UPOV
International Union for the Protection of New Varieties of Plants

E

Technical Working Party for Agricultural Crops **TWA/46/7**

Forty-Sixth Session
Hanover, Germany, June 19 to 23, 2017

Original: English
Date: June 7, 2017

USE OF DISEASE AND INSECT RESISTANCE CHARACTERISTICS IN DUS EXAMINATION

Document prepared by the Office of the Union

Disclaimer: this document does not represent UPOV policies or guidance

EXECUTIVE SUMMARY

1. The purpose of this document is to report on developments concerning the use of disease and insect resistance characteristics in DUS examination.

2. The TWA is invited to consider the information to be presented at its forty-sixth session on the use of disease and insect resistance characteristics in DUS examination, as presented in Annexes I to IV to this document.

BACKGROUND

Technical Working Party for Vegetables

3. The TWV, at its fiftieth session, held in Brno, Czech Republic, from June 27 to July 1, 2016, considered documents TWV/50/21 and TWV/50/21 Add. Rev. "Use of disease and insect resistance characteristics in DUS examination" (see document TWV/50/25 "Report", paragraphs 61 to 67).

4. The TWV noted that the use of a characteristic for DUS purposes did not mean that it would need to become a breeding aim, and vice-versa. The use of a disease or insect resistance characteristic for DUS purposes did not require breeders to select for that characteristic in their breeding programs, but would require them to ensure varieties were uniform and stable for the characteristic, in the same way as for other DUS characteristics.

5. The TWV noted the approach by the European Union for their Test protocols, and considered the proposal with regard to the idea of phasing-in asterisked characteristics (which lead to obligatory testing in CPVO Protocols over a period of time) in UPOV Test Guidelines, as presented in document TWV/50/21. The TWV agreed that more time was needed for members of the Union to consider if such an approach would be appropriate.

6. The TWV welcomed the information provided on "MatRef: a national network managing seeds and strains for disease resistance tests", by the expert from France, and "Harmonization of resistance tests to diseases for DUS testing: Harmores 2", by the expert from the Community Plant Variety Office of the European Union (CPVO), as reproduced in document TWV/50/21 Add. Rev. It agreed that it would be useful to have an update on those projects at its fifty-first session and also to present information to the Technical Committee (TC), at its fifty-third session, under the discussion item "Use of disease and insect resistance characteristics in DUS examination".

7. The TWV noted that the approach presented in document TWV/50/21 Add. Rev. was based on the use of molecular data obtained by the DUS examination office to verify information on disease resistance provided by the applicant in the Technical Questionnaire. If the molecular data was consistent with the information provided by the applicant, the DUS examination would be based on the molecular data but if there was a discrepancy, or the applicants did not test, a bioassay would be used for the DUS examination. The TWV considered that it might be necessary to request confirmation from the applicant that the information provided on disease resistance was based on a bioassay and, if that was not the case, a

bioassay would need to be used for the DUS examination. Such an approach could then be proposed for inclusion in the UPOV Test Guidelines.

8. The TWV noted that the above approach was consistent with the model "Characteristic-Specific Molecular Markers", as set out in TGP/15 "Guidance on the Use of Biochemical and Molecular Markers in the Examination of Distinctness, Uniformity and Stability (DUS)". It further noted that the above approach verified the reliability of the link between the molecular marker and the disease resistance characteristic for every candidate variety.

9. The TWV agreed that it would be valuable for the above approach to be presented to the TC, at its fifty-third session, under the discussion item "Use of disease and insect resistance characteristics in DUS examination".

Technical Working Party for Agricultural Crops

10. The TWA, at its forty-fifth session, held in Mexico City, from July 11 to 15, 2016, agreed to invite experts from Brazil, the European Union and France to prepare documents on the use of disease and insect resistance characteristics in DUS examination (see document TWA/45/25 "Report", paragraph 124).

Technical Committee

11. The TC, at its fifty-third session, held in Geneva from April 3 to 7, 2017, noted developments in the TWV and TWA concerning the use of disease and insect resistance characteristics in DUS examination (see document TC/53/31 "Report", paragraph 106).

INFORMATION TO BE PRESENTED AT THE FORTY-SIXTH SESSION OF THE TWA

12. The Annexes to this document contain the following information to be presented at the forty-sixth session of the TWA:

ANNEX I: "Rust Resistance as DUS Characteristics in Wheat", presentation prepared by an expert from Australia

ANNEX II: "Use of disease and insect resistance characteristics in DUS examination: experience of Brazil with soybean", document prepared by an expert from Brazil

ANNEX III: "Harmonization of resistance tests for DUS testing: 'Harmores 2'", presentation prepared by an expert from the European Union

ANNEX IV: "Phasing-in period for asterisked disease resistance characteristics in CPVO vegetable technical protocols", presentation prepared by an expert from the European Union

13. The TWA is invited to consider the information to be presented at its forty-sixth session on the use of disease and insect resistance characteristics in DUS examination, as presented in Annexes I to IV to this document.

[Annexes follow]

Australian Government
IP Australia

Rust Resistance as DUS Characteristics in Wheat

Tanvir Hossain
Senior Examiner
Plant Breeder's Rights
IP Australia

Australian Government
IP Australia

Importance of Rust Resistance in Wheat

- Wheat is economically the most important cereal crop in Australia ($7 billion annually).
- Mostly wheat varieties are spring grown in Australia.
- Rust is the most damaging disease of wheat. Severe yield losses in susceptible varieties. (up to 37% reported). Three types of rust:

  Stem rust - *Puccinia graminis* f. sp. *tritici*
  Leaf rust - *Puccinia recondita* f. sp. *tritici*
  Stripe rust - *Puccinia striiformis* f. sp. *tritici*

- All are airborne pathogens.

Australian Government
IP Australia

Three Types of Rust

Robust intellectual property rights delivered efficiently

Australian Government
IP Australia

Host Plant Resistance

- Developing varieties with high level of genetic resistance is the most logical and economic approach.
- Breakdown of rust resistance is a serious problem in wheat breeding.
- Host plant resistance is regulated by gene-for-gene mechanism.

- For each resistant gene in the wheat plant there is a corresponding virulent gene conditioning virulence in the pathogen.

Robust intellectual property rights delivered efficiently

Australian Government
IP Australia

Host Plant Resistance

- Rust resistance in wheat is an interaction between two biological systems – host and pathogen.
- Complementary genetic system through evolutionary mechanisms.
- Many major wheat genes are known for rust resistance. (*Sr36*, *Lr23*, *Yr17* etc.)
- Wheat varieties grown in Australia possess one or more major rust resistant genes.
- To overcome a resistant gene the rust pathogen must carry the corresponding virulent gene.
- This can be tested by using differential rust pathotypes.

Robust intellectual property rights delivered efficiently

Australian Government
IP Australia

Use in DUS Examination

- Rust resistance could be used in DUS examination.
- Must be carried out by an accredited laboratory using standard protocols.
- University of Sydney, Plant Breeding Institute (PBI), Cereal Rust Laboratory is currently the only accredited laboratory in Australia.
- PBI conducts the rust testing for all cereal breeding programs including wheat.

Robust intellectual property rights delivered efficiently

Australian Government
IP Australia

Types of Resistance

- Rust reaction of a wheat variety is based on two types of resistance.
- **Seedling Resistance** – effective from seedling emergence to maturity provided that the virulent gene in the pathogen is absent. Tested against single resistant gene (QL)

- **Adult Plant Resistance** – effective from the fourth leaf stage to head emergence. Virulent genes in the pathogen may or may not be present. Tested against multiple resistant genes. (QN)

Robust intellectual property rights delivered efficiently

Australian Government
IP Australia

Seedling Resistance (QL)

- Tested in the greenhouse under controlled climatic condition.
- Seedlings are inoculated with specific rust pathotypes with known differentials.
- Host response is recorded by resistant and susceptible infection types.
- All major resistant genes in the host can be detected as absent or present.

- Qualitative Resistance.

Robust intellectual property rights delivered efficiently

Australian Government
IP Australia

Seedling Resistance (QL)

Inoculation Procedure

Seedlings removed from a misting chamber following inoculation with urediospores suspended in mineral oil.

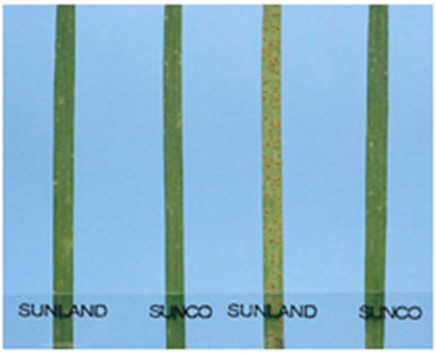
Australian Government
IP Australia

Seedling Resistance (QL)

'Sunvale'(left) carries stripe rust resistant gene *Yr17* while comparator 'Sunco'(right) does not possess this gene. When tested with pathotype 110E 143A+ 'Sunvale' exhibits resistant infection type while 'Sunco' shows susceptible infection.

Australian Government
IP Australia

Seedling Resistance (QL)



Wheat - 'Sunland' and comparator 'Sunco' both exhibiting resistant infection type with the pathotype 104-2,3,6,7 (left) whereas with pathotype 104-2,3,6,7, 8 (right) 'Sunland' is exhibiting susceptible infection type and 'Sunco' is still showing resistant infection type (*Lr28* present)

Australian Government
IP Australia

Adult Plant Resistance

- Adult Plant Resistant (APR) is generally tested in the field or in the net house.
- Varieties under testing are grown as single rows.
- Susceptible varieties are grown as buffer rows.
- High disease pressure is maintained by artificial inoculation and high moisture content.
- Varieties are inoculated with mixture of prevailing virulent rust pathotypes starting from the vegetative stage. Inoculation is carried out 4 times.
- Scoring on APR is done at flag leaf stage.
- Testing is conducted over two growing seasons.

Australian Government
IP Australia

Adult Plant Resistance Inoculation Procedure

Inoculation of adult plants with mineral oil suspension of urediospores in the late afternoon in anticipation of overnight dew.

Australian Government
IP Australia

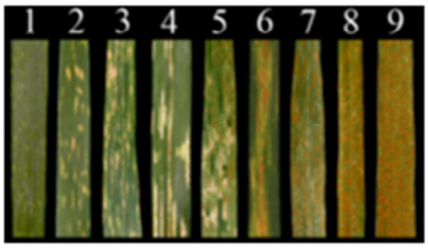
Adult Plant Resistance (QN)

Level of Resistance	Infection Type	Note
Resistant (R)	No rust pustules, yellow chlorotic areas or necrotic areas may present	1
Moderately Resistant (MR)	Small rust pustules surrounded by chlorotic areas or necrotic areas	3
Moderately Resistant/ Moderately Susceptible (MR/MS)	Rust pustules having a range of size with some associated chlorotic areas or necrotic areas	5
Moderately Susceptible (MS)	Moderate sized pustules with some chlorosis	7
Susceptible (S)	Large size pustules with little chlorosis	9

Robust intellectual property rights delivered efficiently

Australian Government
IP Australia

Adult Plant Resistance (QN)



Scoring on APR is done on a 1 to 9 scale at flag leaf stage.

Australian Government
IP Australia

Adult Plant Resistance (QN)

	Beaufort	Mackellar	EGA Wedgetail	Bolac	Preston
Stripe Rust resistance	MR-R	MR	MS-MR	MR-R	MR-R
Leaf Rust resistance	R	S	MS	MS-S	R
Stem Rust resistance	S	MS-MR	MS-MR	MS	-

	Frelon	Preston	Mackellar	Brennan	Revenue
Stem Rust	S	S-VS	MR	MS	R
Stripe Rust	R	R-MR	R	R-MR	R
Leaf Rust	R	R	S	R	R

Adult Plant Resistance in some wheat varieties

Robust intellectual property rights delivered efficiently

Australian Government
IP Australia

Conclusion

- Rust resistance results from a given genotype or combination of genotypes.
- Sufficiently consistent and repeatable in a particular environment.
- Exhibits sufficient variation between the varieties to be able to establish distinctness.
- Capable of precise definition and recognition.
- Allows uniformity and stability requirements to be fulfilled.
- **Rust resistance can be used in DUS testing as additional characteristics. (TG/1/3)**

Robust intellectual property rights delivered efficiently

Australian Government
IP Australia

Thank you

P TM D PBR

Robust intellectual property rights delivered efficiently

[Annex II follows]

ANNEX II

USE OF DISEASE AND INSECT RESISTANCE CHARACTERISTICS IN DUS EXAMINATION: EXPERIENCE OF BRAZIL WITH SOYBEAN

Document prepared by an expert from Brazil

Disease and insect resistance as a characteristic

Document TG/1/3, chapter 4.6.1 states that characteristics based on the response to external factors, such as living organisms, may be used in DUS tests provided that they fulfil the criteria specified for selection of characteristics. Nevertheless, considering the potential variation in such external factors, it is important for those characteristics to be well defined and have an appropriate method established to ensure consistency in the examination.

Considering these points, the Brazilian PVP Office (SNPC) started in 2007 a workplan in order to define disease resistance characteristics to be used in the DUS tests of soybean (*Glycine max* (L.) Merrill).

Variety protection of soybean in Brazil

By the end of 2016, the SNPC had received 1346 PVP applications for Soybean varieties.

Despite the high number of applications, the candidate varieties have a narrow genetic basis, because of the low number of ancestors used in the breeding programs. Thus, from 2005, the assessment of distinctness based on morphological characteristics became a problem.

At that time, disease resistance characteristics used in the national test guidelines contained no harmonized protocol for examination.

Considering that disease resistance is one of the main purposes of breeding programs and the need of increasing the number of characteristics to distinguish the new varieties, the SNPC decided to improve the use of such characteristics for DUS examination.

In this regard, through the indication of breeding companies and invitation of the SNPC, a working group of experts consisting of phytopatologists and breeders was established and a schedule of meetings was planned for a year. The objective was to review and suggest disease characteristics to be used on the DUS test guidelines and to define standard protocols to be used. The work was conducted as follows:

- The SNPC carried out a previous research and conducted a survey with the breeders and phytopatologists to identify which disease characteristics would be appropriate for DUS examination;
- The working group of experts decided to distribute the diseases according to the specialty of each member. A protocol for testing would be suggested based on scientific world-wide knowledge, the genetic inheritance of the characteristic and designed according to the recommendations of UPOV, particularly the guidance provided in document TGP/12 “Guidance on Certain Physiological Characteristics”. The example varieties for each level of expression should also be indicated.
- The designated experts circulated the protocol among themselves for improvements and suggestions before being submitted to the approval of the working group.
- A ring test was organized with laboratories and breeders to harmonize the methodology and compare the results amongst different companies, locations and over time.
- The characteristics were classified as mandatory or optional, regarding the fulfillment of the official variety description, according to the reliability of the methodologies to access distinctness, taking into account the repeatability and distribution of occurrence across the country (in the case of field evaluations).
- The working group also identified the critical points that could compromise the performance of the tests when they were carried out by different laboratories or breeders and concluded that once the protocols and example varieties had been defined, the inoculum should be also standardized, by centralizing its source. Thereby, two institutions were recommended as suppliers of the inoculums to be used in DUS testing.

- The working group meetings resulted on the publication, in September 2008, of the new Brazilian DUS test guidelines with 8 mandatory and 7 optional disease resistance characteristics and their specific protocols.
- The characteristics and protocols can be accessed in these two documents (in Portuguese only):
    - http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/insumos-agricolas/protecao-de-cultivar/arquivos-agricolas/soja_formulario_novo_29out2009_p-2.doc; and
    - http://www.agricultura.gov.br/assuntos/insumos-agropecuarios/insumos-agricolas/protecao-de-cultivar/arquivos-agricolas/soja_anexo_ii_-_protocolos_doen-c7as_p.doc.

Conclusion

Considering the work carried out, we can point out that the use of disease resistance characteristics in DUS is an important tool for the authorities, especially considering its usefulness for establishing distinctness and for grouping varieties on DUS trials.

Nevertheless, its establishment must be carefully considered and analyzed particularly regarding the following points:

- The characteristics shall be defined case by case, considering the peculiarities of the pathogen and the species concerned;
- The protocols shall be harmonized and ring tested to ensure consistency and repeatability;
- The inoculum source shall be as centralized as possible (if tests are carried out by different laboratories);
- The test protocols used shall be widely recognized;
- The cost associated with the use of disease resistance characteristics for DUS examination should be considered.

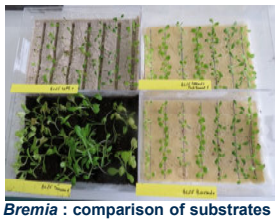
[Annex III follows]

ANNEX III

HARMONIZATION OF RESISTANCE TESTS FOR DUS TESTING: "HARMORES 2"

Document prepared by an expert from the European Union

CPVO
Community Plant Variety Office



***Bremia* : comparison of substrates**

TWA 2017-Hannover

Harmonisation of resistance tests for DUS testing: "Harmores 2"

CPVO

1

Introduction

- CPVO co-funded three year project (2012-2015), coordinated by GEVES
- 7 examination offices (CZ, DE, ES, FR, GB, HU, NL) and 5 European Seed Association (ESA) members involved
- **Aims**: Harmonise at the European Union level, resistance tests to seven vegetable diseases:
  - *Bremia lactucae* in lettuce
  - *Fusarium oxysporum* f. sp. *pisi* race 1 in pea
  - *Ascochyta pisi* race C in pea
  - TMV: 0 in pepper,
  - PMMoV: 1.2 in pepper,
  - PMMoV:1.2.3 in pepper,
  - PVY: 0 in pepper

Harmores 2

2

Why harmonise DUS resistance test protocols

- Better coherence of results between countries
- Better declarations from breeders and official tests
- Better definition and exchange of reference material

Methodology

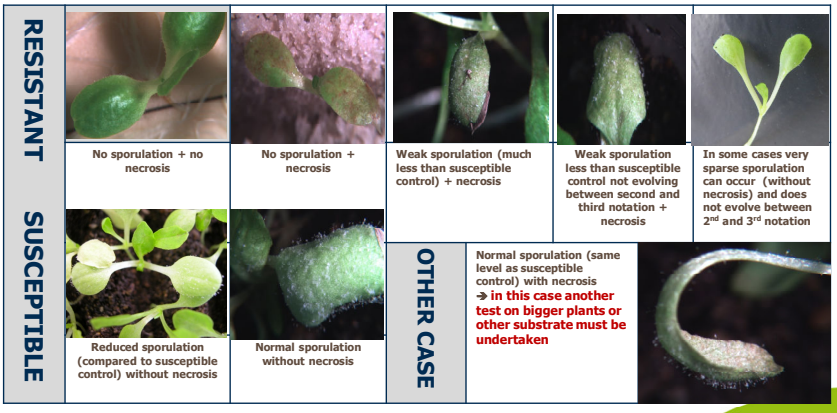
- ✓ updated bibliography on the selected host/pathogen combinations
- ✓ available reference isolates with maintainer laboratories
- ✓ available reference varieties as resistant and susceptible controls
- ✓ optimised culture conditions for all the studied pathogens
- ✓ optimised test conditions
- ✓ harmonised techniques to be proposed to CPVO for implementation

Time frame and deliverables of project

Phase 1	• *Description and comparison of the existing tests*
Phase 2	• *Selection of common reference material*
Phase 3	• *Harmonisation and validation of resistance testing techniques*
Phase 4	• *Implementation of harmonised techniques into corresponding CPVO test protocols and UPOV Test Guidelines*

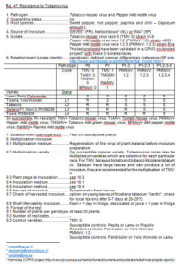
Collaboration and « hands on » approach

- Numerous exchanges during the project's duration
- Annual meetings between the project partners
- Practical workshops to harmonise interpretation of results



Results and implementation

- Presentation of new notation scale to the International Bremia Evaluation Board (IBEB), and scientific posters at Eucarpia congresses 2015-16
- Updated robust test techniques proposed 2016 to CPVO
- CPVO implemented improved techniques in March 2017 via partial revisions to lettuce, pepper and pea protocols
- **Final step**: implementation by UPOV of improved techniques into:
  - ✓ **Revision of the Test Guidelines for lettuce TG/13/11** (approval at TC/53)
  - ✓ **Partial revision of the Test Guidelines for pepper TG/76/8 Rev.** (discussion at TWV/51)
  - ✓ **Partial revison of the Test Guidelines for pea TG/7/10 Rev.** (discussion at TWV/51)



Thank you for your attention!

Any questions ?

Harmores 2

7

[Annex IV follows]

ANNEX IV

PHASING-IN PERIOD FOR ASTERISKED DISEASE RESISTANCE CHARACTERISTICS IN CPVO VEGETABLE TECHNICAL PROTOCOLS

Document prepared by an expert from the European Union

CPVO
Community Plant Variety Office

TWA 2017-Hannover

Phasing-in period for asterisked disease resistance characteristics in CPVO vegetable technical protocols

CPVO

8

Introduction

- 12 CPVO vegetable technical protocols contain a total of 127 disease resistance characteristics
  - 33 of these are asterisked, thus obligatory to observe in the DUS tests to be carried out anywhere within EU, also for listing purposes
  - In corresponding UPOV Test Guidelines, just 24 asterisked characteristics
- **Advantages**:
  - ✓ reflects breeding work being done by seed companies
  - ✓ discriminatory, thus limiting number of comparison varieties in DUS test
- **Problem**: A number of smaller breeders in certain parts of the EU declared that asterisked disease characteristics were prejudicial, since several diseases were not of importance in certain parts of the EU
  - ! Obliged to breed for **uniform** resistance/susceptibility to pass DUS test!

Phasing-in

9

How did CPVO and stakeholders tackle issue

- Provisional moratorium imposed by CPVO Administrative Council in 2013 on any new asterisks being added to disease resistance characteristics
- Analysis of the situation between CPVO, entrusted EU vegetable examination offices, and European Seed Association (ESA)
- Questionnaire formulated by CPVO/ESA addressed to ESA vegetable members to get their feedback on their experiences with disease resistance characteristics
- Review of each of the 33 asterisked vegetable disease resistance characteristic, to see if they were necessary

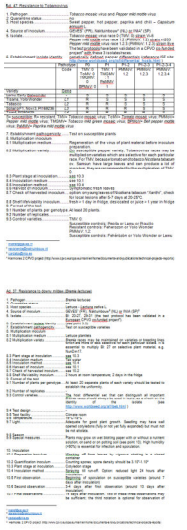
The devised phasing-in period

- ✓ Main proposal emanating from consultation process was to establish a **running-in phase** in the adoption of asterisked disease and insect resistance characteristics in CPVO vegetable protocols
- ✓ Would allow breeders to develop their breeding activity in that area over a **period of time**, as well as permitting examination authorities to establish or build-up the necessary testing facilities
- ✓ CPVO Administrative Council agreed in 2015 to adopt the principle of a normal **5 year** phasing-in period for each **new** asterisked insect or disease resistance asterisked characteristic, although length of time could be varied according to the complexity of each characteristic

Implementation of principle

- Phasing-in principle well received by all CPVO stakeholders
- More thought now given on necessity of the asterisk as well as **how long** stakeholders need to adjust until characteristic becomes obligatory
- Principle first utilised in partial revisons to CPVO lettuce and spinach protocols, with DUS testing as from 2016:
  - Lettuce: **3-year** phasing in period for several *Bremia lactucae* races
  - Spinach: **3-year** phasing in period for several *Peronospara farinosa* races
- After one year of utilising the phasing-in principle in lettuce and spinach, breeders & examination authorities expressed high levels of **satisfaction**
- Further implementation in CPVO protocols foreseen in coming years

Results and implementation



- Presentation of new notation scale to the International Bremia Evaluation Board (IBEB), and scientific posters at Eucarpia congresses 2015-16
- Updated robust test techniques proposed 2016 to CPVO
- CPVO implemented improved techniques in March 2017 via partial revisions to lettuce, pepper and pea protocols
- **Final step**: implementation by UPOV of improved techniques into:
  - ✓ **Revision of the Test Guidelines for lettuce TG/13/11** (approval at TC/53)
  - ✓ **Partial revision of the Test Guidelines for pepper TG/76/8 Rev.** (discussion at TWV/51)
  - ✓ **Partial revison of the Test Guidelines for pea TG/7/10 Rev.** (discussion at TWV/51)

Thank you for your attention!

Any questions ?

Phasing-in

14

[End of Annex IV and of document]