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UPOV

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

# *AD HOC* CROP SUBGROUP ON MOLECULAR TECHNIQUES FOR MUSHROOM

## First Session
## Tsukuba, Japan, September 13, 2002

REPORT

*prepared by the Office of the Union*

Opening of the Session

1. The *Ad hoc* Crop Subgroup on Molecular Techniques for Mushroom (hereinafter referred to as "the Subgroup") held its first session in Tsukuba, Japan, in the afternoon of September 13, 2002. The list of participants is reproduced in the Annex to this report.

2. The session was opened by Mr. Nico van Marrewijk (Netherlands), Interim Chairman of the Subgroup, who welcomed the participants and, in particular, Dr. Teruyuki Matsumoto (Japan) and Dr. Anton S.M. Sonnenberg (Netherlands), who had agreed to deliver presentations.

Adoption of the Agenda

3. The Subgroup adopted the agenda as reproduced in document BMT-TWV/Mushroom/02/1.

Report of Discussions and Developments in UPOV Regarding the Possible Use of Molecular Techniques in DUS Testing

4. The Subgroup received a report from the Office of the Union on the latest developments in the Working Group on Biochemical and Molecular Techniques, and DNA-Profiling in Particular (BMT), the *Ad hoc* Crop Subgroups on Molecular Techniques (Crop Subgroups) and the *Ad hoc* Subgroup of Technical and Legal Experts of Biochemical and Molecular Techniques (BMT Review Group), on the basis of documents BMT/7/19 (Prov.), TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add. The Subgroup noted that the task of the Crop Subgroups was to consider crop-specific biochemical and molecular techniques.

5. The Subgroup also noted the views of the Administrative and Legal Committee (CAJ) and the Technical Committee (TC) at their April 2002 sessions on the three options mentioned in document TC/38/14 Add.-CAJ/45/5 Add.

6. The Subgroup further noted that the Technical Working Party for Vegetables (TWV) had agreed that Option 1 (a) (Use of molecular characteristics which are directly linked to traditional characteristics (e.g. gene specific markers) would be useful for the detection of certain vegetable characteristics, such as disease resistance and male sterility, and could be considered in conjunction with the discussion of individual Test Guidelines documents.

7. The Subgroup recalled the observation made by the TWV that the usefulness of Option 2 (Calibration of threshold levels for molecular characteristics against the minimum distance in traditional characteristics) for the management of reference varieties in DUS testing for vegetable varieties was worthy of examination. However, its examination would depend on the availability of data on both molecular and conventional distances.

8. The Subgroup recalled further the observation made by the TWV that, in the case of mushroom, the small number of available morphological assessment methods might justify the consideration of the introduction of molecular techniques for DUS testing.

AFLP Technique for Strain-typing of *Lentinula edodes* (Shiitake Mushroom) Cultivars (Document BMT-TWV/Mushroom/02/2)

9. Dr. Matsumoto (Japan) made a presentation entitled “AFLP technique useful for strain-typing of *Lentinula Edodes* (Shiitake Mushroom) cultivars,” on the basis of document BMT-TWV/Mushroom/02/2). Dr. Matsumoto reported that shiitake mushroom, *Lentinula edodes* (Berk.) Pegler, was one of the most popular and economically important edible mushrooms cultivated in East Asia. In Japan more than 100 different varieties were registered on the morphological and physiological characteristics and somatic compatibility, and used for commercial production. Shiitake mushroom was also recognized as a functional food with medicinal components and the improvement of such components was becoming one of the

main targets for the breeding of shiitake mushroom. Dr. Matsumoto explained that the purpose of his study was to establish a robust system, using amplified fragment length polymorphism (AFLP), to distinguish shiitake mushroom varieties in order to facilitate their protection. Dr. Matsumoto reported that, by using 6 AFLP primer pairs, 179 polymorphic DNA fragments had been recognized from 15 commercial shiitake mushroom varieties. He stated further that AFLP technique has a high potential and will be able to differentiate all commercial varieties of shiitake mushroom. Dr. Matsumoto showed dendrograms, produced by UPGMA (Unweighted Pair Group Method with Arithmatic Mean), distinguishing at least 50 strains. This system proved to be useful for the identification of heat-dried fruit bodies, especially by using gill tissue. In conclusion, the AFLP technique used in Dr. Matsumoto's study proved to be:

- reproducible, efficient and sensitive,
- useful for discrimination of strains developed by selfing etc.,
- an effective tool for strain typing in heat dried material,
- a tool for determining genetic relatedness among cultivars.

10. Dr. Matsumoto proposed that an AFLP profile database of protected shiitake varieties should be established to facilitate the protection of new varieties of shiitake mushroom.

Strain Identity of Mushrooms (Document BMT-TWV/Mushroom/02/3)

11. Dr. Anton S.M. Sonnenberg (Netherlands) made a presentation entitled "Strain Identity of Mushrooms" on the basis of document BMT/TWV/Mushroom/02/3. Dr. Sonnenberg explained that the species *Agaricus bisporus* ("button" mushrooms) possessed a very small number of phenotypic characteristics for reliable distinction of varieties. It had been expected that almost all commercial strains of the species were derived from the hybrid Horst U1, and this view was supported by DNA finger-printing. The lack of phenotypic characteristics and the ease of propagating existing strains were the main reasons to discourage substantial breeding in button mushrooms. The application of molecular analysis to wild strains showed, however, the existence of a wide rage of genetic variation in the species, indicating the possibility of breeding button mushroom varieties with more genetic variability.

12. In his study Dr. Sonnenberg paid special attention to the technique called "transposon tagging" where a modified AFLP was used for "tagging" different bands indicating repetitive elements (transposon). He concluded that this method (transposon-AFLP) showed a high polymorphism due to the presence of a high number of copies of transposons and could be improved to show a higher reproducibility. Dr. Sonnenberg observed, however, that the necessity for complete sequence information of the transposon would present an additional technical burden and that the modified AFLP might be protected by patent and might not be used freely.

13. Dr. Sonnenberg introduced alternative techniques called "simple sequence repeat" (ISSR) and "microsatellite fraction length polymorphism" (MFLP). For both techniques macrosatellites were used; in the case of ISSR, microsatellites were tagged through amplification using primers anchored at either the 5' or 3' ends whereas, in the case of MFLP, microsatellites were tagged by the modified AFLP. Further study needed to be done to

identify the most appropriate technique for tagging the repetitive elements in genomes of basidiomycetes.

14. Concerning the question of whether methylation on the DNA sequence might lead to different phenotypes, which could not be detected at the DNA level, Dr. Sonnenberg suggested that the MFLP had the possibility of detecting sites where methylation occurred.

15. In relation to the question of the patented molecular technologies, the Subgroup recalled that the UPOV strategy on the inclusion of patented methods in UPOV Test Guidelines adopted by the Administrative and Legal Committee at its forty-fourth session, in October 2001 (see document CAJ/44/9, paragraph 41) should be followed.

Discussions

16. The Subgroup observed a different situation for the application of molecular techniques in shiitake mushroom, on the one hand, and in button mushroom, on the other hand. In the case of shiitake mushroom, a wide range of variability existed. Fifty different strains could be differentiated within 5 to 7 days by using AFLP, and by applying a more detailed approach more than 100 strains could be differentiated. All these strains registered until now could be differentiated by using the conventional strain typing markers such as phenotypic characteristics and somatic compatibility. In the case of button mushroom, neither phenotypic nor molecular characteristics could clearly differentiate commercial varieties available on the world market. In the case of shiitake mushroom where DUS testing was being conducted on the basis of conventional characteristics, the immediate use of molecular techniques might be in establishing a molecular database to facilitate the enforcement of breeders' rights.

17. The subgroup was aware that the purpose of plant variety protection was to give incentives to breeders to continue their breeding activities and that the potential use of molecular techniques to DUS testing for button mushroom should be considered on this basis. This was a different situation compared to other crops where the introduction of molecular characteristics was considered for facilitating or refining the DUS testing, but not for encouraging significant breeding activities.

18. The Subgroup furthermore considered that the introduction of molecular techniques for DUS testing of button mushroom would not be sufficiently covered by any of the options included in documents TC/38/14-CAJ/45/5 and TC/38/14 Add.-CAJ/45/5 Add., given the fact that the genetic variation of commercial button mushroom varieties had proven, both by using phenotypic and molecular characteristics, to be extremely small.

19. Dr. Sonnenberg considered that it was still possible to identify even smaller differences at the molecular level by increasing the discriminatory power of molecular techniques. But this approach would not lead to a substantial improvement of button mushroom varieties unless the difference at molecular level had a link to any new improvement in agronomic or culinary characteristics. Therefore, the Subgroup considered it essential for incentives to be provided to improve substantial features of button mushroom varieties.

20. The Subgroup considered that, understanding that no significant morphological difference existed, breeding objectives for button mushroom might be focussed on increased

disease resistance (viral and fungal diseases) or other agronomic characteristics, such as an enhanced growth rate at a lower temperature. Questions were raised whether such characteristics could be detected easily by the use of molecular characteristics.

21. The Subgroup noted that technical robustness, reproducibility and availability should be further explored and the most appropriate protocol established.

Proposals to the TWV and the BMT

22. The Subgroup agreed that the Chairman should report to the BMT and TWV on the outcome of its discussion and ask for further guidance, especially on how the use of molecular characteristics should be considered in the work of the preparation of Test Guidelines for Mushroom.

[Annex follows]

ANNEX

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