



TGP/12.1.3 Draft 1

ORIGINAL: English

DATE: August 30, 2002

INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

Associated Document
to the
General Introduction to the Examination
of Distinctness, Uniformity and Stability and the
Development of Harmonized Descriptions of New Varieties of Plants (document TG/1/3)

DOCUMENT TGP/12

“SPECIAL CHARACTERISTICS”

**Section TGP/12.1.3: Characteristics Expressed in Response
to Living Organisms: Insect Resistance**

Document prepared by expert from France

to be considered by the

*Technical Working Party for Agricultural Crops (TWA), at its thirty-first session to be held in
Rio de Janeiro, Brazil, from September 23 to 27, 2002*

SECTION 12.1.3

CHARACTERISTICS EXPRESSED IN RESPONSE TO LIVING ORGANISMS: INSECT RESISTANCE¹

1. Among the characteristics which can be used to establish distinctness of a candidate variety, some are the result of the interaction between two living organisms: the plant variety and a fungus; a bacterium; a virus or an insect (designated as L.O. in this paper).
2. In such cases, certain specific conditions must be considered because of the possible variation of the L.O. which interacts with the variety.
3. In comparison with climatic or soil factors, additional sources of variation can change the effect of the L.O. on the variety:
 - the effect of factors, such as temperature, relative humidity and light, on the development or the aggressivity of the L.O.
 - the genetic variability of the L.O. (different races or strains).
4. Due to these sources of variation, the protocols used to obtain the description of candidate variety, or to compare close varieties, must be established with due attention to these sources of variation.
5. Different situations must be considered. In this first draft document, the assessment of insect resistance based on a genetic modification in maize varieties is described as an example.

Corn borer resistance in GM maize varieties

6. The procedure can include two parts:
 - (a) Check on the expression of the transgene: Bioassay
 - (b) Check on the presence of the transgene

The strategy on how to use these two tests can be as follows:

Check on the expression of the transgene: Bioassay

7. The expression of the transgene is directly observed in a test where the plant and the insect interact using pieces of young leaves and corn borer larvae. The protocol is described in the Annex.

¹ Living organisms (or any other wording) is better than external factors because this TGP deals with others factors than climate or soil which are also external factors.

8. This test works well and it enables the efficiency of the genetic transformation to be assessed. Compared to a PCR test, or Elisa test, which only reveal the presence of the protein, the Bioassay brings information on the real effect on the insect.

9. The present experience is that the transgenes which have been developed up until now are efficient whatever the origin of the corn borer.

Check on the presence of the transgene

10. When sufficient experience has been gathered on a given transgenic event² and if no interaction has been observed on the expression of the transgene between the transgene and the plant genetic background, the test to check the corn borer resistance could be done using PCR technique.

11. It is assumed that the specific probe³ is available to recognize the transgenic event.

12. Each time a new transgene is developed, its expression in different genetic backgrounds must be checked before relying on PCR technique alone to assess the characteristic.

13. It is also important to clarify that whatever the transgene or the transgenic event used, only one characteristic is considered to establish distinctness: corn borer resistance. It means that distinctness does not rely on differences in transgenes or transgenic events with the same expression.

[Annex follows]

² transgenic event = a transgene transferred to a given location in the plant genome using an appropriate technique

³ Specific probe means a probe with which the identity of the transgenic event (the transgene and its location in the genome) can be precisely determined.

ANNEX

**PROTOCOL FOR THE BIOASSAY TO CHECK CORN BORER
(*OSTRINIA NUBILALIS* HÜBNER) RESISTANCE OF GM MAIZE VARIETIES**

The protocol is as follows:

- Plants in growth with 8 to 10 leaves
- Larvae at the stage L1 (1st stage of development)

Pieces of leaves are sampled plant-by-plant on 10 plants per variety.

Leaves of each plant are distributed in 5 water-tight plastic boxes of 45 mm of diameter in which a disk of watered filter paper has been placed.

Six larvae are placed in each box; in total 50 boxes and 300 larvae per variety are used.

A susceptible variety is always included in each bioassay.

Conditions and Observations:

The boxes are placed in a chamber at 25° C with a photophase 16 : 8 (16 hours of light and 8 hours of dark) during 4 days with saturated moisture.

Mortality is recorded after 4 days exposure and surviving larvae are recorded on the 5th day.

Expression of the results

The criteria to assess resistance is the death rate of larvae.

The total number of dead larvae per plant is recorded as a percentage.

The average percentage per variety and a standard deviation are computed.

[End of Annex and of document]