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SCREENING FOR POTATO RESISTANCE TO BLACKLEG AND SOFT ROT

ABSTRACT

Two diseases of the potato, blackleg of potato plants and soft rot of tubers, are caused by several species of pectinolytic bacteria which belong to two genera: *Pectobacterium* and *Dickeya.* Resistance to these bacteria is polygenic and the expression of resistance in tubers and plants is only partially related, as well as strongly dependent on the aggressiveness of the bacteria and on environmental factors. Two methods of assessing tuber and stem tissue resistance of potato cultivars and breeding lines are described.

Key words: *Dickeya*, laboratory test, pectinolytic bacteria, *Pectobacterium*

INTRODUCTION

Blackleg

Different symptoms of potato infected with pectinolytic bacteria during growing season are listed by Helias *et al.* (2000): non-emergence of seed tubers, chlorosis, wilting, haulm desiccation and typical blackleg. The development of symptoms depends on such factors as: the susceptibility of the cultivar, the aggressiveness of bacterial isolates (strains), and on environmental conditions, mostly soil moisture and temperature (Ansermet *et al.*, 2016). The resistance of potato cultivars to blackleg can be evaluated in the field or in the greenhouse conditions after planting of tubers inoculated with bacteria. The methods of inoculation differ among laboratories. Tubers are most often inoculated by vacuum infiltration, but even using this method there are differences in the concen-

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tration of inoculum, the duration of bacteria infiltration, or duration of air drying tubers after inoculation before planting (Helias *et al.*, 2000; van der Wolf *et al.*, 2016). The inoculated tubers are planted in the field or in the greenhouse. In the modified Muenzert method (1975) the stems of potted plants grown under a plastic cover in high humidity are inoculated with a toothpick immersed in a bacterial suspension. In studies of Allefs (1996) the resistance of various potato organs to the blackleg were interpreted as components of partial resistance: etiolated sprout resistance, stem tissue resistance and tuber slice resistance. All these components were evaluated in laboratory conditions and compared with the field resistance of potato to blackleg. It was concluded that selection for resistance in breeding programs under laboratory or greenhouse conditions would be most efficient with regard to clonal differences for etiolated sprout infection (stem base resistance).

The method of evaluation potato blackleg resistance described here is the method testing of stem tissue resistance, in greenhouse growing plants, which is one of the components of the potato's partial resistance to blackleg. The incidence of the disease is scored as the percentage of plants showing symptoms of the disease: the stem with blackleg or brown necrosis, yellowing and wilting of the plant. The severity of the symptoms and the infection of progeny tubers can be estimated.

Soft rot

Soft rot of potato tubers can cause direct losses mainly during storage. Differences in the level of resistance to tuber soft rot in potato cultivars have been found, but not immunity. Sources of high resistance have been found in wild and diploid species of *Solanum* (Zimnoch-Guzowska *et al.*, 1999; Lebecka and Zimnoch-Guzowska, 2004; McGrath *et al.*, 2002). There is no standardized method for evaluation of this trait under laboratory conditions. Different methods have been used to inoculate potato tubers: whole tubers, tuber slices, tubers cut in half, fragments cut out from tubers. The manner of inoculation can be different: tubers are submerged in the inoculum, the inoculum is injected with a syringe or with micropipette tips, or it is deposited on the tuber tissue as a drop/ a piece of blotting paper soaked in the inoculum. The conditions of incubation can differ depending on temperature, humidity and the time of incubation. The scoring methods differ, from the diameter of rotting, to the weight of rotted tissue, or to the depth of rotting and the area of rotting. The results are expressed on different scales: from 0 to 3 (where 0 = no rot, 3 = the diameter of rot ≥ 10 mm), from 0 to 4 (0 = no rot, 1 ≤ ¼ of the area is rotted, 2 ≤ ½ of the area is rotted, 3 ≥ ½ of the area is rotted, without rotting of the core of the tuber, 4 ≥ ½ of the area is rotted, with rotting of the core of the tuber) or on a 1 to 9 scale (where 1 = most susceptible) (Austin *et al.*, 1988; Bain and Perombelon, 1988; Bourne *at al*., 1982; Dobias *et al.*, 1976; De Maine *et al.*, 1998; Henniger *et al.*, 1965; Maher and Kelman, 1983; Ratuszniak, 1978). There have been a few attempts to compare results obtained using different methods. For example, three methods of testing whole tubers at single site, infectivity titration and infiltration, gave different ranking of the tested potato cultivars (Bain and

Perombelon, 1988). Inoculation of whole tubers and slices cut from tubers gave the same ranking only in the most resistant and the most susceptible cultivars (Wastie *et al.*, 1988). The point inoculation method of whole tubers with micropipette tips and the use of two standard cultivars, susceptible Bintje and medium-susceptible Desiree, was proposed as a standard method (Priou *et al.*, 1992). Ring test in different European laboratories showed differences in ranking of the tested cultivars using this method, which indicates a big influence of environmental factors on potato tuber soft rot development (Allefs *et al.*, 1993). It was recommended to use standard cultivars and evaluate this trait throughout several growing seasons. The method applied here is also described by Wikens *et al.* (2017), and it is a modification of the method by Laurila *et al.* (2008). For many years two Polish potato cultivars have been used as standards: a susceptible cv. Irys and a medium-resistant cv. Glada.

MATERIALS AND REAGENTS

1) Agar (Sigma Aldrich, cat. No A1296)
2) Box (IKEA, cat. No 801.029.76)
3) Camping gas propan/butan C206 super
4) Cell spreader (Bionovo, cat. No B-4046)
5) Compost soil
6) Elrenmayer flask (Bionovo, cat. No S-1137)
7) Eppendorf Semi-Micro Vis Cuvette (Eppendorf, cat. No 0030079353)
8) Eppendorf tubes 2 ml (Medlab Products, cat. No 25-2000-1)
9) Fine permanent marker (Staedtler permanent, Lumacolor)
10) Fruit spoon baller
11) Glass bottle 1000 ml (Bionovo, cat. No S-2074)
12) Gloves
13) Horticulture peat
14) Inoculation loop and handle (Bionovo, cat. No 1-2128 and 1-2120)
15) Isopropyl alcohol (ALCHEM, cat. No 363-327515001)
16) Lunch box (IKEA, model KULLAR, see Fig.2)
17) Lysogeny Broth (LB, Luria Bertani) (Sigma Aldrich, cat. No L3152)
18) Parafilm (Linegal, cat. No catalog number: H666.1-R)
19) Petri dishes, diameter 9 cm (Medlab, cat. No 51-0091-0SR)
20) Pots (diameter 8 cm)
21) Ruller
22) Sand
23) Scizors
24) Sodium hypochlorite (Chemia Sp. z o.o. Warszawa, CAS cat. No 7681-52-09)
25) Spray bottle Turn'n'Spray 500 ml (Linegal, cat. No PX91.1)
26) Steel rod (see Fig.2)
27) Tips 2-200 µl (Eppendorf, Meranco, cat. No 0030 000.889)
28) Tips 50-1000 µl (Eppendorf, Meranco, cat. No 0030 000.927)
29) Toothpick (wooden)

30) Tunnel foil
31) Vaseline
32) Viabank (BioMaxima S.A. Centrum Mikrobiologii)

EQUIPMENT

1) Autoclave (Prestige Medical, model Extended Plus 2100)
2) Electronic scales (RADWAG, model: PS 210/c/2)
3) Gas burner (WLD-TEC GmbH, model: gasprofi 2 scs)
4) Growth chamber
5) Incubator Shaker (Biosan, model: ES-20, cat. No BS-010111-AAA)
6) Laminar flow cabinet, Biohazard class A (ESCO, model: AC3-3E1)
7) Liquid nitrogen flask (Bionovo cat. No B-4110)
8) Pipette Reference 100-1000 µl (Eppendorf, cat. No 4920.000.083)
9) Pipette Reference 20-200 µl (Eppendorf, cat. No 4920.000.067)
10) Varioklav steam sterilizer (HP Medizintechnik)
11) Water purification system (PURELAB, Elga)

SOFTWARE

1) STATISTICA (StatSoft, Inc. 1997, Tulsa, OK).

PROCEDURE

A. Evaluation of stem tissue resistance in greenhouse growing plants

The method described for the first time by Muenzert (1975), was modified by Pietrak (2001), by using a toothpick for inoculation instead of blotting paper. Tubers are a source of eye-cuts, from which single stem plants are grown in pots (of diameter 8 cm). From 20 to 30 pot-plants in each of the two replicates are tested (40 to 60 pot-plants per genotype).

1) Inoculum preparation (24 h before inoculation):
   a) Bacteria from a frozen culture are grown for 24h at 27°C on LB agar medium plates sealed with a parafilm,
   b) Grown bacteria are washed out with sterilised distilled water and the suspension adjusted to about 10^9 CFU x ml^{-1} (the optical density of 1.0 at the wavelength 600 nm in a spectrophotometer).
2) Greenhouse-grown plants assay
   a) In very early spring, dormant tubers are pre-sprouted for 2-3 weeks at room temperature in medium-intensity light, while in late spring sprouted tubers are used directly,
   b) Eyes are cut from sprouted tubers (40-60 per genotype) and planted in pots with a mixture of soil, sand and peat (pH about 6.3) to obtain single stem plants,
   c) Three-week-old plants are inoculated with a toothpick soaked for

a few minutes in an inoculum, at the base of the stem. The plants are sprayed with water and kept in a plastic tunnel to create high relative humidity. Due to the high influence of environmental conditions on the results of the test (Lebecka and Murawska, 2017) it is recommended to keep the plants in a growth chamber with a constant temperature (around room temperature) and 16 h of light.

d) After 3 weeks the percentage of plants with disease symptoms (Figure 1) is determined and the obtained results are transformed with Bliss transformation before analysis of variance. The results are back-transformed and compared with the results of the standard cultivars: the susceptible cv. Irys and the resistant cv. Glada (with scores 2 and 7 respectively, on a 1 to 9 grade scale, where 9 is the most resistant).

e) The assay is repeated in two seasons.

f) According to The European Cultivated Potato Database Descriptor Dictionary (by Stuart Carnegie), the resistance scale of 1 to 9 is as follows: 1. Very low; 2. Very low to low; 3. Low; 4. Low to medium; 5. Medium; 6. Medium to high; 7. High; 8. High to very high; 9. Very high (https://www.europotato.org/docs/descriptors.pdf)

Fig. 1. Symptoms of blackleg on plants of cultivar Bojar

B. Evaluation of tuber resistance to soft rot in laboratory conditions

1) Inoculum preparation:

As it was described for a stem tissue resistance test.

2) The tuber assay:
   a) Potato tubers are surface-disinfected in 1% sodium hypochlorite solution for 15 min, rinsed in distilled water and air dried at room temperature one day before inoculation,
   b) The tubers are wounded with a steel rode (10 x 2 mm) (Figure 2) and inoculated with 10 µl of bacterial suspension,
   c) The inoculated site is covered with a vaseline and a piece of a parafilm,
   d) tubers are put in boxes, sprayed externally with water and boxes are covered with lids.
   e) After an incubation at a temperature of 26°C for 3 days, the tubers are cut vertically through the inoculation site and either the diameter of the rotten tissue is measured (the scale is presented in Table 1) or the weight of the rotted tissue is determined by the difference in tuber weight before and after the rotted tissue has been removed from each individual tuber. From 3 to 10 tubers per genotype are tested in two replications in two seasons at least. Two standard cultivars are used: the susceptible cv. Irys and the moderately resistant cv. Glada, scored as 3 and 5 respectively, on a 1 to 9 grade scale, where 9 is the most resistant. The resistance of the evaluated cultivars and breeding clones is compared to the reaction of the standards.

Fig.2. Screening for potato resistance to soft rot. From the left: a steel rod for wounding the tubers, inoculated tubers covered with vaseline and a piece of parafilm, tubers of the susceptible cv. Irys and the moderately resistant cv. Glada with the rotten tissue removed three days post inoculation.

Table 1

The scale of resistance of potato tubers to pectinolytic bacteria on root square transformed data for the diameter of rotten tissue in mm.

Resistance score	9	8	7	6	5	4	3	2	1
Diameter of rotten tissue [mm]	2.0 – 2.6	2.7 – 4.6	4.7 – 7.7	7.8 – 11.4	11.5 – 15.4	15.5 – 19.1	19.2 – 22.2	22.3 – 24.2	24.3 – 25.0

Resistance on a scale of 1 – 9, where 9 – the most resistant acc. to Ratuszniak *et al.* (1978).

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