

Deformations of rapeseed *Brassica napus* L. plants in the first progeny after phytoplasma infection

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ABSTRACT

In winter oilseed rape plantations very often are observed shaggy-looking, stuck up the other, deformed plants. With the use of SCAR it was proofed by molecular markers that phytoplasma organisms are responsible for these symptoms. Deformations of infected plants referred morphological changes of blossoms and flowers. Yield of seeds from infected plants is very small. After sowing them into soil some percent of deformed seedlings was observed. These deformations were different from previously observed and symptoms referred mainly with the lack of stem growing point and abnormal shapes of leaves. Nevertheless from some seeds were obtained deformed plants, but capable to flowering. The haploid plants were obtained from deformed plants using microspores culture. All of them were nearing appearance to the deformed donor plants. The mature pollen of deformed plants (2n) was used for cross pollination with normal rapeseed plants. The seeds obtained from all combinations of crossings, were sown in greenhouse conditions. The vernalization of obtained plants was performed during 10 weeks in natural conditions. After vernalization period the plants were transferred into greenhouse. All plants from re-isolations and from crossings were different deformed in degree. It showed negative influence of crosspollination with pollen derived from deformed plants, because already in F₁ generation were observed unfavourable phenotypic changes in the progeny. To make sure that it is impossible to transfer pathogen into offspring by crossings, there were made for them DNA analyses the same as for identification of phytoplasma. The absence of the pathogen was confirmed using primers, which in PCR process amplified 558bp DNA.

Key words: winter oilseed rape – *Phytoplasma* - DNA of pathogen - progeny - deformation

INTRODUCTION

In 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. Morphological changes of inflorescences and single flowers first time was described by Schmidt (1955). In the next years other authors - Lehmann (1969), Horvath (1969), Gundersen et al. (1996) - described the cause of strong deformations of plants as well as ethiology and symptomatology of the pathogen which was responsible for growth aberration and irregular organogenesis. Initially, it was thought that yellow type viruses were responsible for such situation (Valenta, Musil 1963). Further investigations excluded this hypothesis and pointed at mycoplasma-like organisms as the actual perpetrators (Sears, Kirkpatrick 1994). To distinguish them from bacterial pathogens of animals, so called mycoplasmas, bacteria that settle on plants was termed “phytoplasmas”. The vectors of phytoplasmas are insects from Jasside family. Inside the insect the pathogen occurs as inclusions.

MATERIAL AND METHODS

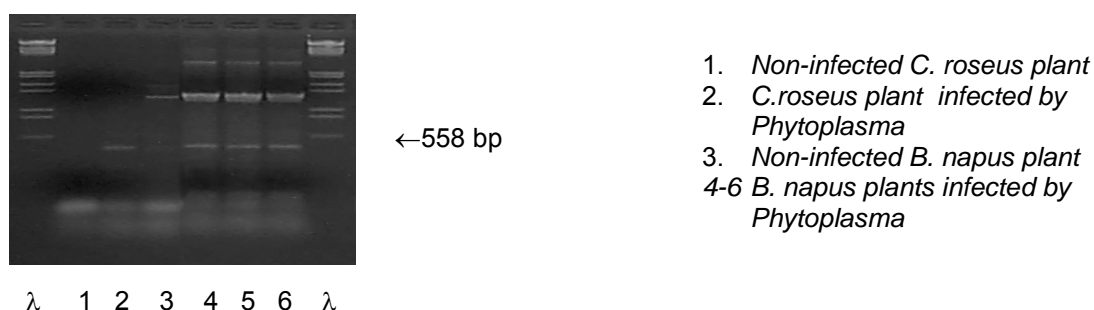
Plants with symptoms of *Phytoplasma* were collected from two different sites: Borowo and Malyszyn. Strongly deformed upper parts of the plants were cut off and transported at the temperature of 20°C to the Laboratory of Resistance Breeding Methods in Poznań. The infected parts of the stems were stored in liquid nitrogen. Isolation of total DNA was done by Doyle method (Doyle J.J., Doyle J.L 1990). One pair of universal primers which amplifies 558 bp: rA16/fA16 (Ahrens, Seemuller 1992

Schneider et al. 1993) was used for identification of *Phytoplasma*. As a standard of phytoplasma from group AAY (Kamińska M., Korbin M. 1999), DNA of infected plant *Catharanthus roseus* L. was used (Kamińska M. i in. 1996).

From some less infected plants there were received seeds, which were sown again. After vernalization period only “shaggy” plants were crossed and isolated in blossom phase and put to haploidyztation (Cegielska-Taras, Szała 1997). Observations of haploids development (1n) were made from seedling stage (*in vitro*) to grown-up stage (*in vivo*). The same treatment was applied to diploid plants (2n) that were descendent from self-pollinated “shaggy” plants. The investigations were conducted for three years.

RESULTS

Observing fields in Borowo and Malyszyn in 1999 a similar amount of *Phytoplasma* rapeseed plants was observed. Average on every 100 m², 2-5 plants with typical morphological deformations of flowers and inflorescences were found. To confirm the occurrence of *Phytoplasma* on rapeseed plants PCR analysis was used (Ryc. 1.). This analysis unanimously confirms the presence of the pathogen (Bertaccini et. all. 1998). In infected plant fragments transported at temperature about 20°C the presence of phytoplasma was detected by PCR analysis (Ryc. 1).



Ryc. 1. PCR analysis specific for *Phytoplasma* fA16/rA16 (558bp), (Ahrens, Seemuller 1992, Schneider et al. 1993).

Seeds from infected plant before flowering time were not obtained. Among the seeds received from plants which were infected in blossom stage and after flowering, 50% were deformed and 50% were normal. Regardless of the fact that the seeds were well shaped or not, 30% “shaggy” and 70% normal progeny plants were received. The further investigations were concerned only with “shaggy” progeny plants. These plants were undergone haploidyztation process and 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.

Table 1. Observations in plants F1 generation received from crossing healthy and deformed plants.

Before vernalization 15 Nov. 2000		After vernalization 23 Feb. 2001	
Total number of plants	Number of deformed plants	Total number of plants	Number of deformed plants
40	0	29	26
20	0	18	10
10	0	10	7
35	0	31	26

23 Mar. 2001 – all plants were deformed

To ensure that transfer of the pathogen into progeny is not possible by over-crossing, the offspring plants were checked up by the same DNA analysis as for identification of phytoplasmas. The lack of the pathogen was confirmed by using primers which in PCR process amplified 558 bp DNA.

Table 2. Observations on plants received from self-pollination of deformed plants (S3)

Before vernalization 15 Nov. 2000		After vernalization 23 Feb. 2001	
Total number of plants	Number of deformed plants	Total number of plants	Number of deformed plants
40	3	29	26
15	0	15	13
45	3	44	43
96	55	93	86
24	9	24	24
53	2	51	46

23 Mar. 2001 – all plants were deformed

DISCUSSION

In winter oilseed rape plantations very often are observed shaggy-looking, stuck up the other, deformed plants. 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. Flowers of infected plants are strongly deformed. Petals are transformed into light-green, leaf-like structures, pistils are 5 cm high, 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 rA16/fA16 primers. The same results were obtained by Bertaccini et al. (1998), (Ahrens, Seemüller 1992). In all cases, the progeny of "shaggy" plants was deformed both in haploid and diploid stage. It is supposed that this state depends on unknown and unidentified phytoplasm's metabolites with mutagenetic effect, which could affect infected plants and their gametes. Deformations of progeny plants which were received from infected parent have a 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.

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