

# Investigations on Resistance of In Vitro Regenerants of Apple (*Malus domestica* Borkh.) ‘Čadel’<sup>®</sup> to Major Diseases

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## Abstract

Decreasing the application of agrochemicals, especially pesticides, in fruit growing practices is one of the most important problems placed by the European Commission of Agriculture with the aim of preserving human health and protecting the environment. The problem is a matter of urgency in the apple crop, which occupies a leading position by the number of pesticide treatments. Solving the problem necessitates the establishment of cultivars resistant or tolerant to the major diseases and pests. The aim of the present study is to test the resistance of in vitro obtained apple regenerants ‘Čadel’<sup>®</sup>, to the most widely distributed phytopathogens in apple – scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*) and fire blight (*Erwinia amylovora*). Varying concentrations of monospore cultures of the respective fungal pathogens isolated from naturally infected leaves and most aggressive bacterial strain from Bulgaria are used in the investigation. The acclimatized in vitro somaclones, 8-10 cm in height or at the 4<sup>th</sup>-5<sup>th</sup> true leaf development stage, are inoculated with the respective phytopathogens following standard or improved methods. It is established that 3 out of the studied clones demonstrated a complex resistance to scab and powdery mildew diseases and low susceptibility/tolerance to the dangerous bacterial disease fire blight.

## INTRODUCTION

Apple breeding is aimed at creating cultivars with high fruit quality, consistently high yields, and durable disease and pest resistance (Fischer, 2000; Kellerhals et al., 2000; Richter and Fischer, 2000; Sansavini et al., 2005). Apparent tissue culture-induced variation, named somaclonal variation, has been the subject of several studies. Somaclonal variation is in general a potentially useful source of genetic variability for plant improvement and could result in new genotypes. Several cases of somaclonal variation for disease resistance traits have been reported in temperate fruit species (Hammerschlag, 1992): apple rootstocks resistant to *Phytophthora cactorum* (Rosati et al., 1990), apple cultivar ‘Greensleeves’ to fire blight resistance (Donovan et al., 1993), peach seedlings resistant to *Pseudomonas syringae* pv. *syringae* (Hammerschlag, 1992) or to *Xanthomonas campestris* pv. *pruni* (Ritchie et al., 1993), pear resistant to *Erwinia amylovora* (Viseur, 1990). In most of these cases, somaclonal variation was spontaneous and observed after regeneration from adventitious buds or somatic embryos. The stability of these variations in the field has rarely been reported (Toyoda et al., 1991; Ritchie et al., 1993).

The apple cultivar ‘Čadel’<sup>®</sup> was obtained from a cross between ‘Golden Delicious’ × ‘Jonathan’, in 1984 in Serbia. A winter apple with high quality medium large to large fruits, suitable for transporting and successful cold storage until May, the cultivar ‘Čadel’<sup>®</sup> is moderate susceptible to *Venturia inaequalis* and *Podosphaera leucotricha* (Misic et al., 1993; Jevtic, 1996).

The aim of the present study is to test the resistance of in vitro obtained regenerants from apple cultivar ‘Čadel’<sup>®</sup> to the most widely distributed phytopathogens in apple – scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*) and fire blight (*Erwinia amylovora*).

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## MATERIAL AND METHODS

### Plant Material

The experiments were carried out with plants obtained through somatic regeneration of leaf segments of the apple 'Čadel'<sup>®</sup> (Gercheva et al., 2009). All regenerants were cloned, propagated, rooted and adapted to in vivo greenhouse conditions. The plants used for the aims of the experiment were 8-10 cm in height or at the 4<sup>th</sup>-5<sup>th</sup> true leaf development stage, potted in separate pots.

### Testing the Regenerants for Disease Resistance

**1. Resistance to the Causative Agent of Scab – *Venturia inaequalis*.** Inoculation was performed with spore suspension of *Venturia inaequalis* fungus extracted from naturally infected leaves. The regenerants were infected by fine pulverizing with suspension of pathogenic spores - CFU  $2.5 \times 10^4$ ,  $2.75 \times 10^4$ , and  $3.25 \times 10^4$  number of spores/ml, respectively. The plants were placed in a phytotron for 48 h at a temperature of 12-14°C, air humidity 80% and a photoperiod 12/12 h. The attack and the level of resistance were reported according to the scale of Chevalier et al. (1991) on the 7<sup>th</sup> day, class 0 corresponding to plants without visual symptoms of the disease and class 4 – strongly susceptible plants with abundant sporulation.

Class 1 – Hypersensitivity

Class 2 – Resistance

Class 3a – Weak resistance

Class 3b – Weak susceptibility

Class 4 – Susceptibility

The plants that had shown high level of resistance were infected with *Podosphaera leucotricha*.

**2. Resistance to the Causative Agent of Powdery Mildew – *Podosphaera leucotricha*.** Suspension of fresh conidia of the pathogen, extracted from the leaves of strongly susceptible apple cultivars with visual symptoms of the attack, was used as infecting material. The plants were inoculated with the suspension (CFU –  $4.6 \times 10^5$ ,  $4.8 \times 10^5$  and  $5 \times 10^5$ ) under the above described conditions, following a similar method and they were cultivated for 48 h at a temperature of 20°C and 80% air humidity. The results were reported on 7<sup>th</sup> day after inoculation, following a five-degree scale, where the zero degree corresponds to healthy plants without stains of powdery mildew; the first degree – very slight susceptibility, up to 10% infected leaves and level 5 – very strong susceptibility with the leaves and apex infected by the pathogen.

The plants that had shown low susceptibility to powdery mildew were infected with *Erwinia amylovora*.

**3. Resistance to the Causative Agent of Fire Blight – *Erwinia amylovora*.** Inoculation was conducted with fresh 48-hour culture of the pathogen *Erwinia amylovora* – a local strain T-21-3, cultivated at 26°C on the nutrient medium NYDA (23 g L<sup>-1</sup> agar, 10 g L<sup>-1</sup> dextrose, 5 g L<sup>-1</sup> yeast extract) or medium King's B (20 g L<sup>-1</sup> proteose, 1.50 g L<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub>, 0.73 g L<sup>-1</sup> MgSO<sub>4</sub> and 15.00 agar). Bacterial suspension of turbidity  $3 \times 10^8$  CFU number of cells/ml determined by comparing with tubes of standard turbidity, was used in the experiments. Infection was performed by cutting the true upper 2-3 leaves of the regenerants with scissors dipped in the bacterial suspension before each cut. The plants were cultivated for 6 days in a growth chamber at 28°C and 80-90% relative air humidity. Reporting was done on 10<sup>th</sup> day and twice in the next 20 days and clones with high degree of visible symptoms of diseases were eliminated. Following the 5-degree modified scale (Zeller et al., 1990) depending on the degree of spread of fire blight in the inoculated plants was used. Class 0 corresponding to plants without visual symptoms of the infection; Class 1 – plants in which the leaf cut has blackened; Class 2 – plants in which the cut and the central nerve have blackened; Class 3 – plants in which the leaf and the leaf petiole have blackened; Class 4 – plants in which the apical shoot and the upper part of the plant have blackened; and Class 5 – the whole plant is dying.

## RESULTS

A total of 137 from the obtained somaclones of 'Čadel'<sup>®</sup> were acclimatized and were transferred into soil in a greenhouse. All appeared phenotypically normal, but show definite differences in plant pathogen response.

After inoculation with *Venturia inaequalis* (Fig. 1) the plants demonstrated good level of resistance (up to class 3a). Most of them (35.6 and 43.8%, respectively) were without visual symptoms of the disease (class 0) or hypersensitive (class 1). On the leaves of some somaclones typical pin-point reaction after infection with *Venturia inaequalis* was observed (Fig. 3).

Regarding to the disease powdery mildew (*Podosphaera leucotricha*) level of resistance of tested apple somaclones ranged from healthy plants without stains of powdery mildew (zero level) to very strong susceptibility with the leaves and apex infected by the pathogen (level 5) (Fig. 2).

After inoculation with *Erwinia amylovora* most of the plants died 20-30 days after inoculation. Ten out of the 137 tested somaclones demonstrated good results after inoculation with scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*) and fire blight (*Erwinia amylovora*) (Table 1). Three somaclones (199-1; 211-3 and 3/1-B) with complex resistance to scab and powdery mildew diseases and low susceptibility/tolerance to fire blight were selected for future evaluation.

Our approach of generating somaclones from cultivars with moderate disease resistance and good fruit quality proved to be useful for germ plasm improvement of apple. We manipulated the culturing conditions in vitro so that the majority of regenerants were phenotypically normal and regenerated somaclones without any selection pressure. Somaclones with higher resistance to plant pathogens than source cultivars were identified in subsequent greenhouse studies. The best genotypes are to be studied by means of additional molecular, phytopathological and field tests and will be hand over to apple breeders.

## CONCLUSION

- Ten out of the 137 in vitro obtained somaclones of apple cultivar 'Čadel'<sup>®</sup> demonstrated good results after inoculation with scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*) and fire blight (*Erwinia amylovora*).
- Three somaclones (199-1; 211-3 and 3/1-B) with complex resistance to scab and powdery mildew diseases and low susceptibility/tolerance to fire blight are selected for future evaluation.

## ACKNOWLEDGEMENTS

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## Tables

Table 1. Somaclones with good level of resistance after inoculation with scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*) and fire blight (*Erwinia amylovora*).

Accession number of somaclones	Degree of resistance after inoculation with <i>Venturia inaequalis</i> (scab)	Degree of resistance after inoculation with <i>Podosphaera leucotricha</i> (powdery mildew)	Degree of resistance after inoculation with <i>Erwinia amylovora</i> (fire blight)
115-6-A	2	0	1
188-1	1	0	2
189-7	3	0	0
189-7 A	0	3	1
173-1	2	1	2
199-1	3	0	2
211-3	0	0	1
200-2	3	0	1
3-1	0	3	2
3/1-B	1	0	2

## Figures

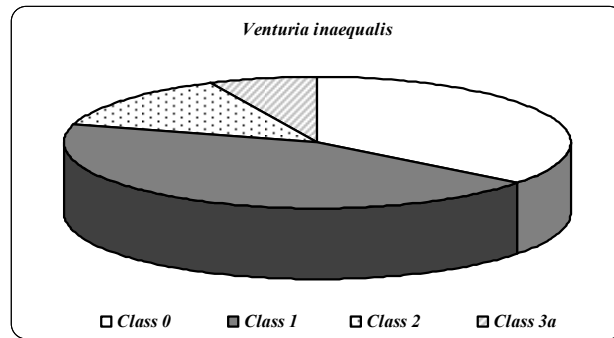


Fig. 1. Reaction (level of resistance) of apple somaclones after inoculation with *Venturia inaequalis*.

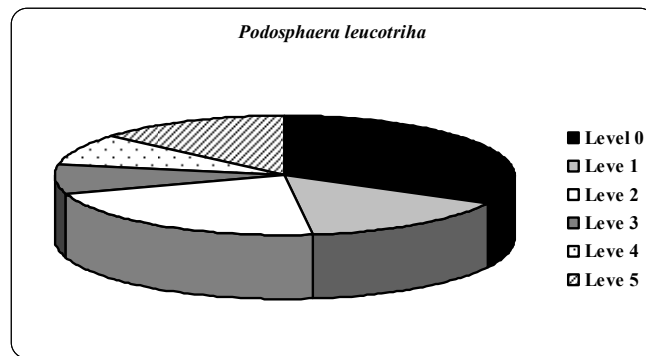


Fig. 2. Reaction (level of resistance) of apple somaclones after inoculation with *Podosphaera leucotricha*.

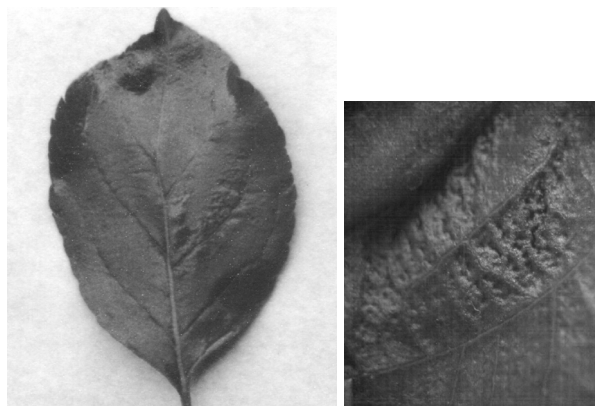


Fig. 3. Pinpoint reaction after infection with *Venturia inaequalis*.

