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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS

GENEVA

DRAFT

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 1: Development of Characteristics Based on a Response to an External Factor

Document prepared by the Office of the Union

Technical Working Party for Vegetables (TWV), at its fortieth session, to be held in Guanajuato, Guanajuato State, Mexico, June 12 to 16, 2006

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1. Introduction

1.1 The General Introduction (document TG/1/3, Chapter 2, Section 2.5.3) states that:

> "The expression of a characteristic or several characteristics of a variety may be affected by factors, such as pests and disease, chemical treatment (e.g. growth retardants or pesticides), effects of tissue culture, different rootstocks, scions taken from different growth phases of a tree, etc. In some cases (e.g. disease resistance), reaction to certain factors is intentionally used (see TG/1/3 Chapter 4, Section 4.6.1) as a characteristic in the DUS examination. However, where the factor is not intended for DUS examination, it is important that its influence does not distort the DUS examination. Accordingly, depending on the circumstances, the testing authority should ensure either that:
>
> (a) the varieties under test are all free of such factors or,
>
> (b) that all varieties included in the DUS test, including varieties of common knowledge, are subject to the same factor and that it has an equal effect on all varieties or,
>
> (c) in cases where a satisfactory examination could still be undertaken, the affected characteristics are excluded from the DUS examination unless the true expression of the characteristic of the plant genotype can be determined, notwithstanding the presence of the factor."

1.2 The General Introduction (document TG/1/3, Chapter 4, Section 4.6.1) further states that "Characteristics based on the response to external factors, such as living organisms (e.g. disease resistance characteristics) or chemicals (e.g. herbicide resistance characteristics), may be used provided that they fulfil the criteria specified in [document TG/1/3, Chapter 4] Section 4.2. In addition, because of the potential for variation in such factors, it is important for those characteristics to be well defined and an appropriate method established which will ensure consistency in the examination." It should also be noted that, notwithstanding the fact that varieties may exhibit such traits, special tests for characteristics based on response to external factors do not need to be used where the routine characteristics resolve distinctness.

1.3 The following table presents the basic requirements that a characteristic should fulfill before it is used for DUS testing or producing a variety description together with some particular considerations with regard to characteristics based on the response to external factors:

Basic requirements that a characteristic should fulfill (document TG/1/3 Chapter 4, Section 4.6.1)	**Particular considerations with regard to characteristics based on response to external factors**
The basic requirements that a characteristic should fulfill before it is used for DUS testing or producing a variety description are that its expression:	
(a) results from a given genotype or combination of genotypes;	knowledge of the nature of genetic control of the response is important
(b) is sufficiently consistent and repeatable in a particular environment;	(i) important to standardize, as far as possible, the conditions in the field, greenhouse or laboratory, as appropriate, and the methodology used; (ii) the methodology should be validated, e.g. by a ring test; and (iii) the key requirements should be set out in a protocol.
(c) exhibits sufficient variation between varieties to be able to establish distinctness;	the response and suitable states of expression should be described (see (d) below)
(d) is capable of precise definition and recognition;	(i) the external factor should be clearly defined and characterized [(e.g. disease inoculum, chemical, race of insect etc.)] / [(e.g. disease inoculum, chemical, fungal race, virus pathotype, insect biotype etc.)] [1]; (ii) the type of response to the external factor (e.g. resistant, ~~tolerant~~, [intermediate resistant][1], susceptible etc.) and suitable states of expression (e.g. resistant or susceptible (qualitative characteristic); or levels of resistance / susceptibility (quantitative or pseudo-qualitative characteristic)) should be clearly defined. [The term "tolerant" should only be used for abiotic traits.][1]
(e) allows uniformity requirements to be fulfilled;	the uniformity requirements for characteristics based on the response to external factors are the same as for other characteristics. In particular, it is necessary for the method to allow the examination of individual plants.
(f) allows stability requirements to be fulfilled, meaning that it produces consistent and repeatable results after repeated propagation or, where appropriate, at the end of each cycle of propagation.	the stability requirements for characteristics based on the response to external factors are the same as for other characteristics.

[1] Text proposed by Mr. Kees van Ettekoven (Netherlands), drafter of the section on disease resistance

2. Disease Resistance

2.1 Introduction

Resistance to pests and diseases in an important breeding aim, particularly in vegetable breeding. Where there is particular focus on breeding for such resistances, the use of disease resistance characteristics in the examination of DUS may be important. However, such characteristics pose particular challenges, in particular with regard to the precise definition and recognition of characteristics and ensuring sufficient consistency and repeatability. The following sections address those requirements and the other requirements that a characteristic is required to fulfill.

2.2 Criteria for use of disease resistance characteristics

2.2.1 As with other characteristics (see Section 1.3: Table and the General Introduction, Chapter 4.2.1), the basic requirements that a disease resistance characteristic should fulfill before it is used for DUS testing or producing a variety description are that its expression:

(a) results from a given genotype or combination of genotypes;
(b) is sufficiently consistent and repeatable in a particular environment;
(c) exhibits sufficient variation between varieties to be able to establish distinctness;
(d) is capable of precise definition and recognition;
(e) allows uniformity requirements to be fulfilled;
(f) allows stability requirements to be fulfilled, meaning that it produces consistent and repeatable results after repeated propagation or, where appropriate, at the end of each cycle of propagation.

2.2.2 In general these requirements can be fulfilled but a number of requirements pose specific problems:

2.2.3 Ad (d) is capable of precise definition and recognition.

I. The definition of the disease itself usually does not create problems, for the proper denomination internationally accepted standards may be used such as that of the American Phytopathological Society (APS) for fungi and bacteria and the International Committee for Taxonomy of Viruses (ICTV).

2.2.4 Ad (d) is capable of precise definition and recognition.

II. The definition and denomination of the races and strains per disease pose a specific, more complicated problem as almost no longer any scientific work is done on this subject. This can result in confusing situations where the same race / strain could be named differently in Europe and the USA e.g. *Fusarium oxysporum* f.sp. *lycopersici* (Fol) in tomato where race 1 in the USA is identical to race 0 in Europe. Also different races / strains may have the same name e.g. *Fusarium oxysporum* f.sp. *lycopersici* (Fol) in tomato where race 2 in the USA is different from race 2 in Europe. At the moment a joint effort is made by ISF on this subject with the aim to create one clear system of definition and nomination. The core of this system is the precise definition of a set of host differential lines/varieties with which the races / strains can be determined. The seed industry is willing to cooperate by maintaining the necessary stocks of seed for this purpose.

2.2.5 In Section 2.3 *[cross ref.]* the definition of the various terms as developed and used by ISF is given. In Annex (II) a list of diseases is reproduced where it is known that resistance breeding has been carried out. Comments on this list are welcome on the ISF website.

2.2.6 Ad (a) The cooperation with breeders also results in better knowledge on the genetic background of the various forms of disease resistance. Knowing which genes are responsible for resistance and if it concerns a single gene or a combination of genes gives valuable information that will help to properly observe and evaluate the resistance.

2.2.7 Ad (b) is sufficiently consistent and repeatable in a particular environment.
Repeated tests and ring tests have shown that the stability of disease resistance, provided this was established on race / strain level is very good. In fact, as disease resistance is of crucial importance for the marketing of varieties, it is a primary selection criteria for companies to check the varietal stability.

2.2.8 Ad (e) allows uniformity requirements to be fulfilled.
Testing for disease resistance characteristics means introducing more variables in the trial; not only the development of the plants is subject to the environment, but also the quality of the inoculum, the inoculation and the interaction between symptom and development of the plant may cause variation within the trial. It has to be avoided that the heterogeneity introduced through the trial is blamed to the candidate variety.

2.2.9 Ad (d) is capable of precise definition and recognition.
Following the provided explanations in the test protocols, ring tests have shown to give deviating results. These deviations were caused by variation in the climatic conditions under which the trials were carried out. Also different interpretation of the symptoms by different observers was noted. The conclusion of these trials was that only if a correct set of standards was included in the trial, the observations and evaluation of the results was harmonized. It was however observed that slight differences in the standards (between lot differences) could cause problems. The advise here is to develop a centralized set of standards per disease or per strain to avoid problems. The seed industry is willing to cooperate by maintaining the necessary stocks of seed for this purpose.

2.2.10 Ad (c) exhibits sufficient variation between varieties to be able to establish distinctness.
Disease resistance characteristics, properly tested, give per definition a clear differentiation in the variety collections. Therefore disease resistance characteristics are often used as grouping characteristics. The differentiation usually may take place even on race / strain level as many collections of varieties are known to show different resistance reactions to different races / strains of the disease. Also on race / strain level grouping may be done, provided the races / strains are properly identified. A specific problem are those diseases or races / strains of diseases, where the difference between susceptible and resistant is not discontinuous, but in fact a scale of resistance can be observed ranging from absent to very weak to very strong. In practice, however, it is not yet possible to define the different levels using example varieties, so in the guidelines diseases that show this phenomenon are usually treated as discontinuous by defining a threshold dividing susceptible from resistant. The threshold is clearly defined using example varieties. It may be expected that in future this practice will be replaced by a more precise description of the different levels of resistance. These levels have to be defined

precisely and standards will have to be included in the tests to enable the differentiation between the different levels.

2.2.11 As additional points for consideration, the following has to be taken into account:

(g) the availability of reliable inoculum and host differential set
(h) quarantine regulations
(i) the costs involved in disease resistance testing

2.2.12 Ad (g) the availability of reliable inoculum.
In general, a few institutes are still maintaining stocks of inoculum of most of the diseases that are used in breeding programs. In the explanation of the methods in the guidelines, the available information on these sources will have to be indicated. If inoculum from another source is used, a defined host differential set will have to be used to clearly identify the inoculum.

2.2.13 Ad (h) quarantine regulations.
With a worldwide organization as UPOV, it is unavoidable that diseases that are of importance in a certain area, are unknown to cause problems in another part of the world and are there considered as quarantine diseases. Usually this means that the import of inoculum and the test itself is not possible. A good way to solve this kind of problems is to contact a DUS test authority elsewhere and ask them to carry out the test.

2.2.14 Ad (i) The costs and technical requirements of disease tests are for some DUS testing authorities impassable barriers to carry out these tests. Two options may be considered to overcome these problems:

- Another DUS testing authority may be asked to perform the necessary disease test(s).

- The applicant / breeder may be requested to carry out a blind disease test with coded samples including the candidate variety and a number of also coded control samples as susceptible and resistant controls on the basis of a clear control.

2.2.15 In order to take into account the given points of consideration, the explanation of the disease resistance characteristics, included in the guidelines have to be extended with the necessary information on

- the address(es) where inoculum may be obtained,
- the host differential set of varieties / lines to use to check the inoculum on correctness regarding the races / strains used,
- the address(es) where the differential set may be obtained
- the race / strain specific standard varieties to be included in the test
- the address(es) where the set of standard varieties may be obtained

2.3 Terminology in Disease Resistance (Definition of the Terms Describing the Reaction of Plants to Pests or Pathogens and to Abiotic Stresses)

2.3.1 Preamble

Differing degrees of specificity exist in the relations between plants and pests or pathogens. Identification of such specificity generally requires the use of highly elaborate analytical methods. Recognizing whether a plant is subject to a pest or pathogen or not may depend on the analytical method employed. It is important, in general, to stress that the specificity of pests or pathogens may vary over time and space, depends on environmental factors, and that new pest biotypes or new pathogen races capable of overcoming resistance may emerge.

2.3.2 Definitions[2]

Immunity: Not subject to infection by a specified pest or pathogen.

Resistance is the ability of a plant variety to restrict the growth and development of a specified pest or pathogen and/or the damage they cause when compared to susceptible plant varieties under similar environmental conditions and pest or pathogen pressure. Resistant varieties may exhibit some disease symptoms or damage under heavy pest or pathogen pressure.

Susceptibility is the inability of a plant variety to restrict the growth and development of a specified pest or pathogen.

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[2] The TC requested that the definitions provided in Section 2.3.2 should be compared with the definitions previously agreed by the TC, which are as follows (see document TC/32/7, paragraph 17(i)):

"Definition of the terms describing the reaction of plants to pests and pathogens

- The definitions below concern exclusively the specific host-parasite pairs between which there exists compatibility. They do not concern non-recognition between partners amounting to incompatibility.
- There exist differing degrees of specificity in the host-parasite relations. The identification of that specificity generally requires the use of highly elaborate analytical means.
- Recognizing whether a plant is subject or not to parasites may depend on the analytical method.
- It is important, in general, to stress that the specificity of pests or pathogens may vary over time and space and that new pathogen races or new pest biotypes capable of overcoming a resistance may emerge.

"The following terminology has been adopted by UPOV:

Resistance: The ability of a variety or of a mono-specific population to limit the activities of a given pest of pathogen throughout the whole or a part of a growing cycle. Several resistance levels may generally be defined.

Susceptibility: Susceptibility corresponds to a zero-resistance level of a variety or population with respect to a given pest or pathogen.

Tolerance: Ability of a variety or population to tolerate the development of a pest or pathogen whilst displaying disorders that are without serious consequences for their growth, appearance or yield."

Mr. Kees van Ettekoven, drafter of the Section on disease resistance comments that "In particular the term tolerance is difficult to establish in DUS trials. As also in practice the use of the term tolerance is leading to interpretation difficulties, in this document it is proposed to accept the terminology for DUS purposes as presented in Section 2.3.2.. Further the use of the proper states of expression is indicated."

The Vegetable Section of ISF recommends, as it pertains to biotic stress, that its members use the terms immunity, high/standard or moderate/intermediate resistance and susceptibility and to avoid the term tolerance in communications with their customers.

Tolerance is the ability of a plant variety to endure abiotic stress without serious consequences for growth, appearance and yield. Vegetable companies will continue to use tolerance for abiotic stress.

2.4 Developing characteristics for disease resistance

In general, disease resistance characteristics are qualitative or quantitative characteristics:

2.4.1 Qualitative characteristics

Disease resistances which are expressed as absent or present, where those states are discontinuous, are qualitative characteristics.

Example

	English	français	Deutsch	español	Example Varieties	Note
39. (+)	**Resistance to downy mildew (*Bremia lactucae*)**	**Résistance au mildiou (*Bremia lactucae*)**	**Resistenz gegen Falschen Mehltau (*Bremia lactucae*)**	**Resistencia al mildiú (*Bremia lactucae*)**		
39.1	**Isolate Bl 2**	**Isolat Bl 2**	**Isolat Bl 2**	**Aislado Bl 2**		
QL	absent	absente	fehlend	ausente	[…]	1
	present	présente	vorhanden	presente	[…]	9

2.4.2 Quantitative characteristics

Disease resistances for which there is a continuous range of levels of susceptibility / resistance across varieties, are quantitative characteristics. In general, it is not possible to define the nine states of resistance which would be necessary in order to apply the standard "1-9" scale. Therefore, the condensed "1-3" scale may be the most appropriate way in which to present such characteristics.

Example

		English	français	Deutsch	español	Example Varieties	Note
70. **(+)**	**VG**	**Resistance to *Sphaerotheca fuliginea (Podosphaera xanthii)* (Powdery mildew)**	**Résistance à *Sphaerotheca fuliginea (Podosphaera xanthii)* (oïdium)**	**Resistenz gegen *Sphaerotheca fuliginea (Podosphaera xanthii* (Echter Mehltau)**	**Resistencia a *Sphaerotheca fuliginea (Podosphaera xanthii)* (Oidio)**		
70.1		**Race 1**	**Pathotype 1**	**Pathotyp 1**	**Raza 1**		
QN		susceptible	sensible	anfällig	susceptible	[…]	1
		moderately resistant	moyennement résistant	mäßig resistent	moderadamente resistente	[…]	2
		highly resistant	hautement résistant	hochresistent	altamente resistente	[…]	3

3. Chemical Response

3.1 Introduction

Plant growth can be significantly influenced by a number of chemical compounds. When applied to plants, such chemicals can affect the phenology, physiology and change phenotypic characteristics. They include herbicides, plant growth regulators, defoliants, rooting compounds, and compounds used in tissue culture media. The effect of herbicides and plant growth regulators on plant characteristics are discussed below:

3.2 Herbicides

3.2.1 Herbicide Tolerant Varieties

3.2.1.1 The breeding of herbicide resistant varieties is now commonplace. When such varieties are treated with herbicide, their level of “tolerance” is manifested by some phenotypic expression(s). Subject to the fulfillment of the requirements for a characteristic to be used in DUS testing (TG/1/3 Section 4.2) these characteristics can be useful in assessing distinctness.

3.2.1.2 Herbicide tolerance can either be an inherent characteristic of a plant variety or can be introduced by conventional plant breeding, mutation, or genetic modification. For example, some grasses are inherently tolerant to 2,4-D (2-4 phenoxyaliphatic acid) and other growth hormone mimics. Selection within these grass species has resulted in tolerant varieties. In contrast, other crops may not possess natural tolerance, even at very low levels and genetic modification is required to introduce herbicide tolerance (eg to phosphinothricin or glyphosate).

3.2.2 Case Study on the Use of Herbicides in the Expression of Plant Characteristics and Assessing Distinctness

3.2.2.1 Glyphosate tolerance in genetically modified cotton varieties can be used as a study case of the way in which morphological characteristics expressed in response to a particular chemical compound can be used to assess distinctness.

3.2.2.2 It has been reported (Australian PBR trials, 2000-2004) that certain phenotypic characteristics with different states of expressions were noticeable when different cotton varieties were treated with commercial concentrations of glyphosate. Examples of "additional characteristics"[3] specially developed to record the states of expression of various morphological/phenological traits in cotton in response to the application of *glyphosate* are presented in Table 1:

Table 1: Example of additional characteristics specially developed to reflect the states of expression of various morphological/phenological traits in cotton in response to the application of *glyphosate*

Characteristics	**States of Expression**	**Notes**
HERBICIDE EFFECT†: Young leaf folding	Very low effect	1
	Low effect	3
	Medium effect	5
	Strong effect	7
	Very strong effect	9
HERBICIDE EFFECT†: Leaf blotching	Very low effect	1
	Low effect	3
	Medium effect	5
	Strong effect	7
	Very strong effect	9
HERBICIDE EFFECT†: Terminal chlorosis	Very low effect	1
	Low effect	3
	Medium effect	5
	Strong effect	7
	Very strong effect	9
HERBICIDE EFFECT†: Plant wilting	Very low effect	1
	Low effect	3
	Medium effect	5
	Strong effect	7
	Very strong effect	9
HERBICIDE EFFECT†: Plant death	Absent	1
	Present	9

† = response to the application of *glyphosate*
Note: The scores on leaf blotching, terminal chlorosis and plant wilt are taken both at 3 and 7 days after the treatment. The scores on young leaf folding are taken at 7 days after herbicide treatment. The scores on plant death are assessed 14 days after spraying.

[3] These characteristics are additional to the "standard characteristic" included in the UPOV Test Guidelines for a species and are often specifically developed for particular circumstances.

3.2.2.3 Table 2 shows data on herbicide induced plant characteristics from a cotton trial in Australia that had been sprayed with *glyphosate* (using the additional characteristics included in Table 1)

Table 2: Comparison of cotton varieties on the basis of *glyphosate* tolerance

Variety:	'NuPearl RR'	'DP 5690 RRi'	'DeltaPEARL'
HERBICIDE EFFECT†: Young leaf folding (1- 9 scale)*			
**DAS 7 mean	1	1	6
HERBICIDE EFFECT†: Leaf blotching (1- 9 scale)*			
DAS 3 mean	1	1	5
DAS 7 mean	2	2	8
HERBICIDE EFFECT†: Terminal chlorosis (1- 9 scale)*			
DAS 3 mean	1	1	1
DAS 7 mean	1	1	5
HERBICIDE EFFECT†: Plant wilt (1- 9 scale)*			
DAS 3 mean	1	1	2
DAS 7 mean	1	1	5
HERBICIDE EFFECT†: Plant death (1- 9scale)***			
DAS 14 mean	1	1	9

† = response to the application of *glyphosate*
* = very low effect, 3 = low effect, 5 =medium effect, 7 = strong effect, 9 = very strong effect.
** = DAS = days after spraying; scoring was done at 3, 7 and 14 days after herbicide application (eg DAS 7 means 7 days after spraying).
*** = plants alive, 9 = plants dead.

3.2.2.4 The above data shows that, following *glyphosate* treatment, the differences between the tolerant and susceptible varieties become evident within a week for all characteristics mentioned above. Both 'NuPearl RR'and 'DP 5690 RRi' varieties were tolerant to *glyphosate*, showing very little effect, while the variety 'DeltaPEARL' was completely susceptible and was dead from the treatment by day 14. Without using these additional characteristics it would be difficult to distinguish between those varieties, which are morphologically almost indistinguishable.

3.3 Plant Growth Regulators

3.3.1 Chemicals which act as plant growth regulators are often structurally similar to plant hormones. However, the basic difference between plant growth regulators and plant hormones is that growth regulators are exogenous (not made within the plant) whereas plant hormones are produced within the plants *per se* as a part of the biological process.

3.3.2 Plant growth regulators are commonly used to control plant height, lateral branching, flowering etc. Plant growth regulators (eg. growth retardants) can simultaneously modify many plant characteristics and significantly alter the phenotype of a plant variety, e.g. the use of gibberellic acid (GA_3) in the production of 'Thompson Seedless' grape. This seedless grape is widely used as a premium table grape. 'Thompson Seedless' is the result of GA_3 treatment of the grape variety named 'Sultana' (or 'Sultania'), which is commonly used for the dry fruit market as raisins. However, when the variety 'Sultana' is treated with GA_3 (20-40ppm) at the early stage of fruit development the resulting fruits tend to elongate and the size of the fruit also increase and 'Sultana' is then marketed as the table grape 'Thompson Seedless'.

3.3.3 Responses to plant growth regulators could, in certain circumstances, be used a characteristic if the requirements set out in Sections 1.2 and 1.3 are met. However, where this is not the case, it may be difficult to ensure that the use of plant growth regulators in a DUS trial would not distort the DUS examination (see Section 1.1). In particular, it would be difficult to ensure that a plant growth regulator would have an "equal effect" on all varieties included in the DUS test, including varieties of common knowledge. Furthermore, as plant growth regulators may have subtle effects on a range of plant characteristics, special care would be needed to ensure that the description of 'standard characteristics' included in the Test Guidelines were not distorted.

4. Insect Resistance

4.1 Introduction

4.1.1 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).

4.1.2 In such cases, certain specific conditions must be considered because of the possible variation of the L.O. which interacts with the variety.

4.1.3 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 agressivity of the L.O.

- the genetic variability of the L.O. (different races or strains).

4.1.4 Due to these sources of variation, the protocols used to obtain the description of a candidate variety, or to compare close varieties, must be established with due attention to these sources of variation.

4.1.5 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.

4.2 Example: Corn borer resistance in GM maize varieties

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:

4.2.1 Check on the expression of the transgene: Bioassay

4.2.1.1 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 Section 4.2.3 *[cross ref.]*.

4.2.1.2 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.

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

4.2.2 Check on the presence of the transgene

4.2.2.1 When sufficient experience has been gathered on a given transgenic event[4] 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.

4.2.2.2 It is assumed that the specific probe[5] is available to recognize the transgenic event.

4.2.2.3 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.

4.2.2.4 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.

4.2.3 Protocol for the Bioassay to check Corn Borer (Ostrinia Nubilalis Hübner) Resistance of GM Maize Varieties

4.2.3.1 The protocol is as follows:

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

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

[5] 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.

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.

4.2.3.2 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 5^{th} day.

4.2.3.3 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 document]