Plant Extracts as the Source of Physiologically Active Compounds Suppressing the Development of Pathogenic Fungi

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Abstract

It is shown that aqueous extracts from bird cherry tree *Padus avium* L., aspen *Populus tremula* L., and celandine *Chelidonium majus* L., effectivily suppress the germination of *Puccinia triticina* Eriks uredospores. Fungitoxic activity of the extracts is supposed to be determined by high content of phenolic compounds and high peroxidase activity in the leaves of these plants. Fungitoxic activity of the extracts from the leaves, inflorescences, roots, and stems of siberian cowparsnip *Heracleum sibiricum* L., was also correlated with the content of phenolic compounds in these organs. Treatment of healthy wheat *Triticum aestivum* L., cv. Lyubava, with the extracts from common comfrey *Symphytum officinale* L., cowparsnip *Heracleum sibiricum* L., and giant knotweed *Reynoutria sashalinensis* (F. Schmitt) Nakai, stimulated the photosynthetic activity in treated leaves.

Keywords: brown rust; wheat; plant extracts; phenols; peroxidase; photosynthesis; chlorophyll uorescence; thermoluminescence

INTRODUCTION

A high content of physiologically active compounds is characteristic for many plants. In some cases, these substances can stimulate the physiological and biochemical processes, in other cases, they play the role of inhibitors, antibiotics, and fungicides. Recently, extracts prepared from certain plant species were proposed as biological agents for crop protection against pathogenic fungi. According to the literature, about 30% of tested plant extracts show the antifungal activity in the range of 50%, and 5% of the extracts have an efficacy over 80% (BLAESER & STEINER 1999). The identification of active substances of the extracts is not so easy task. The antifungal properties of *Glycine max* are due to lecitin (KLINGAUF & HERGER 1990). The high antifungal activity of *Caryophyllus aromaticus* is due to high content of essential oil and its main component eugenol (SCHOLZ *et al*. 1999). Other examples of antifungal compounds are saponins (JURZYSTA & BIALY 1999). It is important that plant extracts may serve as inducers of resistance in the plants. The most famous example of the inducer is the extract from giant knotweed, *Reynoutria sachalinensis*. It does not significantly inhibit germination of *Sphaeroteca fuliginea* fungi, but effectively protects cucumber plants from powdery mildew when applied two-three days before inoculation (HERGER & KLINGAUF 1990). Protective properties of this extract are ascribed to phytoalexins that are built up in infected leaves (DAAYF *et al*. 1997). High interest to the application of plant extracts for biological control of pathogenic fungi is excited by the possibility to protect the crops without any negative consequences for the environment.

The objectives of this study were: (i) to test a number of plant extracts and to find the extracts with strongly pronounced fungitoxic activity against brown rust of wheat; (ii) to search for the correlation between the fungitoxic activities of the extracts on the one hand,

and the content of phenolic compounds in them on the other hand; (iii) to study the effect of treatment with fungitoxic extracts on photosynthetic apparatus of healthy plants. Besides traditional physiological techniques, we used two biophysical methods allowing to monitor the photosynthetic processes *in situ*. These methods are based on the registration of slow fluorescence induction (SFI), and also of the thermoluminescence (TL) of the leaves.

MATERIALS AND METHODS

To prepare the extracts (with the exception of *Reynoutria sachalinensis*), 10 g of biomass were thoroughly ground, then added to 100 ml of a tap water and passed through several layers of cheese cloth one day later. Extract from *R. sachalinensis* was prepared fresh prior to spraying, by adding 2 g of plant powder to 200 ml of distilled water at 50°C. The suspension was stirred for one hour without further heating. After that the plant particles were filtered off with a help of gauze bandage.

The resulting extracts were used for spraying (two treatments at an interval of 2–3 days) the seedlings of wheat *Triticum aestivum* L. cv. Lyubava. Extracts were used to treat healthy wheat plants and also the plants, leaves of which were inoculated with a suspension of *Puccinia triticina* Eriks uredospores. In the later case, the treatment with the extracts was started 6 h after the infection. Control plants were treated with a tap water. Extracts from wheat leaves were used as the biological control. All measurements were carried out on the first leaves of treated plants, at the seventh day after the inoculation or at the third day after the last treatment (in the case of healthy plants). Direct antifungal activity of the extracts was estimated from the data of uredospores germination on the surface of 2% water agar. Content of chlorophyll and phenols, rate of photosynthetic O_2 evolution and peroxidase activity were determined by standard methods as described in our previous publication (YURINA *et al*. 1996). Reaction activity of cell sap was determined by the method of chemical models with the addition of 3,4-dioxyphenylalanine (TAMBIEV 1984).

To measure SFI, the leaf was initially adapted to darkness for 5 min and then exposed to wide-band blue light (50 W/m^2). Fluorescence intensity was recorded at the wavelength $\lambda = 686$ nm (the maximum of chlorophyll fluorescence band).

The TL curves were recorded as described previously (SOLNTSEV *et al*. 1998). At first, the leaf was pre-illuminated with 725 nm red light for 1 min at room temperature to oxidize the electron carriers between photosystems I and II. Then the sample was chilled to –30°C and illuminated for 3 min with saturating white light (30 W/m^2). After illumination, the sample was rapidly cooled down to –70°C and then heated at an average rate 30 degrees per min. The TL signal was recorded during the heating of the sample up to the temperature +80°C.

RESULTS AND DISCUSSION

Fungitoxic activities of eleven plants extracts tested in the work varied from 0 to 100% (Table 1). It was found that the extracts from bird cherry tree *Padus avium* L., aspen *Populus tremula* L., and celandine *Chelidonium majus* L. completely suppressed the germination of *P. triticina* uredospores. Spraying of wheat seedlings with the extracts effectively protected them from brown rust infection (data not shown). Data presented in Table 2 show that high antifungal activity of the extracts from bird cherry tree, aspen, and celandine is obviously determined by high content of phenolic compounds as well as by high peroxidase and reaction activities in the leaves of these plants. This is in agreement with a view about the important role of oxidative processes in plant defences (RUBIN *et al*. 1975). Products of the oxidation of phenolic compounds are known to have a high and diverse physiological activity (ZAPROMETOV 1993), and this

Table 1. Effect of plant extracts on the germination of *P. triticina* uredospores

Plant	Germinated uredospores (%)	Hyphae length (μm)
Control (H_2O)	92	750
Aconitum excelsum	64	470
Aegopodium podagraria	74	510
Chelidonium majus	0	–
Padus avium	0	–
Polygonum weyrichii	94	760
Populus tremula	0	–
Ranunculus acris	12	135
Sambucus racemosa	72	512
Sinapis arvensis	15	25
Syringa vulgaris	80	570
Urtica dioica	75	540

Table 2. Comparative physiological characteristics of plants differing in antifungal activity of leaf extracts

Padus avium	*Populus tremula*	*Chelidonium majus*	*Aegopodium podagraria*	*Polygonum weyrichii*
Antifungal activity of the extracts in respect to *P. triticina*				
High	High	High	Low	Low
Content of phenolic compounds (rel. units per g fr wt)				
40.9	39.4	40.1	28.6	27.2
Peroxidase activity (extinction units)				
21.6	19.8	20.3	8.3	7.9
Reaction activity of cell sap (extinction units)				
21.1	20.6	19.4	0.95	0.90

is obviously the reason for high reaction activity of cell sap.

In the second part of our work, we have used aqueous extracts from different parts of siberian cowparsnip *Heracleum sibiricum* L. We have shown that fungitoxic activity of these extracts was slightly diminished in the direction: leaves → inflorescences → roots → stems (Table 3). Spraying of wheat seedlings with the extracts effectively protected them from fungal infection; this protective action was correlated with the direct suppression of fungi development. It must be noted that there was a high positive correlation between the fungitoxic activity of the extracts, on the one hand, and the content of phenolic compounds, as well as peroxidase and reaction activities in various parts of the plants, on the other hand (Table 3). These data confirm the conclusion about the important role of phenol metabolism in fungitoxic action provided by plant extracts.

In the third part of our work, we studied the effect of plant extracts on the photosynthetic apparatus of healthy plants. Spraying of wheat seedlings with fungitoxic extracts from comfrey *Symphytum officinale* L., and cowparsnip *Heracleum sibiricum* L. caused a

Table 3. Antifungal activities of aqueous extracts from the leaves, in orescences, roots, and stems of cowparsnip plants and physiological characteristics of these organs

Control (H_2O)	Leaves	In orescences	Roots	Stems
Number of germinated *P. triticina* uredospores (%)				
75	6	9	12	25
Protective properties of the extracts (average number of pustules per leaf)				
85	16	20	35	50
Content of phenolic compounds (rel. units per g fr wt)				
–	22.3	18.0	12.8	8.8
Peroxidase activity (extinction units)				
–	23.5	21.3	6.8	7.4
Reaction activity of cell sap (extinction units)				
–	17.6	13.8	5.3	4.5

Table 4. Impact of treatment with aqueous extracts from *S. officinale*, *H. sibiricum*, and *R. sachalinensis* on physiological and biophysical characteristics of wheat leaves

Control (H_2O)	*Symphytum officinale*	*Heracleum sibiricum*	*Reynoutria sachalinensis*
Chlorophyll content (mg/g of fresh biomass)			
0.80 (100%)	0.90 (113%)	0.85 (106%)	0.84 (105%)
O_2 evolution (ml O_2 per mg of chlorophyll in 1 h)			
1.10 (100%)	1.28 (116%)	1.22 (111%)	1.37 (125%)
Relative uorescence decrease $(F_M - F_T)/F_T$ (rel. units)			
0.90 (100%)	1.07 (119%)	1.02 (113%)	1.14 (127%)
Relative light sum S_A/S_{tot} of TL band A (rel. units)			
0.40 (100%)	0.58 (146%)	0.63 (158%)	0.52 (130%)
Relative light sum S_C/S_{tot} of TL band C (rel. units)			
0.20 (100%)	0.11 (56%)	0.09 (45%)	0.13 (67%)

slight but well reproducible increase in the chlorophyll content; the same result was obtained for the extract from giant knotweed *Reynoutria sachalinensis* (F. Schmidt) Nakai (Table 4). We also demonstrated an explicit increase in the photosynthetic activity (O_2 evolution per mg chlorophyll) in the leaves treated with the extracts (Table 4). Biophysical methods revealed the stimulation of photosystem (PS) II activity and the increase in the rate of electron transport between the photosystems in treated leaves (see below).

The typical pattern of the SFI curve was presented in KRAUSE and WEISS (1991) and LAZAR (1999). It is known that the fluorescence originates from the chlorophylls of the light-harvesting antenna of PS II (LAZAR 1999). The ratio $(F_M - F_T)/F_T$ was used as the SFI parameter, where F_M is the fluorescence corresponding to the second maximum of the induction curve and F_T is the steady state fluorescence achieved after 8–10 min of illumination. Earlier, we showed that this parameter was correlated with the photosynthetic activity (KARAVAEV *et al*. 1998). The treatment of plants with the extracts caused the increase in $(F_M - F_T)/F_T$ ratio (Table 4) due to the enhancement of the F_M values. The increase in F_M, in its turn, can be associated with lower ΔpH values across the thylakoid membrane (KRAUSE & WEISS 1991) as the result of more active ATP synthesis at the first seconds of illumination. An active synthesis of ATP leads to the increase both in the rate of electron transport and in the total photosynthetic activity.

Typical TL curve was given in (SOLNTSEV *et al*. 1998). This curve is characterized by three bands: A (from –30 to 0°C), B (from 0 to 40°C), and C (from 40 to 80°C). Bands A and B arise due to the recombination of electrons from reduced acceptors of PS II with the "holes" in the oxidized states in O_2-evolving system (DEMETER & GOVINDJEE 1989). Earlier, we have shown that the relative light sum of band A, S_A/S_{tot}, correlates with the photosynthetic activity (YURINA *et al*. 1992). The treatment of plants with the extracts caused the increase in the TL band A (Table 4). This result is in accordance with the data obtained by the method of SFI. Band C is supposed to reflect the destruction of chloroplast membranes during freezing of the samples; the intensity of this band was shown to increase under unfavourable growth conditions (SOLNTSEV 1989). Thus, lower values of S_C/S_{tot} in treated plants indicate on their augmented ability to resist against unfavourable environmental conditions.

Thus, we conclude that the extracts from *S. officinale*, *H. sibiricum*, and *R. sachalinensis* can produce a well-pronounced stimulant effect on the photosynthetic activity of wheat seedlings. This, obviously, is not a common feature of all fungitoxic plant extracts, because we could not register any changes in SFI curves in the case of the extracts from *P. avium*, *P. tremula*, and *Ch. majus* leaves. It must be also noted that high concentrations of fungitoxic extracts can produce some damage of leaf tissue. This effect

was revealed for the extracts from *S. officinale*, *H. sibiricum*, and *Ch. majus* (SCHMITT, pers. commun.). Thus, practical use of plant extracts needs thorough selection of optimal conditions for their preparation and application.

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