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BACTERIAL DISEASE OF SUNFLOWER IN HUNGARY CAUSED BY *Erwinia carotovora*

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SUMMARY

During the period 1984–86 the symptoms of an unusual, previously unknown disease were observed on sunflower hybrids and a variety in different parts of the country.

Soft rot symptoms appeared on stalks, petioles and heads. The frequency and severity of the disease varied greatly on the different organs and areas.

It was presumed, that a bacterium is the causal agent of the disease. Isolations were performed and biochemical tests carried out. The identification procedure including artificial inoculation tests proved, that two *Erwinia carotovora* subspecies, namely *E.c. subsp. carotovora* and *E.c. subsp. atroseptica* are present which are able to infect sunflower causing a severe disease and loss of yield.

Evaluating the behaviour of inbred materials and commercial hybrids under natural infection conditions, it can be stated that there are remarkable differences in resistance of the genotypes tested. This phenomenon can be a basis for breeding for resistance as an effective means of control ling this destructive disease.

Key words: Sunflower, *Erwinia carotovora*, bacterial disease, identification

INTRODUCTION

Sunflower is an important crop in Hungary, which occupies about 350 000 ha annually. In 1984, 1985, 1986, the symptoms of a previously unknown soft rot appeared on petioles, stalks and heads of sunflowers in commercial production fields in many counties. The severity of the disease varied greatly: on petioles of the lower leaves of nearly all the plants in the field, and on the stalks the infection reached up to 5% of the population, and the percentage of the diseased heads reached 20–25%. The observed fields were sown with NS–H–26, NS–H–27, IH–56, HNK–81, DK–Solaris and Iregi szürke csíkos hybrids and the open pollinated variety, respectively.

The symptoms on the stalks appeared in oil–green, black coloured long spots. The stem pith showed a watery break–down, odourless but in the advanced stage of the development of the disease with an unpleasant odour. The stalk later dried, became empty, and often broke. The above described symptoms appeared mostly near to the base and the middle part, but sometimes also at the top of the stalk. The symptoms appeared on the petioles after heavy rains, when they split and show a water–soaked soft rot appearance. After a while the diseased petioles and the leafblades dried up and died. The symptoms appeared on the heads, too, as water–soaked patches, which enlarged in a wedge–shaped form. The diseased heads or parts of them can fall out. Due to the activity of the pathogen and the secondary microorganisms, different aromatic metabolites are produced attracting the insects, which can play a role in the distribution of the bacteria during the vegetation period (Arsenijević, 1970).

The first paper about the bacterial disease of sunflower with similar symptoms was published by Arsenijević (1970) in Yugoslavia. The same disease has been reported by Mazzuchi and Bazzi (1979) in Italy, by Richeson (1981) and Gudmestad et al. (1984) in the U.S.A. The pathogen in these cases has been identified as *Erwinia carotovora*.

MATERIALS AND METHODS

The isolations were performed on nutrient and Paton's pectate agar media (PATON, 1959). Fragments of diseased stems, petioles and heads were washed carefully in running water. Pieces of pith tissues, petioles and heads were macerated and the resulting suspensions were streaked onto agar plates by 1 μl plastic inoculating loops (to avoid scratching the pectate plates). The inoculated plates were incubated at 25°C for 2 days, then different types of colonies from nutrient agar were transferred onto agar slants. From Paton's pectate medium pectolitic colonies from deep, cuplike pits were selected. Single colonies were obtained by repeated streaking on nutrient agar plates.

The pectolitic activity of isolates was checked on potato slices placed in moisture chambers inoculated with drops of bacterial suspension from 24–h old nutrient agar cultures.

The following tests were used for the identification of isolates found to be pectolitic on the basis of potato soft rot test: Gram stain was determined using 24–h old cultures nutrient agar cultures, fuorescent pigment production was checked on King's B agar slants, mode of glucose metabolism was detected in Difco OF medium. To test production of acetoin, reducing substance from sucrose, and of gas from glucose, the methods described by Dye (1968) were used. Indole production was checked with Kovács' reagent, lecithinase activity was recorded on nutrient agar supplemented with eggyolk (1 hen's egg yolk was added to 100 ml nutrient agar). Phosphatase production was determined as described by Kelman and Dickey (1980). Alkali production from malonate and acid production from organic compounds were detected on the OY medium of Dye (1968). 0.2% malonate, 0.5% other carbon sources were added to the medium, both filter sterilized (0.2 μm millipore).

As authentic strains *Erwinia carotovora subsp. carotovora (Ecc), E.c. subsp. atroseptica (Eca)* and *E. chrysanthemi* isolates originating from potato were used.

All tests were carried out in duplicate.

Inoculation

Sunflower cultivar and hybrids, Iregi szürke csíkos, NS–H–26 and HNK–81 respectively were grown on an experiment field. The stalks and petioles were inoculated at the full blossoming stage, the heads 3 weeks after flowering. Plants were inoculated with the suspensions of bacteria prepared from 24–h old cultures of *Ecc* and *Eca* in sterile tap water supplemented with TWEEN 20 in 0.1% of the final concentration. The different organs were injured by scalpel, then suspension was pipetted the into wounds, which were covered with wet cotton and aluminium foil. Sterile tap water was used as the check.

Pathogenicity was evaluated 10–15 days after inoculation by the soft rot symptoms. Reisolations from inoculated plants were carried out as described above.

Isolation of the pathogen from the seed

Seeds were harvested from infected heads. Fifty seeds from each lot were surface sterilized by immersing in 0.1% solution of NaOCl for 15 min, then washed 3 times in sterile tap water. The treated and other 50 seeds without disinfection were cracked and

put into 0.5–0.5 ml sterile tap water in Wasserman tubes, and kept for 24–h at 4°C in refrigerator, after that shaken on a rotary shaker at 200 ppm for 15 min.

10–10 μl from each suspension prepared from seeds were placed on Paton's pectate medium. The inoculated plates were incubated at 25°C. If after 24–48–h the pectate degradation could be observed, a loopful of fliud or bacteria was streaked on Paton's pectate medium to get separate colonies from pectate active bacteria for further characterization.

RESULTS AND DISCUSSION

Twenty one *Erwinia carotovora* isolates were collected from the diseased plants originating from different parts of the country during the period 1985–86.. Table 1 shows the results of identification tests. Concerning the results of comparative investigations carried out according to the diagnostic tests of Dye (1969) and Dickey and Kelman (1988), 13 isolates proved to be *Ecc*, 8 strains *Eca*.

On the basis of the characteristics studied it can be concluded that *Erwinia* isolates from sunflower are identical with those of the standard *Ecc* and *Eca* isolates originating from potato. These results agreed with those of Fucikovsky et al. (1978) in the respect, that both *E. carotovora* subspecies are present on and are able to infect sunflower.

In the inoculation test of potato slices, the pectolitic activity of the isolates proved to be moderate or high; symptoms of soft rotting always appeared within 24 hours.

In the inoculation test the 5 *Ecc* and 4 *Eca* isolates tested developed similar symptoms to those registered on the original diseased stalks, petioles and heads. In the case of some strains the infection became so heavy that the stalks broke and the heads fell off. In other cases the long, water–soaked spots extended for a few cm only.

Reisolation of bacteria was successful in each case.

The experiment for detection of bacterial infection/contamination of seeds remained unsuccessful; bacteria could not be isolated either internally or externally from the seeds. These results show, that patogenic *Ecc* and *Eca* from the soft rot tissues of the head smeared on the seed surface can lose viability in a few weeks.

Evaluating the correlation between weather conditions and the occurrence of the disease, there is a tendency that shorter or longer dry and hot periods followed by heavy rains promote the development of stalk rot epidemics. This finding can be explained with the quick change in turgidity of plant tissues, which causes splitting of the epidermis of petioles and stalks opening an entrance for the invasion by the pathogens existing on the surface of the plant as members of the epiphytic microflora.

According to our observations the stalk and head rot of sunflower occur mainly after flowering time, which is especially true for the head rot form of the disease, which was observed after the yellow ripening stage only, so it seems that the disposition of plants to infection increases toward the ripening. These data confirm the results of Gudmestad et al. (1984) who found that sunflowers approaching maturity became more susceptible to *Ecc* infection; however they came to this conclusion on the basis of inoculation tests.

Although screening for resistance with inoculation was not carried out, it can be stated, on the basis of evaluation of different inbred materials and commercial hybrids under natural infection conditions, that there were considerable differences in the susceptibility of genotypes. Comparing the data of the literature and the above obser-

vations it seems, that further study of the biology and host–parasite relationship of these bacteria is necessary for successful breeding for resistance as an effective means of controling these harmful polyphagous pathogens.

Table 1. Tests used for identification of pectolitic *Erwinia* strains isolated from sunflower

Tests	*Erwinia carotovora subsp. carotovora* (13 strains from sunflower)	*Erwinia carotovora subsp. atroseptica* (8 strains from sunflwer)	*Erwinia chrysanthemi* (1 strain from potato)
potato soft rot	+	+	+
fluorescent pigment on King's B medium	–	–	–
Gram reaction	–	–	–
O/F test /glucose/	F	F	F
acetone	+	+	+
phosphatose	–	–	+
gas from glucose	–	–	+
indole	–	–	+
lecithinase	–	–	+
reducing substance from sucrose	–	d	–
acid from:			
trehalose	+	+	–
D–lactose	+	+	+
α–methyl glucoside	–	+	–
palatinose	–	+	–
maltose	–	+	–
alkali from malonate	–	–	+

+: positive reaction –: negative reaction
d: different isolates give different reactions F: fermentative metabolism of glucose

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ENFERMEDAD BACTERIAL DE GIRASOL EN HUNGRIA CAUSADA POR *Eerwinia Carotovora*

RESUMEN

Durante el periodo de 1984–86 los síntomas de una inusual, previamente desconocida, enfermedad fué observada en híbridos de girasol y una variedad en diferentes partes del país.

Síntomas de pudrimiento blando aparecieron sobre tallos, peciolos y capítulos. La frecuencia y severidad de la enfermedad varió grandemente sobre los diferentes órganos y áreas.

Es presumible que una bacteria es el agente causal de la enfermedad. Los aislamientos fueron llevados a cabo así como test bioquímicos. El procedimiento de identificación incluyendo inoculación artificial demostró que dos subespecies de *Erwinia* están presentes y son capaces de infectar el girasol causando una severa enfermedad y pérdida de rendimiento.

Evaluando el comportamiento de material consanguineo e híbridos comerciales bajo condiciones de infección natural puede constatarse quo hay importantes diferencias en resistencia de los genotipos. Este fenómenos puede ser la base para mejora para resistencia como un medio efectivo para controlar esta importante enfermedad.

UNE MALADIE BACTÉRIENNE DU TOURNESOL EN HONGRIE CAUSÉE PAR *Erwinia carotovora* .

RÉSUMÉ:

Au cour de la période 1984-1986, les symptômes d'une maladie inhabituelle et jusqu'à présent inconnue, ont été observés sur des hybrides et des variétés de tournesol dans différentes parties du pays. Des pourritures molles sont apparues sur les tiges, les pétioles et les capitules. La fréquence d'apparition et la sévérité de la maladie ont considérablement varié en fonction des organes atteints et des régions géographiques. Il était plausible que l'agent pathogéne de cette maladie soit une bactérie. Des isolements et des tests biochimiques ont été pratiqués. La procédure d'identification comportant des tests d'inoculation a prouvé que deux sous espéces d'*Erwinia carotovora* , *Erwinia carotovora* subsp. *carotovora* et *Erwinia* subsp. *atroseptica* étaient présentes et étaient capables d'infecter le tournesol, causant une infection grave et des pertes de rendements. Compte tenu du comportement des hybrides et des lignées, nous pouvons affirmer qu'il existe des différences remarquables concernant la résistance. Ce phénomène peut être le point de départ pour une sélection d'une résistance à cette maladie, moyen efficace pour le contrôle de cette maladie destructrice.