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| International Union for the Protection of New Varieties of Plants |  |

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| Technical Working Party for Vegetables  Fifty-Fifth Session Antalya, Turkey, May 3 to 7, 2021 | TWV/55/15  Original: English  Date: April 8, 2021 |

Partial revision of the Test Guidelines for PEA

Document prepared by an expert from France

Disclaimer: this document does not represent UPOV policies or guidance

The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Pea (document TG/7/10 Rev. 2).

The Technical Working Party for Vegetables (TWV), at its fifty-fourth session hosted by Brazil and organized by electronic means, from May 11 to 15, 2020, agreed that the Test Guidelines for Pea (*Pisum sativum* L.) (document TG/7/10 Rev. 2) be partially revised for characteristics 58 “Resistance to *Fusarium* *oxysporum* f. sp. *pisi*”, 59 “Resistance to *Erysiphe pisi* Syd.” and 60 “Resistance to Ascochyta pisi – Race C” (see document TWV/54/9 “Report”, Annex III).

The following changes are proposed:

1. Revision of explanation Ad. 58 “Resistance to *Fusarium oxysporum* f. sp. *pisi*” in Chapter 8.2 “Explanations for individual characteristics”;
2. Revision of Characteristic 59 “Resistance to *Erysiphe pisi* Syd.”;
3. Revision of explanation Ad. 59 “Resistance to *Erysiphe pisi* Syd.” in Chapter 8.2 “Explanations for individual characteristics”;
4. Revision of explanation Ad. 60 “Resistance to *Ascochyta pisi*, Race C (Ascochyta Leaf and Pod Spot)” in Chapter 8.2 “Explanations for individual characteristics”.

The proposed changes to are presented below in highlight and underline (insertion) and ~~strikethrough~~ (deletion).

## Proposed revision of explanation Ad. 58 “Resistance to *Fusarium oxysporum* f. sp. *pisi*” in Chapter 8.2 “Explanations for individual characteristics”

*Current wording*

Ad. 58: Resistance to *Fusarium oxysporum* f. sp. *pisi* race 1 (Near wilt)

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Fusarium oxysporum* f. sp. *pisi* (race 1) |
| 2. | Quarantine status | no |
| 3. | Host species | Pea – *Pisum sativum* L. |
| 4. | Source of inoculum | GEVES[[1]](#footnote-2) (FR), INIA[[2]](#footnote-3) (ES) or SASA[[3]](#footnote-4) (GB) |
| 5. | Isolate | *Fusarium oxysporum* f. sp. *pisi* race 1 strain MATREF 04-02-01-01 (the test protocol has been validated with this isolate/race) |
| 6. | Establishment isolate identity | genetically defined pea controls (See ISF website: http://www.worldseed.org) |

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| Differentials host |  |
| susceptible: | M410, Bartavelle, Little Marvel |
| resistant: | New Era, Mini 93, Dark Skin Perfection, Vantage, WSU 23, New Season, WSU 31, 74SN5, Sundance II, Grant |

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| 7. | Establishment pathogenicity | Test on susceptible plants |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | Multiplication on agar medium: malt Agar or PDA for example |
| 8.4 | Inoculation medium | Multiplication on agar medium: water for scraping agar plates.  Multiplication on liquid medium: Potato Dextrose Broth, Kerrs broth or Czapek-Dox (3 to 7 days old aerated culture) for example. |
| 8.6 | Harvest of inoculum | see 10.1 |
| 8.7 | Check of harvested inoculum | see 10.2 |
| 8.8 | Shelflife/viability inoculum | between 4 and 8 hours, keep cool to prevent germination of spores. Viability of spores should be more than 3 years if stored at -20°C. |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | At least 20 plants and 5 non inoculated plants per variety. |
| 9.2 | Number of replicates | - |
| 9.3 | Control varieties | Susceptible controls: Bartavelle  Resistant controls: New Era and Nina |
| 9.5 | Test facility | Climate room or greenhouse. |
| 9.6 | Temperature | 20-25°C |
| 9.7 | Light | 12 hours or longer |
| 9.9 | Special measures | It is important to compare the inoculated plants with the negative non inoculated control plants of the same sample. This allows interpretation of symptoms of root rot, senescence or 'wilting' caused by the stress of having roots cutted and not caused by *F. oxysporum* infection. |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | For agar plates, remove hyphen fragments by filtering solution through muslin.  For liquid medium, filter through muslin. |
| 10.2 | Quantification inoculum | 106 spores/ml |
| 10.3 | Plant stage at inoculation | seeds or 2 weeks old seedlings (2-3 node stage). |
| 10.4 | Inoculation method | For seeds:  sowing in contaminated substrate (soil based substrate), 750 ml of suspension of spores at 106sp/ml for 5 l of substrate.  For 2 weeks seedlings:  Sowing in a mix of vermiculite + soil or soil based substrate  Cut the apical 2/3 of the roots with scissors, dip the root of the seedling in the spores suspension for 1 to 5 minutes and transplant in clean soil based substrate in a new tray. |
| 10.7 | Final observations | 28 days post-inoculation. |
| 11. | Observations |  |
| 11.1 | Method | Visual |
| 11.2 | Observation scale | susceptible:  Class 2: Range from most of the plant wilted/dried but still alive, to plants brown and dead with stem collapsed.  resistant:  Class 0: No symptoms or equivalent to negative control, 1 or 2 wilted/dried lower leaves and slight reduction in growth compared to negative control of same variety are acceptable.  Class 1: Range from a few chlorotic or wilted/dried leaves not present on, or more than on the negative control, up to many leaves with symptoms of senescence or wilting, some leaf drop, upper part of the plant still green and growing. |

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| Pea Fop note 0.JPG | Pea Fop note 1.JPG | Pea Fop note 2-2.JPG | Pea Fop note 2.JPG |
| Class 0  resistant | Class 1  resistant | Class 2  susceptible | |

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|  |  | Varieties with the same or higher level of resistance as New Era will be interpreted as resistant. Varieties with a lower level of resistance than New Era will be interpreted as susceptible. Nina will be highly resistant, Bartavelle will be highly susceptible. New Era expresses weak symptoms and variation can occur in these weak symptoms depending on the agressivity of the test conditions. |
| 11.3 | Validation of test | evaluation of variety resistance should be calibrated with results of resistant and susceptible controls. |
| 12. | Interpretation of data in terms of UPOV characteristic states |  |
|  | absent [1] | susceptible |
|  | present [9] | resistant |
| 13. | Critical control points | Each lab has to define the best method of inoculation in its lab depending on controls results.  Inoculation by sowing in contaminated soil can in some cases lead to germination problems. No conclusion can be done in this case, and the test should be repeated. |

*Proposed new wording*

Ad. 58: Resistance to *Fusarium oxysporum* f. sp. *pisi* race 1 (Near wilt)

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Fusarium oxysporum* f. sp. *pisi* (race 1) |
| 2. | Quarantine status | No |
| 3. | Host species | Pea – *Pisum sativum* L. |
| 4. | Source of inoculum | GEVES[[4]](#footnote-5) (FR), INIA[[5]](#footnote-6) (ES) or SASA[[6]](#footnote-7) (GB) |
| 5. | Isolate | *Fusarium oxysporum* f. sp. *pisi* race 1 strain MATREF 04-02-01-01  The test protocol has been validated in a CPVO co-funded project[[7]](#footnote-8) with this isolate/race. |
| 6. | Establishment isolate identity | genetically defined pea controls  See ISF website  <https://www.worldseed.org/our-work/plant-health/differential-hosts/>  Version July 2019 |

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| ~~Differentials host~~ |  |
| ~~susceptible:~~ | ~~M410, Bartavelle, Little Marvel~~ |
| ~~resistant:~~ | ~~New Era, Mini 93, Dark Skin Perfection, Vantage, WSU 23, New Season, WSU 31, 74SN5, Sundance II, Grant~~ |

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| 7. | Establishment pathogenicity | Test on susceptible plants |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | Multiplication on agar medium: malt Agar or PDA for example |
| 8.4 | Inoculation medium | Multiplication on agar medium: water for scraping agar plates.  Multiplication on liquid medium: Potato Dextrose Broth, Kerrs broth or Czapek-Dox (3 to 7 days old aerated culture) for example. |
| 8.6 | Harvest of inoculum | see 10.1 |
| 8.7 | Check of harvested inoculum | see 10.2 |
| 8.8 | Shelf life/viability inoculum | 4/8 hours, keep cool to prevent germination of spores. Viability of spores should be more than 3 years if stored at -20°C. |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | At least 20 inoculated plants and 5 non inoculated plants per variety. |
| 9.2 | Number of replicates | - |
| 9.3 | Control varieties | Susceptible controls: Bartavelle  Resistant controls: New Era and Nina |
| 9.5 | Test facility | Climate room or greenhouse. |
| 9.6 | Temperature | 20-25°C |
| 9.7 | Light | 12 hours or longer |
| 9.9 | Special measures | It is important to compare the inoculated plants with the ~~negative~~ non inoculated control plants of the same sample. This allows interpretation of symptoms of root rot, senescence or 'wilting' caused by the stress of having roots cut and not caused by *F. oxysporum* infection. |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | For agar plates, remove hyphen fragments by filtering solution through muslin.  For liquid medium, filter through muslin. |
| 10.2 | Quantification inoculum | 106 spores/ml |
| 10.3 | Plant stage at inoculation | seeds or 2 weeks old seedlings (2-3 nodes stage). |
| 10.4 | Inoculation method | For seeds:  sowing in contaminated substrate (soil-based substrate), 750 ml of suspension of spores at 106sp/ml for 5 l of substrate.  For 2 weeks seedlings:  Sowing in a mix of vermiculite + soil or soil-based substrate  Cut the apical 2/3 of the roots ~~with scissors~~, dip the root of the seedling in the spores suspension for 1 to 5 minutes and transplant in clean soil based substrate in a new tray. |
| 10.7 | Final observations | 28 days post-inoculation. |
| 11. | Observations |  |
| 11.1 | Method | Visual |
| 11.2 | Observation scale | ~~resistant:~~  Class 0: No symptoms or equivalent to non-inoculated ~~negative~~ control, 1 or 2 senesced (wilted/dried) lower leaves and slight reduction in growth compared to non-inoculated ~~negative~~ control of same variety are acceptable.  Class 1: Range from a few chlorotic or wilted/~~dried~~/senesced leaves not present on, or more than on the ~~negative~~ non‑inoculated control, up to many leaves with symptoms of senescence or wilting, some leaf drop, upper part of the plant still green and growing.  ~~susceptible:~~  Class 2: Range from most of the plant wilted or senesced ~~/dried~~ but still alive, to plants brown and dead with stem collapsed.  Classes 0 and 1 are generally resistant. Class 2 is generally susceptible. |

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| --- | --- | --- | --- |
| Pea Fop note 0.JPG | Pea Fop note 1.JPG | Pea Fop note 2-2.JPG | Pea Fop note 2.JPG |
| Class 0  ~~resistant~~ | Class 1  ~~resistant~~ | Class 2  ~~susceptible~~ | |

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|  |  | ~~Varieties with the same or higher level of resistance as New Era will be interpreted as resistant. Varieties with a lower level of resistance than New Era will be interpreted as susceptible. Nina will be highly resistant, Bartavelle will be highly susceptible.~~ ~~New Era expresses weak symptoms and variation can occur in these weak symptoms depending on the agressivity of the test conditions.~~ |
| 11.3 | Validation of test | Evaluation of variety resistance should be calibrated with results of resistant and susceptible controls.  New Era expresses weak symptoms and variations can occur in these weak symptoms depending on the aggressivity of the test conditions.  Susceptible: lower level of resistance than New Era (Bartavelle is highly susceptible)  Resistant: same or higher level of resistance than New Era (Nina is highly resistant) |
| 12. | Interpretation of data in terms of UPOV characteristic states |  |
|  | absent [1] | Susceptible |
|  | present [9] | Resistant |
| 13. | Critical control points | Each lab has to define the best method of inoculation in its lab depending on controls results.  Inoculation by sowing in contaminated soil can in some cases lead to germination problems. No conclusion can be done in this case, and the test should be repeated. |

## Revision of Characteristic 59 “Resistance to *Erysiphe pisi* Syd.”

*Current wording*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | English | français | deutsch | español | Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo | Note/ Nota |
| 59.  (+) | VG | Resistance to *Erysiphe pisi* Syd. | Résistance à *Erysiphe pisi* Syd. | Resistenz gegen *Erysiphe pisi* Syd. | Resistencia a *Erysiphe pisi* Syd. |  |  |
| QL |  | absent | absente | fehlend | ausente | Cabro | 1 |
|  |  | present | présente | vorhanden | presente | Stratford, Vivaldi | 9 |

*Proposed new wording*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | English | français | deutsch | español | Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo | Note/ Nota |
| 59.  (+) | VG | Resistance to *Erysiphe pisi* Syd. | Résistance à *Erysiphe pisi* Syd. | Resistenz gegen *Erysiphe pisi* Syd. | Resistencia a *Erysiphe pisi* Syd. |  |  |
| QL |  | absent | absente | fehlend | ausente | ~~Cabro~~, Aladin, Cabree, Ottoman | 1 |
|  |  | present | présente | vorhanden | presente | ~~Stratford~~, Alezan, Ema, Stratagem (JI2302),  Sugar Bon, Vivaldi, | 9 |

## Proposed revision of explanation Ad. 59 “Resistance to *Erysiphe pisi* Syd.” in Chapter 8.2 “Explanations for individual characteristics”

*Current wording*

Ad. 59: Resistance to *Erysiphe pisi* Syd. (Powdery Mildew)

Resistant and Susceptible varieties

Cabro (susceptible = resistance absent (1))

Stratford, Vivaldi (resistant = resistance present (9))

Isolates and isolate identity

No isolates are maintained as infection is natural. There are no known races.

Genetic background

Two recessive genes confer resistance: er1 and er2

er1 er2 = resistant

Er1 Er2 = susceptible

Er1 er2 = susceptible

er1 Er2 = susceptible

Assessment of disease

Infected foliage surfaces are white and powdery. Tissue beneath the infected areas may turn purplish followed by the production of black fruiting structures. Badly infected tissue remains soft and fails to dry out naturally.

In resistant plants, infection is absent or localized in very small patches (pustules). Infestation may overtake resistant plants during senescence.

*Proposed new wording*

Ad. 59: Resistance to *Erysiphe pisi* Syd. ~~(Powdery Mildew)~~

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| --- | --- | --- |
| 1. | Pathogen | Powdery mildew – *Erysiphe pisi* |
| 2. | Quarantine status | No |
| 3. | Host species | Pea – *Pisum sativum* L |
| 4. | Source of inoculum | GEVES[[8]](#footnote-9) (FR) |
| 5. | Isolate | *Erysiphe pisi* e.g. isolate 2430  The test protocol has been validated in a CPVO co-funded project[[9]](#footnote-10)  with this isolate/race. |
| 6. | Establishment isolate identity | Validation by use specific EryF/EryR primers to validate the species of  *Erysiphe* (use ITS primers from Attanayake et al, 2010[[10]](#footnote-11).) |
| 7. | Establishment pathogenicity | use susceptible variety (e.g. Aladin, Cabree or Ottoman) |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | Living plant |
| 8.2 | Multiplication variety | See 7 |
| 8.3 | Plant stage at inoculation | See 10.3 |
| 8.4 | Inoculation medium |  |
| 8.5 | Inoculation method | See 10.4 |
| 8.6 | Harvest of inoculum | For spraying by washing off with demineralized water  For sprinkling by detaching leaves of a susceptible host plant |
| 8.7 | Check of harvested inoculum | Visual check for presence of sporulation |
| 8.8 | Shelf life/viability inoculum | 1-2 hours |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | 20 plants |
| 9.2 | Number of replicates | - |
| 9.3 | Control varieties | Susceptible:  For vegetable crops: Cabree  For agricultural crops: Aladin, Ottoman  Resistant:  For vegetable crop: Ema, Sugar Bon, Vivaldi, Stratagem (JI2302)  For agricultural crop: Alezan |
| 9.4 | Test design | No non-inoculated control due as it is impossible to place them exactly  in the same conditions (risk of contamination). |
| 9.5 | Test facility | green house or climatic room |
| 9.6 | Temperature | 20°C (+5°C) but it is advised not to go below 18°C. In some  conditions it has been observed that increasing the day temperature  up to 27°C allowed a good sporulation on the susceptible control. |
| 9.7 | Light | at least 12h per day |
| 9.8 | Season |  |
| 9.9 | Special measures |  |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | By spraying:  Washing off from leaves by vigorous shaking in a closed container  containing water. Sieve the suspension through muslin cloth.  By sprinkling:  Selection of leaves with strong sporulation. |
| 10.2 | Quantification inoculum | By spraying:  Counting spores; spores density should be 1.105 to 1.106 spores/mL  By sprinkling:  Approximately 1 well-sporulating plant to inoculate 10 plants. |
| 10.3 | Plant stage at inoculation | 3-4 leaf stage |
| 10.4 | Inoculation method | By spraying:  Spraying of the suspension of spores on leaves  By sprinkling:  Shaking of sporulated leaves above the plants to be contaminated. |
| 10.5 | First observation |  |
| 10.6 | Second observation |  |
| 10.7 | Final observations | Between 14-21 dpi, when sporulation is well expressed on the  susceptible control. |
| 11. | Observations |  |
| 11.1 | Method | Visual |
| 11.2 | Observation scale |  |
|  | | |
| 11.3 | Validation of test | Evaluation of variety resistance should be calibrated with results of resistant and susceptible controls.  Susceptible:  sporulation on leaves. Symptoms can be observed on stem and tendril (not always on the whole plant).  Resistant:  No sporulation or few mycelial pustules only on the lower leaves in case of high disease pressure, no evolution of the symptoms |
| 11.4 | Off-types | - |
| 12. | Interpretation of data in terms of UPOV characteristic states | Absent (susceptible) [1]  ~~sporulation on leaves. Symptoms can be observed on stem and tendril (not always on the whole plant).~~  Present (resistant) [9]  ~~No sporulation or few mycelial pustules only on the lower leaves in case of high disease pressure, no evolution of the symptoms~~. |
| 13. | Critical control points | Watering on the substrate (no spraying) to avoid washing the spores  off the surface of the leaves.  It is not possible to freeze spores. Need to maintain on plants. |

## Revision of explanation Ad. 60 “Resistance to *Ascochyta pisi*, Race C (Ascochyta Leaf and Pod Spot)” in Chapter 8.2 “Explanations for individual characteristics”

*Current wording*

Ad. 60: Resistance to *Ascochyta pisi*, Race C

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Ascochyta pisi* |
| 2. | Quarantine status | no |
| 3. | Host species | Pea – *Pisum sativum* L. |
| 4. | Source of inoculum | GEVES[[11]](#footnote-12) (FR) or SASA[[12]](#footnote-13) (GB) |
| 5. | Isolate | *Ascochyta pisi* race C strain 21A.13. (the test protocol has been validated with this isolate)[[13]](#footnote-14). |
| 6. | Establishment isolate identity | genetically defined pea controls (Physiological races of *A. pisi* and differentials, adapted from Gallais et Bannerot, 1992) |

|  |  |
| --- | --- |
| Physiological race  (Dr Hubbeling)  Strain | C  Tézier  21A.13 |
| Gullivert | S |
| Rondo | R |
| Finale | R |
| Kelvedon Wonder | S |
| Dark Skin Perfection | S |
| Arabal, Cobri, Starcovert, Sucovert, Vitalis | S |

R = resistant; S = susceptible

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| 7. | Establishment pathogenicity | test on susceptible plants |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | V8 agar or Mathur medium or Potato Dextrose Agar or a synthetic medium. |
| 8.4 | Inoculation medium | water, option: add Tween 80 (wetting agent to aid dispersal of spores, e.g. 0.4%) |
| 8.6 | Harvest of inoculum | see 10.1 |
| 8.7 | Check of harvested inoculum | see 10.2 |
| 8.8 | Shelflife/viability inoculum | between 4 and 8 hours, keep cool to prevent spores’ germination |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | at least 20 plants and 5 non inoculated plants per variety |
| 9.2 | Number of replicates | - |
| 9.3 | Control varieties |  |
|  | Susceptible | Crecerelle, Kelvedon Wonder |
|  | Resistant | Nina and Madonna or Rondo |
| 9.4 | Test design | - |
| 9.5 | Test facility | climate room or greenhouse |
| 9.6 | Temperature | 20°C |
| 9.7 | Light | 12 hours or longer |
| 9.8 | Season | - |
| 9.9 | Special measures | high humidity or watering by spraying 2 or 3 times per day |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | remove hyphen fragments by straining solution through muslin |
| 10.2 | Quantification inoculum | 106 spores/mL (to adapt depending conditions of tests) |
| 10.3 | Plant stage at inoculation | 2 weeks old seedlings (i.e. 2-3 node stage) |
| 10.4 | Inoculation method | spraying on green leaves without surface moisture |
| 10.5 | First observation | - |
| 10.6 | Second observation | - |
| 10.7 | Final observations | 10-18 days post-inoculation |
| 11. | Observations |  |
| 11.1 | Method | visual |
| 11.2 | Observation scale | Class 0: no symptoms  Class 1: few small superficial necrosis  Class 2: bigger darker and deep necrosis  Class 3: necrosis on all parts of the plant or serious symptoms surrounding the stem  Madonna, Nina and Rondo will be resistant controls; varieties with same level of resistance as Madonna/Rondo and/or Nina will be interpreted as resistant. Crecerelle and Kelvedon Wonder will be susceptible controls, varieties with a lower level of resistance than Nina as well as Madonna/Rondo will be interpreted as susceptible. |

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| --- | --- |
| Class 0: | Class 1: |
| Class 2: | Details on Class 2: |
| Class 3 : | Details on Class 3 |

Resistant

Crecerelle

Madonna

Nina

Susceptible

Kelvedon Wonder

Rondo

|  |  |  |
| --- | --- | --- |
| 11.3 | Validation of test | evaluation of variety resistance should be calibrated with results of resistant and susceptible controls |
| 11.4 | Off-types | - |
| 12. | Interpretation of data in terms of  UPOV characteristic states |  |
|  | absent [1]  present [9] | susceptible (classes 2 and 3)  resistant (classes 0 and 1) |
| 13. | Critical control points | - |

*Proposed new wording*

Ad. 60: Resistance to *Ascochyta pisi*, Race C (Ascochyta Leaf and Pod Spot)

|  |  |  |
| --- | --- | --- |
| 1. | Pathogen | *Ascochyta pisi* |
| 2. | Quarantine status | No |
| 3. | Host species | Pea – *Pisum sativum* L. |
| 4. | Source of inoculum | GEVES[[14]](#footnote-15) (FR) or SASA[[15]](#footnote-16) (UK) |
| 5. | Isolate | *Ascochyta pisi* race C strain 21A.13.  The test protocol has been validated in a European CPVO co-funded project[[16]](#footnote-17) with this isolate. |
| 6. | Establishment isolate identity | Genetically defined on Pea controls ~~(Physiological races of~~ *~~A. pisi~~* ~~and differentials, adapted from Gallais et Bannerot, 1992)~~  see ISF website  <https://www.worldseed.org/our-work/plant-health/differential-hosts/>  Version July 2019 |
| |  |  | | --- | --- | | ~~Physiological race~~  ~~(Dr Hubbeling)~~  ~~Strain~~ | ~~C~~  ~~Tézier~~  ~~21A.13~~ | | ~~Gullivert~~ | ~~S~~ | | ~~Rondo~~ | ~~R~~ | | ~~Finale~~ | ~~R~~ | | ~~Kelvedon Wonder~~ | ~~S~~ | | ~~Dark Skin Perfection~~ | ~~S~~ | | ~~Arabal, Cobri, Starcovert, Sucovert, Vitalis~~ | ~~S~~ |   ~~R = resistant; S = susceptible~~ | | |
| 7. | Establishment pathogenicity | Test on susceptible plants |
| 8. | Multiplication inoculum |  |
| 8.1 | Multiplication medium | V8 agar or Mathur medium or Potato Dextrose Agar or a synthetic  medium. |
| 8.2 | Multiplication variety | - |
| 8.3 | Plant stage at inoculation | - |
| 8.4 | Inoculation medium | water, option: add Tween 80 (wetting agent to aid dispersal of spores, e.g. 0.4%) |
| 8.5 | Inoculation method | - |
| 8.6 | Harvest of inoculum | See 10.1 |
| 8.7 | Check of harvested inoculum | See 10.2 |
| 8.8 | Shelf life/viability inoculum | 4/8h, keep cool to prevent spores’ germination |
| 9. | Format of the test |  |
| 9.1 | Number of plants per genotype | At least 20 plants and 5 non-inoculated plants per variety. |
| 9.2 | Number of replicates | - |
| 9.3 | Control varieties | Susceptible controls: Crecerelle, Kelvedon Wonder  Resistant controls: Madonna or Rondo (lower resistance thresholds) and Nina |
| 9.4 | Test design | - |
| 9.5 | Test facility | Climatic room or greenhouse. |
| 9.6 | Temperature | 20°C |
| 9.7 | Light | 12 hours or longer |
| 9.8 | Season | - |
| 9.9 | Special measures | High humidity or watering by spraying 2 or 3 times per day. |
| 10. | Inoculation |  |
| 10.1 | Preparation inoculum | Remove hyphen fragments by straining solution through muslin. |
| 10.2 | Quantification inoculum | 106 spores/mL (to adapt depending on conditions of tests). |
| 10.3 | Plant stage at inoculation | 2 weeks old seedlings (i.e. 2-3 node stage). |
| 10.4 | Inoculation method | Spraying on green leaves without surface moisture. |
| 10.5 | First observation |  |
| 10.6 | Second observation |  |
| 10.7 | Final observations | 10-18 days post-inoculation. |
| 11. | Observations |  |
| 11.1 | Method | Visual |
| 11.2 | Observation scale | Class 0: no symptom~~s~~  Class 1: few small superficial necrosis  Class 2: bigger darker and deep necrosis  Class 3: necrosis at each level of the plant or serious symptoms surrounding the stem  ~~Madonna, Rondo, and Nina are resistant controls. Varieties with the same or higher level of resistance than Madonna or Rondo will be interpreted as resistant.~~  ~~Crecerelle or Kelvedon Wonder are susceptible controls. Varieties with a lower level of resistance than Nina as well as Madonna or Rondo will be interpreted as susceptible.~~ |
|  | | |
| 11.3 | Validation of test | Evaluation of variety resistance should be calibrated with results of  resistant and susceptible controls.  Susceptible:  Crecerelle or Kelvedon Wonder are susceptible controls. Varieties with a lower level of resistance than Madonna or Rondo will be interpreted as susceptible.  Resistant:  Madonna, Rondo, and Nina are resistant controls. Varieties with the same or higher level of resistance than Madonna or Rondo will be interpreted as resistant. |
| 11.4 | Off-types |  |
| 12. | Interpretation of data in terms of UPOV characteristic states | absent .............................. [1] susceptible ~~(classes 2 and 3)~~  present ............................. [9] resistant ~~(classes 0 and 1)~~ |
| 13. | Critical control points | - |

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1. [matref@geves.fr](mailto:matref@geves.fr) / [www.geves.fr](http://www.geves.fr) [↑](#footnote-ref-2)
2. [resistencias@inia.es](mailto:resistencias@inia.es) [↑](#footnote-ref-3)
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4. matref@geves.fr [↑](#footnote-ref-5)
5. resistencias@inia.es [↑](#footnote-ref-6)
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16. Harmores 2 CPVO project: (<https://cpvo.europa.eu/sites/default/files/documents/vem15_7_b_harmores_2_final_report.pdf> [↑](#footnote-ref-17)