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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

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to be considered by the

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SECTION 12.1.2

CHARACTERISTICS EXPRESSED IN RESPONSE TO EXTERNAL FACTORS: CHEMICAL RESPONSE

Plant growth activities can be highly influenced by a number of chemical compounds. When applied on plants, these chemicals can affect the growth of the plant and change its phenotypic characteristics. These chemicals, which are mainly synthetic compounds, include: plant growth regulators, herbicides, defoliants, rooting compounds, and compounds used in tissue culture media. For the purpose of TGP/12 "Special Characteristics", the effect of plant growth regulators and herbicides on plant characteristics is discussed. Growth regulators are discussed in relation to ornamental and horticultural crops, while herbicides are discussed in the context of agricultural crops.

1. Plant Growth Regulators (PGRs)

These chemicals often possess structural similarity to plant hormones. However, the basic difference between PGRs and plant hormones is that PGRs are exogenous (not made within the plant) whereas plant hormones are produced within the plants *per se* as a part of the biological process. PGRs could be used to control the expression of various plant characteristics outlined below:

1.2 PGRs for plant height control

Certain PGRs are known as "growth retardants" for their anti-gibberellic acid activity. Growth retardants are commonly used in the greenhouse to regulate the shoot development of bedding plants, chrysanthemums, poinsettias and other container plants. Growth retardants are commercially known by various brand names: B-Nine (daminozide), Cycocel (chlormequat chloride), A-rest (ancymidol), Bonzi (paclobutrazol), Sumagic (unionazole) etc. These PGRs reduce plant height by inhibiting the production of gibberellins, the primary plant hormone responsible for cell elongation. Therefore, their effects are primarily on stem, petiole and flower stalk tissues. Lesser effects are seen in the reduction of leaf expansions, resulting in thicker leaves with dark green colour. There are some benefits using these PGRs in plant production, which include improved plant appearance by maintaining plant size and shape in proportion with the pot. Plant growth retardants also increase the stress tolerance of the plants during shipping and handling and retail marketing of the plants and thereby improve shelf life and extend the plant marketability.

1.3 PGRs for lateral branching

Another group of chemicals used in floriculture crops are those that enhance branching. These include Florel (ethephon), Atrimmec (dikegulac sodium), Off-Shoot–O (methyl esters of fatty acids) etc. These chemicals inhibit the growth of the terminal shoots and enhance the growth of the terminal buds, thereby increasing the development of lateral branching. They can be used to replace mechanical pinching of many crops. Often this increased branching reduces the overall height of the plants but increases the width of the plant. The overall growth habit of the plant can be changed due to the effect of these chemicals.

1.4 PGRs for controlling flowering

Certain chemicals can be used to enhance flowering (GibGro) or to remove flowers (Florel). To improve flowering, GibGro, which contains the growth promoter gibberellic acid, can be used to substitute for all or part of the chilling requirement of some ornamentals such as azaleas, hydrangea etc. Flower removal is especially desirable for stock plants for cuttings of vegetatively propagated ornamentals like geraniums, fuchsia, begonias etc. Florel (ethephon) is the primary compound used for flower removal. Once ethephon is absorbed by the plant it is converted to gaseous ethylene. Ethylene is the primary plant hormone responsible for flower senescence and fruit ripening. Therefore, the duration of flowering can be controlled by these chemicals.

1.5 PGRs for modifying other plant characteristics

The use of certain PGRs is common in some horticultural practices especially in viticulture. In some cases these PGRs are used to modify some characteristics of a plant variety to suit the market demand. One classic example is the use of gibberellic acid (GA_3) in the production of the grape variety 'Thompson Seedless'. This seedless variety is widely used as a premium table grape. 'Thompson Seedless' is the product of GA_3 treatment of the original 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, the size of the fruits also increases and 'Sultana' is then marketed as the table grape variety 'Thompson Seedless'. In other seedless grape varieties such as 'Reliance', GA_3 application also results in increased berry size, larger clusters and advanced fruit maturation. Also in some other grape varieties (e.g. 'Concord') the uneven ripening of fruits can be treated with GA_3 application. When GA_3 is applied to fruits, it increases in the rate of photosynthate translocation into the berries, and increases number of berries per cluster and sugar accumulation.

In Avocado the fruit size of the variety 'Hass' can be increased by the application of synthetic urea cytokinin complex. Also, in olive varieties 'Ascolana Tenera' and 'Santa Caterina' the average fruit size and weight can be increased with CPPU (a cytokinin complex) application.

The use of PGRs is not common in agricultural crops. However, in agricultural crops such as beans, oats, peas, cotton, rye, soybeans and wheat – GA_3 can be used as a seed treatment to promote rapid seedling emergence. The seedlings of the treated varieties often elongate more than normal due to GA_3 application. Also, in sugarcane varieties GA_3 application as a foliar spray can result in an increase in sugar production.

2. Herbicides

Along with the increased use of herbicides, the breeding of herbicide resistant varieties is now commonplace. 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) these expressions can be useful in assessing the differences between the varieties for the purpose of establishing distinctness.

2.1 Breeding Herbicide Tolerant Varieties

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

2.1.1 Herbicide Tolerance Introduced by Conventional Plant Breeding

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.

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.

2.1.2 Herbicide Tolerance Introduced by Genetic Modification

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 that make no distinction between crops and weeds. By genetic modification, crops can be given the ability to tolerate the presence of phosphinothricin or glyphosate.

The table 1 is a summary of commercialised 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 efficient selection system in the laboratory phase of genetic modification to identify transgenic plants.

2.2 Use of Herbicides in the Expression of Plant Characteristics and Assessing Distinctness

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, 2000) 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 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
	medium effect	3
	strong effect	4
	very strong effect	5
Plant death	absent	1
	present	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.

The 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.

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).

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

3. Conclusions

3.1 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. Like any other characteristic the response to an applied chemical characteristics must also meet the criteria for uniformity and stability.

3.2 However, where the chemical treatment is not intended to test distinctness, it is important that its influence does not distort the DUS examination. Accordingly (see also document TG/1/3: Section 2.5.3), 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 are subject to the <u>same treatment</u> and that it has an <u>equal effect</u> 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, despite the presence of the chemical factor.

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