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The Effect of Soybean Mosaic Virus on the Activity of Carbohydrases in Leaves of Sensitive and Resistant Soybean Plants

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Influence of soybean mosaic virus (SMV) on activity of 1,3-β-D-glucanase, α-D-mannosidase, β-D-galactosidase and β-D-glucosidase in leaves of extremely resistant (cv. Devamusume) and sensitive (cv. Primorskaya 529) young soybean plants was studied. Enzymes can be put in order of increase of their activities as follows: β-D-glucosidase, β-D-galactosidase, α-D-mannosidase and 1,3-β-D-glucanase independently of a cultivar.

High level of 1,3-β-D-glucanase activity was detected in leaves of the healthy resistant plants in comparison to the healthy sensitive plants. In response to systemic invasion with SMV, the activity of 1,3-β-D-glucanase in leaves of resistant cultivar did not change. Infection of the sensitive cultivar with SMV caused a considerable increase in activity of 1,3-β-D-glucanase in the leaves above the inoculated leaves.

After infection with SMV no difference had been found in the level of activity of glycosidase between the soybean cultivars, though the virus caused mosaic symptoms and deformation and roll of leaves of the sensitive cultivar.

Keywords: 1,3-β-D-glucanase, α-D-mannosidase, β-D-galactosidase and β-D-glucosidase, soybean mosaic virus, soybean.

The interaction between plant and virus can result in a wide diversity of responses depending both on genomes and conditions of the environment. In the sensitive plant, the virus spreads from primary infected cells over the whole plant, multiplies considerably and causes characteristic symptoms. In the extremely resistant plant, infection is blocked at an early stage and its effects are undetectable.

Carbohydrases or O-glycoside hydrolases participate practically in all important processes in organisms. 1,3-β-D-glucanases (EC 3.2.1.39) are absent in animal but widely spread in plants. Isoforms of this enzyme are constitutionally present in their cells, cell-walls and extracellular space (Stintzi et al., 1993; Beffa and Meins, 1996). They functioning in growing plants during the whole ontogenesis. The enzymes play specific role in the reparation of injured tissues and participate in process of seedling, growth maturing of fruits, and senescence (Hrmova et al., 1995). 1,3-β-D-glucanases are induced in response of the different stresses including attack by different pathogens and so are the representatives of the group of pathogenesis-related (PR) proteins (Stintzi et al., 1993). The role of 1,3-β-D-glucanases during fungal invasion has been best studied. The basic

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vacuole 1,3-β-D-glucanases of class 1 hydrolyze cell-walls of fungi with liberation of oligosaccharides or so-called oligosaccharines. Linking with specific receptors they include mechanisms providing viability of plant (Albersheim et al., 1992; Okinaka et al., 1995), which lead to synthesis of phytoalexins toxic to fungi. However 1,3-β-D-glucanases working together with another PR-protein (chitinase) can hydrolyze fungal cell walls *in vitro* (Kim and Hwang, 1997; Cheong et al., 2000).

The virus-induced hypersensitive response of plants is also accompanied by activation of 1,3-β-D-glucanases (Stintzi et al., 1993; Beffa et al., 1996; Lusso and Kuć, 1996). However, the role of the enzymes for virus pathogenesis remains to be elucidated. Recently, Meins and colleagues, working with a mutant tobacco (cv. Havana 425), proposed the hypothesis that activation of 1,3-β-D-glucanase in response to infection with TMV (tobacco mosaic virus) or TNV (tobacco necrosis virus) facilitate virus transport through hydrolyzing of callose (1,3-β-D-glucans) which forms a mechanical barrier of virus moving (Beffa and Meins, 1996; Iglesias and Meins, 2000). In mutant tobaccos deficient of class 1 antifungal 1,3-β-D-glucanase, TMV and TNV transport was limited. The authors proposed that viruses can use a defense response of the host against fungal infection for their own spread.

The glycosidases (for example β-D-glucosidases, β-D-galactosidases and α-D-mannosidases) could be involve in the metabolism of callose and other glycoconjugates of cell-walls. These hydrolases may modify carbohydrates providing solidity and elasticity to the cell-wall. They can also participate in regulation of biological functions of such carbohydrates of cell wall as gemicellulose, callose, pectin polysaccharides, glycoproteins, glycosidese of flavonoids and other glycoconjugates (Srisomspar et al., 1996; Bagatharia and Chanda, 1998). There is no effect of virus infection on activities of these enzymes in soybean plants.

The aim of this study was to examine the changes in the level of activity of carbohydrases, such as 1,3-β-D-glucanase, α-D-mannosidase, β-D-galactosidase and β-D-glucosidase in leaves of two soybean cultivars different in their resistance to soybean mosaic virus (SMV). Participation of these enzymes in the leaf growth processes was also investigated.

Materials and Methods

Soybean plants [*Glycine max.* (L.) Merr.] cv. Primorskaya 529 (sensitive host of SMV) (Krylov, 1995) and cv. Dewamusume (extremely resistant to SMV) (Ishikawa et al., 1979) were used in this work. The pathogenic strain C of SMV was kindly presented by Prof. R. V. Gnutova.

Soybean plants were grown in the soil culture under nonregulated conditions of greenhouses in spring-summer seasons. The 14–16 days old plants were divided in two parts: one was infected by SMV, another was used as control. Unifoliate leaves were dusted with carborundum and gently rubbed with a sap from leaves of SMV-intected plants diluted with three volumes (w/v) of 0.01 M phosphate buffer, pH 7.0. The unifoliates of control plants

were treated with a sap prepared from healthy plant. Unifoliates were washed by distilled water immediately after inoculation.

Unifoliates (as 0 tier) and trifoliate of each tier (1, 2, 3, 4, 5 and 6 tier), were sampled from 5–6 plants in 1, 7, 10, 14, 21 and 28 days after inoculation (1 g fresh tissue of leaves without midvein). The samples were kept under –10 ºC.

One gram tissue samples were homogenized in a mortar with a pestle in 10 ml 0.05 M Na-succinate buffer, pH 5.2. The extracts prepared in a cool room at –4 ºC temperature were centrifuged at 8,000 g for 20 min and supernatants were tested for enzymatic activity.

1,3-β-D-glucanase was detected with using laminaran as substrate. Laminaran was obtained by the method of Zvyagintseva (Zvyagintseva et al., 1999). The standart mixture for determination of hydrolytic activity contained 10–20 µl of the extract in 0.05 M Na-succinate buffer (pH 5.2) and 480–490 µl of laminaran solution (1 mg/ml). The activity was assayed by determining the reducing sugar liberated after 2.5 hours of incubation at 37 ºC (Nelson, 1944).

Glycosidase activity was detected using the corresponding p-nitrophenyl glycopyranosides as substrates. The reaction mixture containing 20–50 µl of the enzyme solution in 0,05 M succinate buffer (pH 5.2) and 450–480 µl of corresponding substrate (1 mg/ml) was incubated for 2.5 h at 37 ºC. The reaction was stopped by addition of 2.0 ml of 1M Na_2CO_3 and the absorbancy of p-nitrophenol liberated was measured at 405 nm (Zvyagintseva et al., 1997). p-Nitrophenyl-β-D-gluco-, -β-D-galacto- and -α-D-mannosides were commercial products (Sigma, USA). The incubation time was choosed on linear part of dependence of product concentration from reaction time.

The enzyme unit corresponds to the enzyme quantity which catalyzes the formation of 1 µmol of Glc or p-nitrophenol in minute/1 g fresh tissue at the reaction conditions. All experiments were conducted in biological replicates. Figures represent the means of two experiments. All results were analysed statistically using the Student's *t*-test.

Results and Discussion

Two soybean cultivars with different reactions to infection by the strain C of SMV have been used in this study: a susceptible soybean cultivar (Primorskaya 529) (Krylov, 1995) and a resistant one of Japanese selection to many pathogens (Dewamusume) (Ishikawa et al., 1979) which reacts to infection by producing of local lesions on primary, unifoliate leaves. The early period of plant development (before blossom) has been chosen to synchronize the growth of the cultivars. The resistant Dewamusume differs from the susceptible cultivar by more late blossom period and production of more number of the additional branches (8–12 in number per one plant). Changes of enzyme activities during four weeks after inoculation of the primary leaves (unifoliates) of soybean seedlings should coincide with the period of distribution and accumulation of the virus.

The level of the activity of four enzymes of carbohydrate metabolism: 1,3-β-D-glucanase, α-D-mannosidase, β-D-galactosidase and β-D-glucosidase in leaves has been investigated after inoculation with the virus. The results are shown in *Figures 1–5*. Each

Figures 1(b–g)–4(b–g) show the changes in activity of the tested enzymes in process of development of six tiers of trifoliates. *Figures 1a–4a* shows changes in the enzyme activity of soybean unifoliates which have been inoculated with SMV. The diagrams show that the soybean seedlings have only one trifoliate one day after inoculation of unifoliates. Seven days after infection simultaneously with growth of the first tier leaf, the second trifoliate expands on the plants, etc.

In the leaves of any tier of healthy resistant soybean cultivar, the level of activity of 1,3-β-D-glucanase was more than 2 times higher in comparison with the susceptible cultivar *(Fig. 1).* During the growth and development of soybean trifoliates, activity of 1,3-β-D-glucanase in both cultivars increases (see each of five tiers, *Fig. 1).* Thus, increasing the activity of 1,3-β-D-glucanase is obviously seen on the example of trifoliate of first tier. It is observed simultaneously with leaf growth: from 0.45 unit of activity in the first day of experiment up to 1.86 unit of activity in four weeks for the resistant soybean cultivar and from 0.16 unit activity up to 0.86 unit of activity for susceptible cultivar. The tendency to the enhancing of the 1,3-β-D-glucanase activity is observed in every growing higher trifoliates of both cultivars *(Fig. 1b–g).*

In the developed primary leaf of the resistant cultivar, the high activity of 1,3-β-D-glucanase has been observed. The level has not changed practically during four weeks of the experiment *(Fig. 1a).*

The infection of the resistant cultivar by SMV does not cause substantial changes in the activity of 1,3-β-D-glucanase in the unifoliates, in which only single local lesions were developed. On the contrary, infection of the susceptible cultivar by SMV results in intensive enhancing of activity of 1,3-β-D-glucanase. Thus, enzyme activity in the growing trifoliate of the second tier increased 2-fold as compared to the control plants. The significant increase of 1,3-β-D-glucanase activity in the trifoliate of the first tier has been demonstrated in four weeks of the experiment. As for the unifoliates infected by SMV, the significant stimulation of enzyme activity has been registered in two weeks and it is of high level *(Fig. 1).*

In accordance with the data on SMV distribution (Iwai and Wakimoto, 1990) the transport of SMV is connected to the attractive capacity of developing plant organs, which in our experiment was presented by the expanding soybean trifoliates. The comparison of the data, obtained before infection and SMV antigen distribution in soybean cv. Primorskaya 529 leaves (Sapotsky et al., 2002), shows that enhancing of 1,3-β-D-glucanase activity and accumulation of SMV in the leaves of the susceptible infected soybean are closely related phenomena.

The decrease of 1,3-β-D-glucanase activity in the trifoliates of the second and third tiers, two weeks after the peak of activity is, probably, connected with reduction in SMV transport from these trifoliates.

The increase of 1,3-β-D-glucanase activity occurred at the same time as the virus distribution occurs in the leaves of the sensitive soybean cultivar. First, the protective reaction delays in time. The virus has enough time in this case to spread up into the whole plant. Second, 1,3-β-D-glucanase has a role in the protective reactions, showing adaptation to the pathogene. The high level of 1,3-β-D-glucanase activity in the resistant cultivar

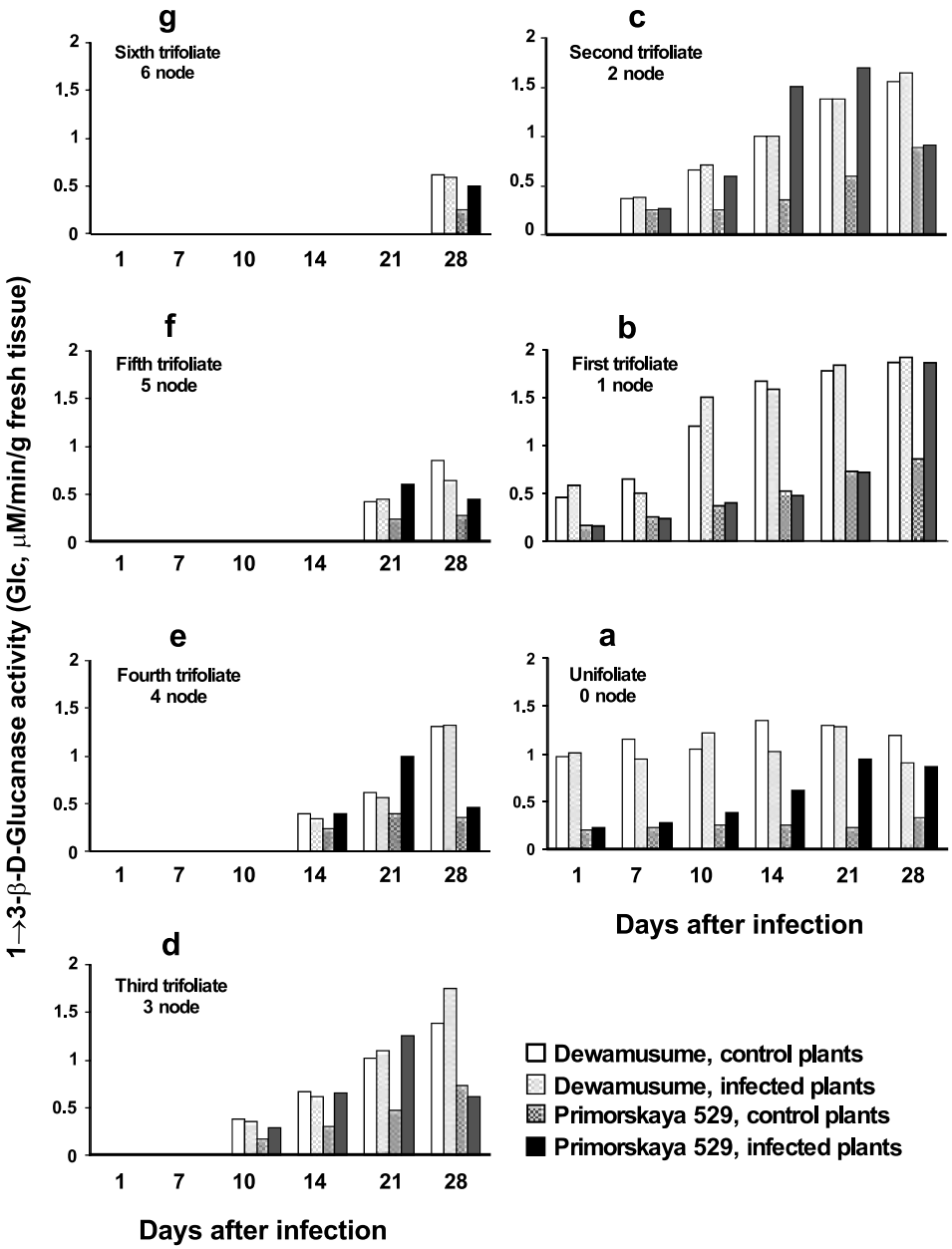

Fig. 1. Influence of SMV on the activity of 1→3-β-D-gucanase in leaves of sensitive and extremely resistant soybean plants

probably shows that these enzymes contribute to the constitutive immunity of the resistant soybean cultivar. Probably, other isoforms of 1,3-β-D-glucanase are synthesized in the resistant cultivar, as compared to the susceptible one. It has been shown (for tobacco) that different groups of this multifunctional enzyme react both to stress and pathogen infection in various ways. For instance, the acidic isoforms activated in response to the action of salicylic acid and viruses, and the basic ones possess antifungal activity and are activated after ethylene treatment and in response to damages (Beffa et al., 1996; Ji and Kuć, 1995; Lusso and Kuć, 1996).

Interestingly, there was no noticable difference between the cultivars in the level of activity of peroxidase (Andreeva, 1989), and another enzyme of the PR-protein group (Stintzi et al., 1993).

Correlation between the growth of leaves and the increase in activity of 1,3-β-D-glucanase does not depend on the cultivar. This may indicate the necessity of active photosynthesizing leaves. This corresponds to the data of other authors on the role of 1,3-β-D-glucanase in processes of plant growth and development (Hrmova et al., 1995).

In leaves of both cultivars activities of other carbohydrases (α-D-mannosidase, β-D-galactosidase, β-D-gucosidase) were on lower levels, as compared to the activity of 1,3-β-D-glucanase *(Fig. 2).* β-D-Glucosidase has the lowest level of activity among glycosidases *(Fig. 3).* Activity of β-D-galactosidase occupies the middle position among the tested glycosidases *(Fig. 4).* α-D-Mannosidase has the highest activity among glycosidase which is comparable with the activity of 1,3-β-D-glucanase *(Fig. 5).* In contrast to 1,3-β-

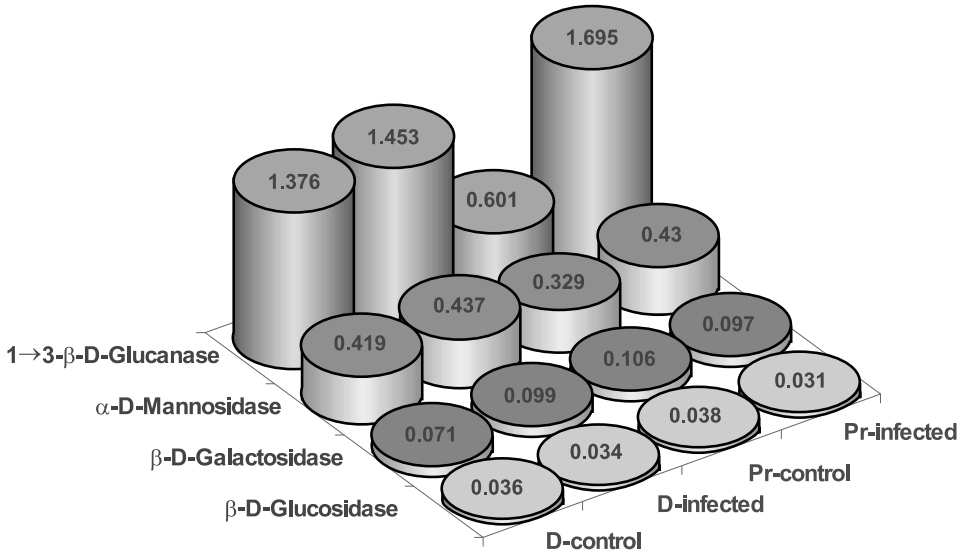

Fig. 2. Comparison of carbohydrase activities from the second trifoliate of the healthy and infected soybean cultivars
D – cv. Dewamusume
Pr. – cv. Primorskaya 529

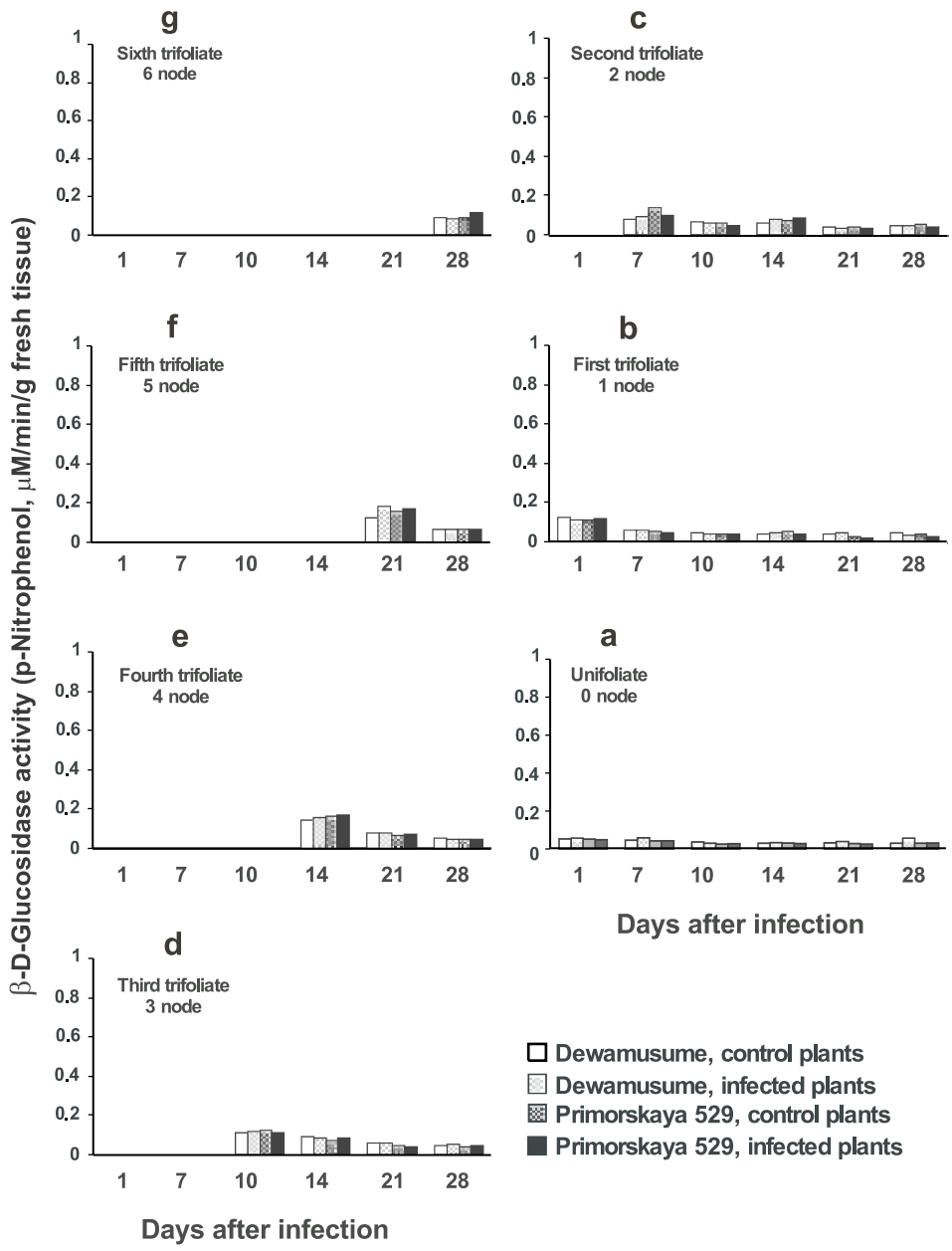

Fig. 3. Influence of SMV on the activity of β-D-gucosidases in leaves of sensitive and extremely resistant soybean plants

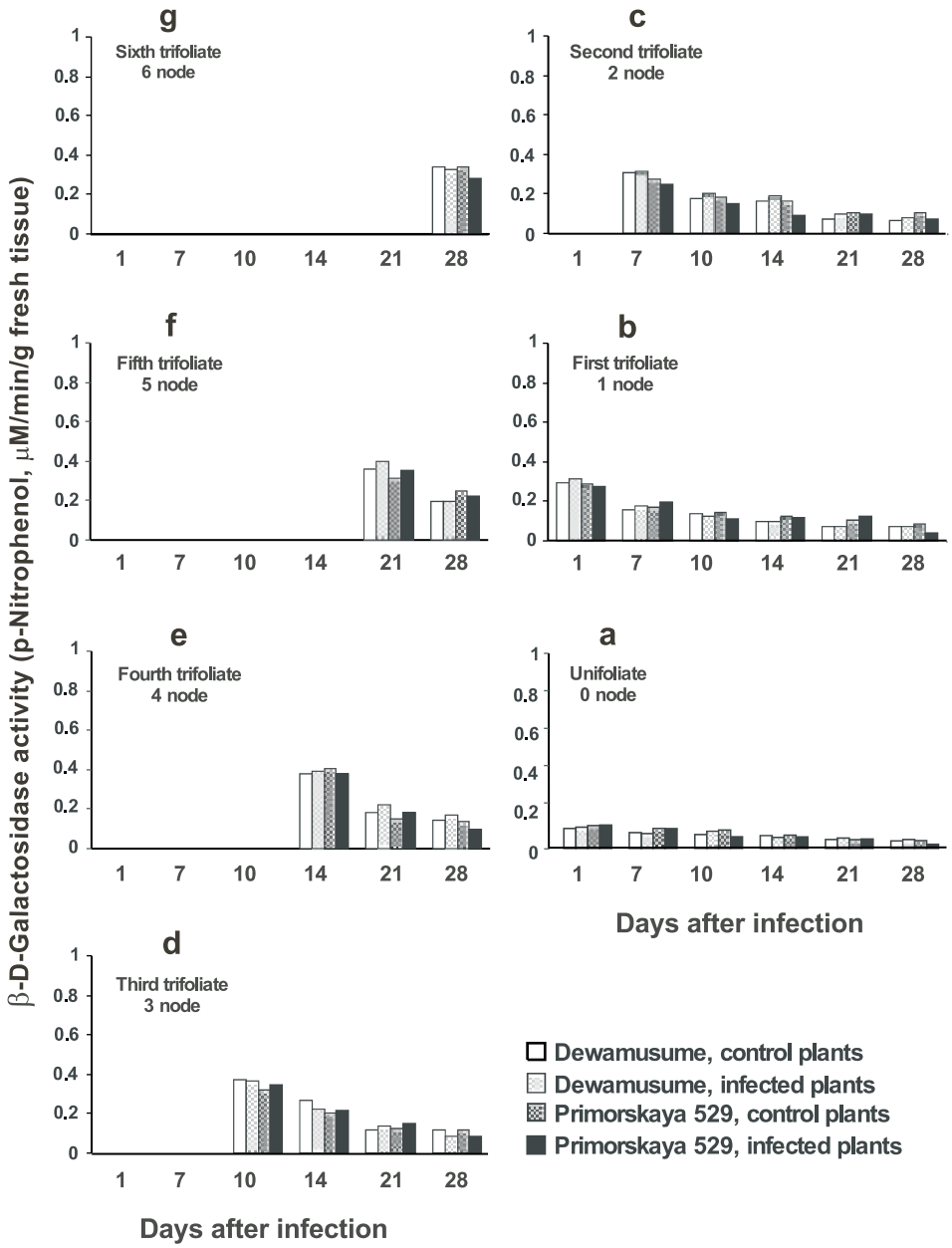

Fig. 4. Influence of SMV on the activity of β-D-galactosidases in leaves of sensitive and extremely resistant soybean plants

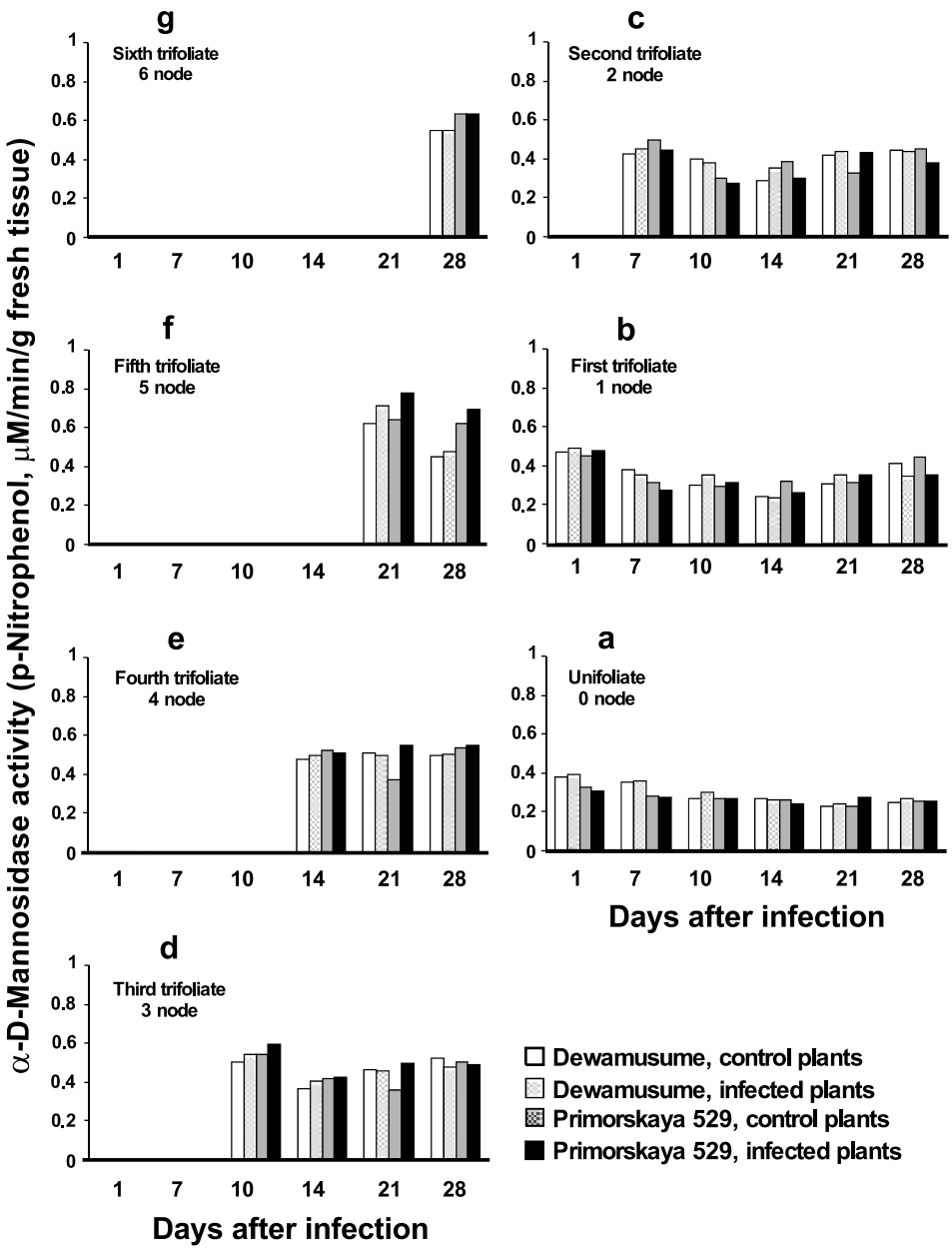

Fig. 5. Influence of SMV on the activity of α-D-mannosidases in leaves of sensitive and extremely resistant soybean plants

D-glucanase, activities of these enzymes have decreased during the development of leaves with the exception of activity α-D-mannosidase that has not been changed. Activities of these carbohydrases also have not been changed after infection by the virus in leaves of both cultivars. Activities of β-D-galactosidase and β-D-glucosidase in developed leaves have been reduced substantially. This type of changes shows that the oligosaccharide-hydrolases actively participate in the process of growth and development of the youngest soybean leaves.

The data of our investigations do not correspond to the results obtained with the bean hypocotiles (Bagatharia and Chanda, 1998). These authors deny the role of hydrolases as wall loosening enzymes. However, according to other literary data, the lytic potential (level of activity of the various hydrolytic enzymes, as RNKase, protease, acidic phosphatase) in the growing plant tissues is much higher than in the developed ones (Reunov and Laphshina, 1985; Laphshina et al., 1991).

Relations between the activity of the glycosidases and SMV infection has not been found, despite the fact that soybean mosaic virus causes strong symptoms and deformations of the leaf in the susceptible soybean cultivar.

Thus, the higher level of activities of α-D-mannosidase, β-D-galactosidase and β-D-glucosidase in the expanding youngest leaves, represent the high level of lytic processes in the actively growing youngest soybean leaves. The 1,3-β-D-glucanase has a role in the answer of soybean leaves to virus infection as well as to the growing process.

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