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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 1.3: Characteristics Expressed in Response to External Factors: Chemical Response

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to be considered by the
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CHARACTERISTICS EXPRESSED IN RESPONSE TO EXTERNAL FACTORS: CHEMICAL RESPONSE

Plant growth can be significantly influenced by a number of chemical compounds. When applied on 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.

1. Herbicides

The breeding of herbicide resistant or tolerant varieties is now commonplace. When such varieties are treated with a herbicide, their level of "tolerance" is manifested by some phenotypic expression(s). Subject to the fulfilment 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.

1.1 Breeding Herbicide Tolerant Varieties

Herbicide tolerance can either be an inherent characteristic of a plant variety or can be introduced by, for example, conventional plant breeding, mutation, or genetic modification.

1.1.1 <u>Herbicide Tolerance Introduced by Conventional Plant Breeding</u>: Some plant species have long been known to be highly varied 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.

1.1.2 During the 1980s, plant breeders sought 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.

1.1.3 <u>Herbicide Tolerance Introduced by Genetic Modification</u>: 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. By genetic modification, crops can be given the ability to tolerate the presence of phosphinothricin or glyphosate.

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

1.2.1 Glyphosate resistance in genetically modified cotton varieties could be used as an example of the range of morphological characteristics expressed in response to a particular chemical compound. It has been reported (Australian PBR trials, 2000-2004) 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 levels of expression are presented in Table 1:

Table 1: 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	3
	medium effect	5
	strong effect	7
	very strong effect	9
Leaf blotching	very low effect	1
	low effect	3
	medium effect	5
	strong effect	7
	very strong effect	9
Terminal chlorosis	very low effect	1
	low effect	3
	medium effect	5
	strong effect	7
	very strong effect	9
Plant wilting	very low effect	1
	low effect	3
	medium effect	5
	strong effect	7
	very strong effect	9
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.

Table 2 shows data on herbicide-induced plant characteristics from a cotton trial in Australia that had been sprayed with glyphosate

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

	'NuPearl RR'	**'DP 5690 RRi'**	**'DeltaPEARL'**
HERBICIDE EFFECT*: YOUNG LEAF FOLDING (1- 9 scale)*			
[1]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

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

1.2.3 The above data shows that, following glyphosate treatment, differences between tolerant and susceptible varieties become evident within a week for all characteristics mentioned above. Both 'NuPearl RR' and 'DP 5690 RRi' were tolerant to glyphosate, showing very little effect, while 'DeltaPEARL' was completely susceptible and was dead from the treatment by day 14.

2. Plant Growth Regulators

Chemicals which act as plant growth regulators often possess a structural similarity 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.

Plant growth regulators are commonly used to control the expression of various plant characteristics outlined below.

2.1 Plant growth regulators for plant height control

Certain plant growth regulators 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, for example, 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 plant growth regulators 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 expansion, resulting in thicker leaves with dark green colour. There are some commercial benefits from using these plant growth regulators in plant production, which include improved plant appearance by maintaining plant size and shape in proportion with the pot. Plant growth retardants can also increase the stress tolerance of the plants during shipping and handling and retail marketing of the plants and thereby improving shelf life and extending the plant marketability.

2.2 Plant growth regulators 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 lateral and axillary buds, thereby increasing the development of lateral branching. These can be used to replace mechanical pinching of the primary axis on 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.

2.3 Plant growth regulators for controlling flowering

Certain chemicals can be used to enhance flowering e.g. (GibGro) or to remove flowers (e.g. 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 timing and duration of flowering can be controlled by these chemicals.

2.4 Plant growth regulators for modifying varietal characteristics

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

2.4.2 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.

2.4.3 In agricultural crops such as beans, cotton, oats, peas, rye, soybeans and wheat – GA_3 can be used as a seed treatment to promote rapid seedling emergence. The seedlings of the treated varieties are often more elongated 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.

3. Conclusions

3.1 The General Introduction explains the following in respect to factors that may affect the expression of a characteristic of a variety:

> "2.5.3 Factors That May Affect the Expression of the Characteristics of a Variety
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> 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 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."

3.2 With plant growth regulators it is difficult to ensure that all varieties included in the DUS test, including varieties if common knowledge , will have an "equal effect". Therefore, it is recommended that Plant Growth Regulators should not be used in DUS examination.

3.3 In some cases (e.g. herbicides etc.) the responses to the chemicals can be used to examine distinctness. Like any other characteristic the response to an applied chemical characteristic must also meet the criteria for uniformity and stability as explained in Section 1.1 Introduction.

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