant grown from inoculated seed produced the spores that were put into storage in 1968. If the original collection from Woodstock was heterozygous for

Figure 1. Healthy barley spike (left), spike containing albino teliospores of *U. nuda* (centre), and spike containing teliospores of wild-type (olive-brown) color (right).

spore color, segregation for the albino character should have been noticed during the previous increases and screenings. However, since the number of differential lines infected were few and the infection levels were low, it is possible that segregants may not have appeared. More likely, the original collection contained only a small proportion of spores that were heterozygous for spore color, and this genotype was preserved when spores from several plants were bulked together at each harvest. Alternatively, a mutation could have occurred more recently in #56-25. The spores in one plant are usually produced only by one dikaryon (Fullerton & Nielsen 1974, Thomas 1978). Therefore, the mutation would have occurred at some stage before the formation of the dikaryon that infected the plant producing the spores whose progeny segregated.

Few spores, none of them viable, remained of the original inoculum of #56-25 after the inoculation of cv. Regal. Therefore it was impossible to isolate haploids to test for heterozygosity of the spores, to reinoculate to verify the segregation of spore color, or to examine the spores to see if a small proportion, previously unnoticed, were albino. However, after the increase of this strain, 17 of the 24 plants with brown spores produced sufficient inoculum for further study. Spores from each of these 17 isolates were inoculated into florets of cv. Regal and the resulting seed was grown to the heading stage. Seven of the 17 isolates produced only brown spores; 50-92% of the plants in this group had sori. The remaining 10 isolates produced sori in 39-77% of the plants with 4-23% having only albino spores. Two of these 10 isolates each produced one plant with both spore types in one spike. If the spores of #56-25 were heterozygous for albinism, and if this albinism is controlled by one gene, we expect 11.3 of the 17 brown spored offspring to have progeny that segregate for the two spore types. The observation of 10 out of 17 is a very good fit to this expectation ($\chi^2 = 0.471$, $P \cong 0.5$). Therefore, the spores of strain #56-25 that were put into storage in 1968 probably were heterozygous for a recessive gene for albinism.

Since both this gene and the gene in the Bulgarian strain are recessive, spores of hybrids between strains carrying these genes would be expected to be

albino if the character were controlled by the same gene in both strains, and brown if controlled by different genes. The two haploid cultures isolated from the albino spore from #56-25 therefore were hybridized with two compatible cultures derived from the Bulgarian strain. Both gave albino spores, as did the selfed (intra-strain) controls. This indicated that the gene controlling albinism in the Canadian strain is the same as that in the Bulgarian strain. In culture the growth type of the haploid lines from #56-25 was different from that exhibited by all haploid lines derived from the Bulgarian strain; in particular, the growth rate of the colonies was much slower both on agar and in liquid medium. Cultures of the *A* mating type were even more restricted than those of the *a* mating type. This further verifies that the albino spores produced by strain #56-25 did not originate from contamination by the Bulgarian strain.

The albino strain from Canada gives a relatively high level of infection after artificial inoculation of teliospores into florets of susceptible host cultivars, e.g. cvs. Regal and Trebi gave 44 and 80% of plants with sori, respectively. Haploid cultures can be utilized in studies if allowance for their slow growth is made. The new albino strain is now available as an alternative to the Bulgarian strain. Such strains can be used in studies on gene linkage, as checks against contamination, and where visual differentiation of strains is important, e.g. where mixtures of two strains are used as inoculum.

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