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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/12.1.2: Characteristics Expressed in Response to External Factors: Chemical Response

Document prepared by expert from Australia

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 12.1.2

CHARACTERISTICS EXPRESSED IN RESPONSE TO EXTERNAL FACTORS: CHEMICAL RESPONSE

1. Introduction

1. Along with the increased use of herbicides, the breeding of herbicide resistant varieties is now commonplace. Different varieties behave differently in their response to a herbicide or group of herbicides. When varieties are treated with a herbicide, their level of “tolerance” is manifested by some phenotypic expression(s). Subject to meeting the UPOV rules relating to characteristics (document TG/1/3 “General Introduction to the Examination of Distinctness, Uniformity and Stability and the Development of Harmonized Descriptions of New Varieties of Plants”) these expressions can be useful in assessing the differences between the varieties for the purpose of examining distinctness.

2. Breeding Herbicide Tolerant Varieties

2. Herbicide tolerance can either be a ‘natural’ characteristic of a plant variety or can be introduced by conventional plant breeding, mutation, or genetic modification.

<u>2.1 Herbicide Tolerance Introduced by Conventional Plant Breeding:</u>

3. Some plant species have long been known to be highly variable in their response to herbicides. For example, some grasses are very tolerant to 2,4-D (2-4 phenoxyaliphatic acid) and other growth hormone mimics, while other broad-leaved species shrivel and die when exposed to it. Soybeans can tolerate trifluralin, but maize plants become stunted and never reach their reproductive phase.

4. During the 1980s, plant breeders tried to take advantage of natural variability to develop tolerant varieties. It has been reported that wheat varieties tolerant to imidazolinone and canola varieties tolerant to triazine and imidazolinone have been developed through conventional plant breeding techniques. However, attempts to conventionally breed glyphosate-tolerant crops were not successful. Such failure is not surprising; after many years of glyphosate use, plant resistance in the field has been noted in only two grass species.

<u>2.2 Herbicide Tolerance Introduced by Genetic Modification:</u>

5. This currently involves two main herbicides: *phosphinotricin* (or glufosinate) commercially known by various brand names such as *Basta*, *Finale*, and *Liberty*; and *glyphosate* (N-phosphono-methyl glycine) often marketed under the brand name *Roundup*. Both chemicals are broad-spectrum herbicides which make no distinction between crops and weeds. By genetic modification, crops can be given the ability to tolerate the presence of phosphinothricin or glyphosate.

6. Table 1 is a summary of commercialized transgenic herbicide tolerant crops:

Table 1: Commercial transgenic herbicide tolerant crops

Common name	**Botanical name**	**Herbicide**
Canola/Oilseed Rape	*Brassica napus* var. *oleifera*	Phosphinothricin
		Glyphosate
Chicory	*Cichorium intybus*	Phosphinothricin
Cotton	*Gossypium hirsutum*	Glyphosate
Maize/Corn	*Zea mays*	Phosphinothricin
Soybean	*Glycine max*	Phosphinothricin
Sugar Beet	*Beta vulgaris* var. *crassa*	Phosphinothricin

Source: OECD, 2002.

Such transgenic herbicide tolerance is often used as an efficient selection system in the laboratory phase of genetic modification to identify transgenic plants.

3. Use of Herbicides in the Expression of Plant Characteristics and Assessing Distinctness

7. Glyphosate resistance in genetically modified cotton varieties could be used as an example of the array of morphological characteristics expressed in response to a particular chemical compound. It has been reported (Australian PBR trials, 2001) that certain phenotypic characteristics with different states of expressions were noticeable when cotton varieties were treated with commercial concentrations of glyphosate. These characteristics with their level of expression are presented in table 2:

Table 2: The expression of various morphological/phenological characteristics in cotton in response to the application of glyphosate

Characteristics	**States of Expression**	**Notes**
Young leaf folding	very low effect	1
	low effect	2
	medium effect	3
	strong effect	4
	very strong effect	5
Leaf blotching	very low effect	1
	low effect	2
	medium effect	3
	strong effect	4
	very strong effect	5
Terminal chlorosis	very low effect	1
	low effect	2
	medium effect	3
	strong effect	4
	very strong effect	5
Plant wilting	very low effect	1
	low effect	2
	medim effect	3
	strong effect	4
	very strong effect	5

Characteristics	States of Expression	Notes
Plant death	absent present	1 9

The scores on leaf blotching, terminal chlorosis and plant wilt were taken both at 3 and 7 days after the treatment. The scores on young leaf folding were taken at 7 days after herbicide treatment. The scores on plant death were assessed 14 days after spraying and all non-tolerant varieties were found dead while the tolerant varieties were still alive.

8. Table 3 shows some actual data from a cotton trial in Australia conducted in 2000.

Table 3: Comparison of cotton varieties on the basis of glyphosate tolerance

	'NuPearl RR'	**'DP 5690 RRi'**	**'DeltaPEARL'**
HERBICIDE EFFECT*: YOUNG LEAF FOLDING (1- 5 scale)*			
[1]DAS 7 mean	1.00	1.00	3.60
HERBICIDE EFFECT: LEAF BLOTCHING (1- 5 scale)*			
DAS 3 mean	1.50	1.40	2.50
DAS 7 mean	2.40	2.20	4.05
HERBICIDE EFFECT: TERMINAL CHLOROSIS (1- 5 scale)*			
DAS 3 mean	1.00	1.00	1.40
DAS 7 mean	1.00	1.00	3.40
HERBICIDE EFFECT: PLANT WILT (1- 5 scale)*			
DAS 3 mean	1.00	1.00	1.70
DAS 7 mean	1.00	1.00	2.75
HERBICIDE EFFECT**: PLANT DEATH (1- 9scale)**			
DAS 14 mean	1	1	9

[1]DAS = days after spraying; scoring was done at 3, 7 and 14 days after herbicide application.
*1 = very low effect, 2 = low effect, 3 =medium effect, 4 = strong effect, 5 = very strong effect.
** 1 = plants alive, 9 = plants dead.

9. The above data shows both 'NuPearl RR'and 'DP 5690 RRi' are tolerant to herbicide while 'DeltaPEARL' is completely susceptible and is dead from the herbicide treatment by day 14. Even the tolerant varieties 'NuPearl RR' and 'DP 5690 RRi' show some degree of differences in their phenotypic expressions in response to glyphosate (see leaf blotching).

10. For data of this type a number of non-parametric procedures are available, while the use of ANOVA is usually not appropriate. Document TGP/8, "Use of Statistical Procedures in DUS Testing", details the statistical procedures for different data types used in DUS testing.

4. Conclusions

11. The expression of a characteristic or several characteristics of a variety may be affected by chemical treatments (e.g. herbicides, growth retardants etc.). These expressions can be used legitimately to establish distinctness. In this particular instance herbicide resistance is

used as an example to establish distinctness between the varieties. Like any other characteristics these characteristics must also meet the criteria for uniformity and stability.

12. However, where the chemical treatment is not intended to test distinctness, it is important that its influence does not distort the DUS examination. Accordingly, depending on the circumstances, the testing authority should, in accordance with section 2.5.3 of the General Introduction, "ensure either that: (a) the varieties under test are all free of such factors or, (b) that all varieties included in the DUS test are subject to the same treatment 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."

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