Plant Deformation in *Brassica napus* L. after *Phytoplasma* Pathogen Infection

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Summary

On winter oilseed rape plantations in Poland sporadically some „shaggy" looking plants can be observed. These deformations are usually caused by phytoplasmas, that settle the sieve tubes (phloem) of affected plants. The pathotypes of phytoplasmas occurring in Poland have not been classified yet. In the field conditions other morphological deformations, apart from shaggy-like plants, are observed. The pathogen has been identified in winter oilseed rape plants by using two pairs of primers, which are able to recognize phytoplasma's DNA. Seeds were obtained from infected plants. Most of seeds received from infected plants were misshaped and covered with cracks. After seeding and growing in glasshouse conditions 70 per cent healthy plants and 30 per cent shaggy-like plants were received. Shaggy-like plants were self-pollinated and their microspores were put into *in vitro* culture. All received 200 haploid plants were strongly misshaped without distinguishable apical growing points. The same symptoms were observed on 453 diploid plants received from self-pollination. Healthy clones were obtained from phytoplasmas infected plants by growing on medium with antibiotics.

Key words: winter oilseed rape, *Phytoplasma*, DNA of pathogen, deformation of haploid and diploid *B. napus*

Introduction

Morphological changes of inflorescences and single flowers was described for the first time by Schmidt (1955). In the next years other authors - Lehmann (1969), Horvath (1969), Gundersen et al. (1994) - described the cause of strong deformations of plants as well as ethiology and symptomathology of the pathogen

which was responsible for growth aberration and irregular organogenesis. Initially, it was thought that yellow type viruses were responsible for such a situation (Valenta, Musil 1963). But further investigations excluded this hypothesis and pointed at mycoplasma-like organisms as the actual perpetrators (Sears, Kirkpatrick 1994, Gundersen et al., 1994). To distinguish them from bacterial animal pathogens, so called mycoplasms, bacteria that settle on plants was termed "phytoplasmas". The vector of phytoplasmas are insects of *Jasside* family. In the insect the pathogen occurs as inclusions. At present the identification of possible phytoplasmas and their pathotypes is possible with the use of molecular techniques. Every year on the rapeseed plantations sporadically some "shaggy" looking plants can be observed. In order to confirm the presence of *Phytoplasma* in *B. napus* plants the preliminary tests by DNA-PCR analysis were done.

Material and methods

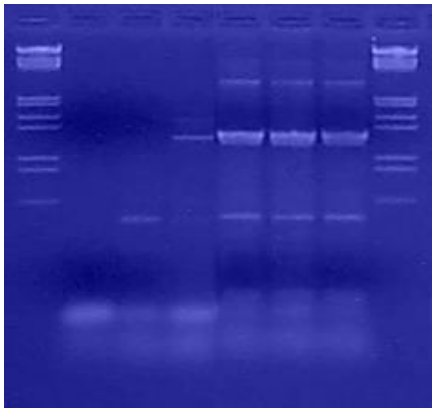
Plants with symptoms of *Phytoplasma* were collected from three different sites: Borowo, Małyszyn and Bąków. Strongly deformed upper parts of the plants were cut off with a scalpel and transported in polyethylene bags to the Laboratory of Resistance Breeding Methods in Poznań. The samples of plants collected in fields situated near Poznań (Borowo and Małyszyn) were transported in the temperature of 20^{o}C, and samples from Bąków were transported in the temperature of 0^{o}C. The infected parts of the stems were stored in liquid nitrogen. Isolation of total DNA was done by Doyle and Doyle method (1990). For PCR analysis two pairs of universal primers for identification of *Phytoplasma* were used: rU3/fU5 that amplifies about 880 bp (Lorenz et al., 1995) and rA16/fA16 (Ahrens, Seemuller 1992, Schneider et al. 1993) that amplifies 558 bp. As a standard of phytoplasma from group AAY (Kamińska, Korbin, 1999) DNA of infected plant *Catharanthus roseus* L. was used (Kamińska et al., 996).

For cloning of infected rapeseed plants agar medium B_5 (Gamborg) enriched by of carbencilinum (800 mg/l) was used.

From some of less infected plants seeds were received, which were sown again. After vernalisation period only "shaggy" plants were isolated in blossom phase and put to haploidysation (Cegielska-Taras, Szała 1997). Observations of haploids development were made from seedling stage (*in vitro*) to grown-up stage (*in vivo*). The same treatment was applied to diploid plants received from seeds that were descendent from self-pollinated "shaggy" plants. The investigations had been conducted for three years.

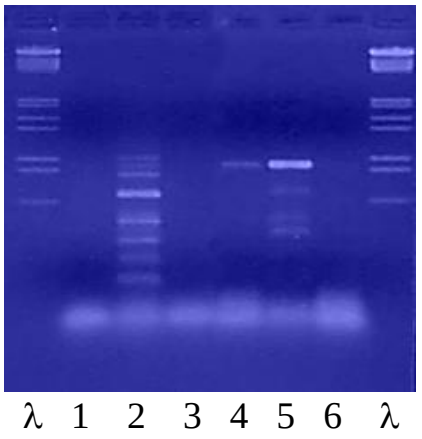
Results

Comparing fields of three sites mentioned above: Borowo, Bąków and Małyszyn in 1999 a similar amount of infected with *Phytoplasma* rapeseed plants was observed. 2-5 plants with typical morphological deformations of flowers and inflorescences were found on the area of 100 m^2. To confirm the occurrence of *Phytoplasma* on rapeseed plants was used PCR analysis which unanimously confirms the presence of the pathogen (Bertaccini et al., 1998). In infected plant fragments transported both in higher (about 20^{o}C) and lower (about 0^{o}C) temperatures the presence of phytoplasm was detected by PCR analysis (Ryc. 2,3). The exception is the sample No 6 on picture 3 (Ryc. 3), where DNA fragment 880 bp long was not found.



1. *Non-infected C. roseus plant*
2. *C.roseus plant infected by Phytoplasma*
3. *Non-infected B. napus plant*
4-6. *B. napus plants infected by Phytoplasma*

Ryc. 2. PCR analysis specific for *Phytoplasma* fA16/rA16 (558bp), (Ahrens, Seemuller 1992, Schneider et al. 1993).



1. *Non-infected C. roseus plant*
2. *C.roseus plant infected by Phytoplasma*
3. *Non-infected B. napus plant*
4-6. *B. napus plants infected by phytoplasma-like organisms*

Ryc. 3. PCR analysis specific for *Phytoplasma* fU5/rU3, (880 bp), (Lorenz et al. 1995).

All plants in which the presence of the pathogen was confirmed by PCR method, after cloning on agar medium (Gamborg B_5 with antibiotic), after 12 months of growing in *in vitro* conditions did not indicate any pathological changes. After carrying them into soil, their growth was typical with normal yielding.

From plants which were infected before flowering time seeds were not received. The seeds received from plants which were infected in blossom and after flowering were half deformed and normal half. Whether the seeds were well shaped or not, 30% "shaggy" and 70% normal plants were received. The further investigations were concerned only with "shaggy" plants. These plants were undergone haploidyzation and were isolated. All 200 plants received from microspore embryos were strongly misshaped and had no established growing-points. The same symptoms were observed in 453 diploid plants derived from seeds of isolated "shaggy" plants.

Discussion

Morphological symptoms of *Phytoplasma* disease are visible enough to, identify sick plants in field conditions. Plant distortions appear usually before flowering as well as during the flowering or after flowering of rapeseed. Seed development depends on the time of infection. If the symptoms of disease are present before flowering time the seeds are usually not formed. In the case when the symptoms appear later a small amount of normal as well as deformed seeds can be expected. Sometimes "shaggy" plants, similar to the ones infected by mycoplasma, but with normal flowers, can be found in fields.

Flowers on infected plants are strongly deformed. Petals are transformed into light-green, leaf-like structures, pistils are 5 cm heigh, empty and distended. The similar symptoms connected with *Phytoplasma* infection were described by Bertaccini et al. (1998), but the analysis of sick plants progeny was not carried out. In their investigations a few methods were used by the authors e.g. electron microscopy, RFLP and sequencing fragments derived from restriction enzymes operation, PCR analysis with primers P1 and P7 (Deng, Hiruki 1991; Kirpatrick et al. 1994).

In this paper the presence of *Phytoplasma* in rapeseed plants was confirmed with two primers: rU3/fU5 (Lorenz et al. 1995), rA16/fA16 (Ahrens, Seemuller 1992). After 880 bp DNA amplification in one case characteristic stripes were absent while for the same sample 558 bp DNA amplification was successful. This situation has not been explained. (Kamińska 1998 – oral information).

In all cases the progeny of "shaggy" plants was deformed both in haploid and diploid generation. It is supposed that this state depends on unknown and unidentified phytoplama's metabolites with mutagenetic effect, which could affect infected plants and their gametes.

Symptoms similar to *Phytoplasma* infection were observed by rapeseed growers on plants after chemical treatment of plantations (for example after herbicide Command – Pszczoła J. oral information).

During the research works on developing *in vitro* microspore embryos or dihaploids embryos on media with high content of phytohormones (IAA, BAP, 2,4D), a small per cent of *B. napus* plants, similar to the progeny of plants infected with phytoplasma was observed.

Deformations of progeny plants received from infected plants have permanent character, despite the lack of the pathogen. That means that genetic changes (mutations) have occurred. This hypothesis should be verified with techniques of molecular biology.

The fact of cure of infected by phytoplasma plants seems to be remarkable (Borecki 1996). After one year of growing in conditions *in vitro* on Gamborg B_5 with carnebicilin, from misshaped stems that were cut off from infected plants normal plants were obtained, which do not differ from the healthy ones.

Conclusions

1. The PCR analysis presented in this paper is useful for detection of *Phytoplasma* presence in winter rapeseed plants.
2. The lack of data on resistance of spring and winter types of rapeseed to *Phytoplasma* infection, and the danger of decreasing the seed yield, is a new challenge for scientists and breeders.
3. The fact of permanent deformations of the progeny derived from plants infected by *Phytoplasma* is a strong impulse for further research works on physiological and genetic reasons of this phenomena.

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