Proceedings

Glutathione is Involved in Resistance of Oilseed Rape to Powdery Mildew †

Elena Mikhaylova *, Michael Shein and Valentin Alekseev

Institute of biochemistry and genetics UFRC RAS, Prospekt oktyabrya 71, Ufa 450054, Russia
* Correspondence: mikhele@list.ru
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Abstract: Oilseed rape (*Brassica napus*) is an economically important crop. In temperate climate powdery mildew *Erysiphe crucifertaum* can reduce its yields dramatically. Nevertheless, cultivars resistant to this fungal disease have not been selected yet. Plants, grown in the field and in the light chamber, were infected with powdery mildew. After 30 days there were severe signs of infection on most of the plants, however several individuals were less susceptible to it. Leaves of B. *napus* were used to measure the level of GSH, GSSG and GST by spectrophotometric assay. The level of total glutathione was higher in plants with increased resistant to powdery mildew than in both severely infected plants and control plants that were not subjected to the infection. The GST activity in resistant and control plants was at the same level, however in susceptible plants it was lower. Therefore, oilseed rape with naturally increased glutathione content can be used in the selection of resistant cultivars. GST and GSH genes should be also considered as targets for genetic engineering and genome editing.

Keywords: *Brassica napus*; oilseed rape; rapeseed; powdery mildew; *Erysiphe crucifertaum*; GST; GSH; GSSG; oxidative stress

1. Introduction

Brassica napus L. (oilseed rape, rapeseed) is used as a source of vegetable oil and protein, animal feed and biodiesel. It is one of the most important crops in moderate climate zone, due to its remarkable cold tolerance. However, oilseed rape is highly susceptible to fungal deceases, including powdery mildew *Erysiphe crucifertaum* [1,2].

Powdery mildew is an obligate biotrophic pathogen, most common in the end of the growing season [1]. Infection can reduce the yield of rapeseed and its quality, causing chlorosis, necrosis and dehydration. Resistance to this pathogen is very rare in *B. napus* and was only achieved by hybridization with distant relatives [3]. Commercial cultivars resistant to this fungal disease have not been selected yet. The impossibility to grow *E. crucifertaum* in culture media apart from the plant complicates associated studies.

Plant physiological reactions to powdery mildew infection are poorly studied. Glutathione plays an important role in plant resistance to oxidative stress. Reduced Glutathione (GSH) is involved in detoxification of ROS and toxic molecules, catalyzed by glutathione S-transferases (GSTs). Glutathione disulfide (GSSG) is formed during this process [4]. Induction of the level of GSH and GST upon exposure to stress provide better protection of the plant cell. Although GSTs are usually associated with tolerance to heavy metals [5], there are data on the role of several GST genes in resistance to other stress factors such as extreme temperatures [6] and fungal deceases [7–10]. Therefore it is supposed that described mechanisms can also work during plant-fungus interactions, they were never studied in oilseed rape, infected by *E. crucifertaum* and the actual level of GSTs and glutathione in healthy and infected plants were never evaluated.

2. Experiments

2.1. Biological Material

B. napus of cultivar "Ratnik" were used in the experiments. Seeds were sown in the experimental plot in natural conditions and in the laboratory in vessels filled with commercial soil (Geolia, Russia) under 10,000 lux generated by LED grow light.

E. crucifertaum was isolated from the infected *B. napus* plants and distinguished on the basis of the sequencing of ITS DNA marker. Leaves of the experimental plants were rubbed with the leaf of the infected plant to spread infection. Control plants remained untreated.

After 30 days 42 plants with severe signs of infection and 30 control plants, from both laboratory and field experiment, were used for spectrophotometric GSH, GSSG and GST assay. Each plant with increased resistance to powdery mildew was studied individually.

2.2. Spectrophotometric Assay

Fresh leaves of *B. napus* were homogenized and used to measure the level of GSH, GSSG and GST by spectrophotometric assay simultaneously. Levels of glutathione and glutathione disulfide were determined using o-phthalaldehyde (79760, Sigma-Aldrich, St. Louis, MO, USA) as a fluorescent reagent, derivatization of GSH to prevent GSH autooxidation was performed using N-ethylmaleimide (E1271, Sigma-Aldrich) [11]. GST activity was measured using the model substrate 1-chloro-2,4-dinitrobenzene (138630, Sigma-Aldrich) [12]. All measurements were performed in 6 replications in a 96-well plates using Perkin Elmer LS 55 Luminescence Spectrometer (US).

3. Results

Most of the *B. napus* plants demonstrated severe signs of infection within 30 days after treatment, however several individuals were less susceptible to it (Figure 1). In general, 12 plants with increased resistance to powdery mildew were found and examined. Out of them, 6 plants were cultivated in the field, and 6—in the laboratory.

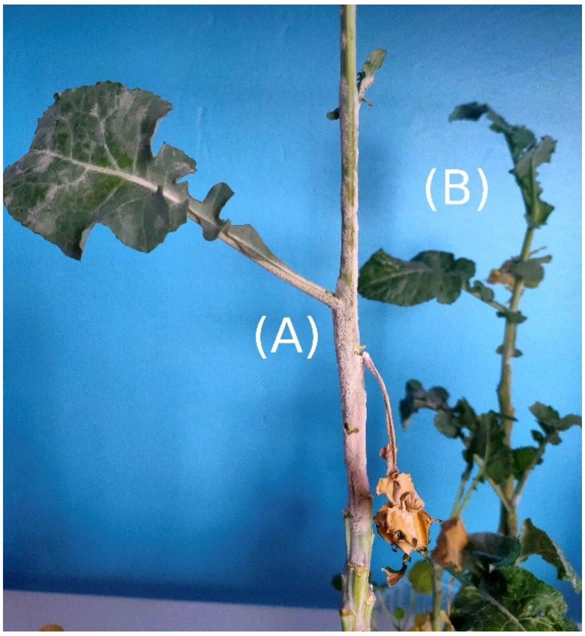

Figure 1. Rapeseed, susceptible (**A**) and resistant (**B**) to powdery mildew.

There was a difference in GSH and GSSG content and GST activity not only between infected and control plants, but also between plants cultivated in laboratory and in the field. All measured parameters were increased in the latter. Therefore, the results were evaluated separately for laboratory and field experiment.

Resistant plants had extremely high glutathione content in comparison with susceptible plants (6–7 times higher in several plants; 2–3 times higher at the average) (Figure 2), however the activity of GST never varied that much. In resistant and control plants it was at the same level, however in susceptible plants it was no more than 3 times lower. In untreated plants GST activity was stable and didn't vary significantly, however some plants had increased glutathione level.

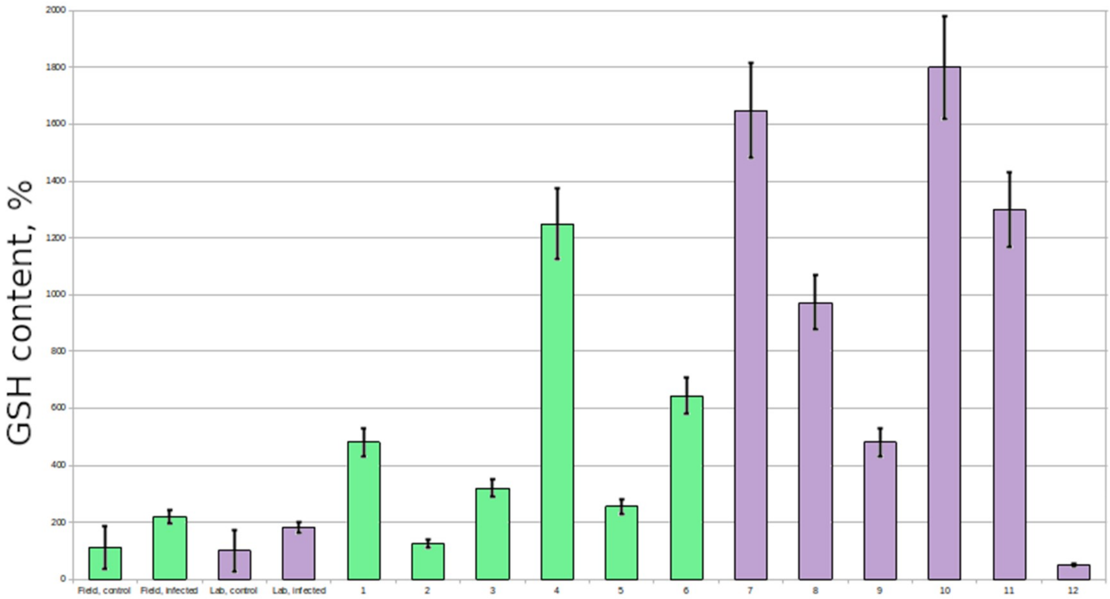

Figure 2. Relative content of glutathione in studied plants. Plants grown in the field (**1–6**) and in the lab (**7–12**) are highlighted in green and purple, respectively.

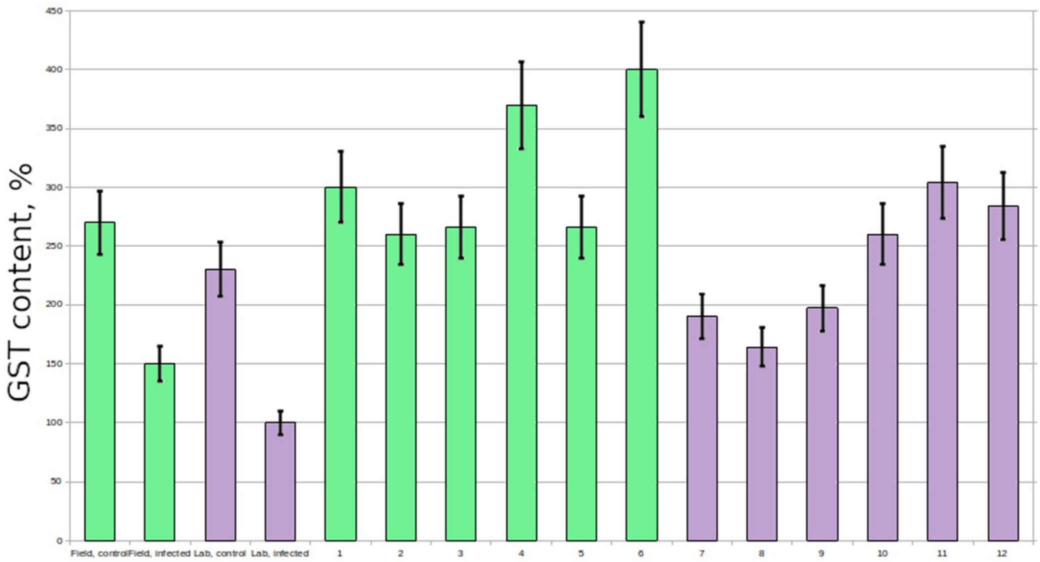

Figure 3. Relative content of GST in studied plants. Plants grown in the field (**1–6**) and in the lab (**7–12**) are highlighted in green and purple, respectively.

GSH:GSSG level in most of the susceptible plants was lower than in resistant plants, which is a sign of heavier stress.

4. Discussion

Our results suggest that increased level of glutathione can give plants an advantage during the infection with powdery mildew. The analysis of control plants showed that there are individuals with physiologically high level of glutathione and they could be the ones showing resistance to the pathogen. These untreated plants also demonstrated higher GSH:GSSG level, witch is characteristic for the lower oxidative stress.

However there is some data on the increased expression of *GST* genes in plants exposed to fungal infection [7–10] in wheat, tomato and barley, in our experiments there was no significant difference between control and resistant plants in GST activity. Many of the susceptible plants had reduced GST activity, therefore this protein is also of research interest.

5. Conclusions

Oilseed rape with naturally increased glutathione content can be used in the selection of resistant cultivars. *GST* and *GSH* genes should be also considered as targets for genetic engineering and genome editing.

Author Contributions: E.M. conceived and designed the experiments, analyzed the data and wrote the paper; M.S. and V.A. performed the experiments.

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Conflicts of Interest: The authors declare no conflict of interest.

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