Volume 55(1):139-141, 2011
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Changes in salicylic acid and polyamine contents following powdery mildew infection of near-isogenic Thatcher-based wheat lines carrying different Lr genes

Magda Pál*, Viktória Kovács, Gyula Vida, Gabriella Szalai, Tibor Janda

Agricultural Research Institute, Hungarian Academy of Sciences, Martonvásár, Hungary

ABSTRACT Changes in endogenous salicylic acid and polyamine contents were investigated following infection with powdery mildew in four near-isogenic Thatcher-based wheat lines. Although the infection could not affect the quantum efficiency of photosystem 2 after 7 days in the investigated genotypes, significant changes were observed in the levels of salicylic acid and polyamines of inoculated plants. Powdery mildew infection caused decrease in salicylic acid content after 3 days, but on the 7th day of infection increased it significantly in line carrying Lr33 gene. Infection also increased the salicylic acid content after 7 days in line carrying Lr19 gene, while the other genotypes were hardly affected. Infection increased the levels of cadaverine, spermidine and spermine in the free form, as well as the levels of spermidine and spermine in the conjugated form. Infection could not affect either of the polyamines associated with various macromolecules (bound form). It was concluded that although salicylic acid and polyamines have important role in plant responses and defence mechanisms during biotic stress, there is no correlation between pathogen induced changes in the levels of them and the level of tolerance to powdery mildew of the investigated four wheat lines **Acta Biol Szeged 55(1):139-141 (2011)**

KEY WORDS

biotic stress
polyamine
powdery mildew
salicylic acid
wheat

Powdery mildew [*Blumeria graminis* (DC.) Speer f.sp. tritici Ém. Marchal] causes infections of varying intensity in wheat cultivars every year. Plants respond to pathogen attack by activating a wide range of protective mechanisms including production of reactive oxygen species, alterations in the cell wall structure, accumulation of secondary metabolites, activation and/or synthesis of defence peptides and proteins (Kotchoni and Gachomo 2006). Salicylic acid (SA), as a component of the signal transduction system, plays important role in defence mechanisms against pathogen attack (Raskin 1992; Catinot et al. 2008; Horváth et al. 2007, Loake and Grant 2007). Increases in endogenous SA level have been reported in pathogen challenged leaves of various plant species, furthermore exogenously applied SA can induce resistance against several biotic stresses (Chaturvedi and Shah 2007). Polyamines (PAs), which are small, positive charged, aliphatic amines and found in all plant cells are able to bind to negatively charged molecules, e.g. nucleic acids, acidic phospholipids and various types of proteins, thus have protective role under stress conditions (Walters 2000a). It was first shown in barley following brown rust infection, that polyamine levels are altered by pathogen infection (Greenland and Lewis 1984). Later it was demonstrated that not only the levels of polyamines increased, but powdery mildew infection also resulted in increased activities of its biosynthetic enzymes in barley plants (Walters et al. 1985).

The aim of the present work to investigate changes in endogenous SA and PA contents after infection with powdery mildew in four wheat lines, and to search correlation between the observed changes and the level of tolerance to powdery mildew.

Materials and methods

Based on our prelimilary results four Thatcher-based near isogenic lines were selected (carrying Lr33, Lr26, Lr19, Lr9 genes, respectively). Plants were grown in pots (2 plants/pot) in a 2:1 mixture of soil and sand under greenhouse conditions. Inoculation was carried out using a mixture of pathotypes with a known virulence spectrum (determined on a differentials carrying genes Pm0, 1, 2, 3a, 3b, 3c, 3d, 3f, 4a, 4b, 5, 6, 7, 8, 17, 2+6, 2+4b+8, 1+2+9, 2+Mld). Plants were inoculated at adult stage (GS45) by shaking conidia onto the leaf surface of the test plants. Throughout the experiment the temperature in the greenhouse was 16–22°C and the relative humidity of the air under the isolation boxes was above 90%. All measurements were performed 3 and 7 days after infection on the leaves of control as well as inoculated plants. The chlorophyll fluorescence from the leaves was determined under growth conditions using a pulse amplitude modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany). Salicylic acid was

Accepted July 11, 2011
*Corresponding author. E-mail: palmagda@mail.mgki.hu

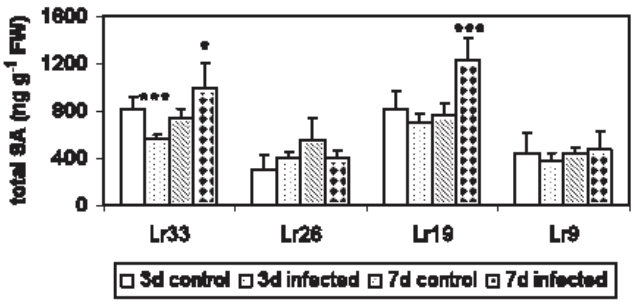

Figure 1. Changes in total salicylic acid content in four Thatcher-based wheat lines on the 3rd and 7th day following powdery mildew infection. Data presented as mean ± SD (*n*=5); * and *** denote significant differences from the control of the same day at the 0.05 and 0.001 levels, respectively.

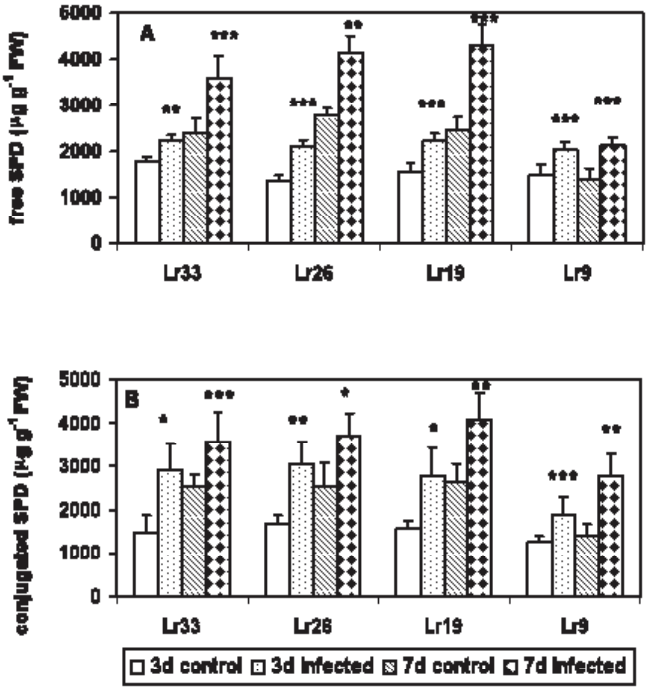

Figure 2. Changes in the levels of free (A) and conjugated spermidine (B) in four Thatcher-based wheat lines on the 3rd and 7th day following powdery mildew infection. Data presented as mean ± SD (*n*=5); * , ** and *** denote significant differences from the control of the same day at the 0.05, 0.01 and 0.001 levels, respectively.

measured according to Meuwly and Métraux (1993) and Pál et al. (2005). SA and oHCA were quantified fluorimetrically (W474 scanning fluorescence detector, Waters, USA) with excitation at 317 nm and emission at 436 nm for oHCA, followed by with excitation at 305 nm and emission at 407 nm for SA. PAs were analyzed as dansylated derivatives via HPLC using a W2690 separation module and a W474 scanning fluorescence detector (Waters, Milford, MA, USA) as described by Németh et al. (2002) and Radyukina et al. (2010). Changes in these parameters were compared to the control for the same day. Five independent repetitions were performed for each experiment. The data were statistically evaluated using the standard deviation and t-test methods.

Results

After infection small, white or gray tufts were appeared on the leaves of all investigated wheat lines. However, significant differences in disease severity and the rate of symptom development were observed between the four lines. Bases on phenotypic testing, we found that lines carrying Lr33 and Lr26 genes were rather susceptible, while lines with Lr19 and Lr9 genes were rather resistant for powdery mildew infection.

The $\Delta F/F_m'$ chlorophyll-a fluorescence induction parameter, which indicates the quantum efficiency of photosystem 2 (PS 2), was measured to detect the damaging effect of the powdery mildew infection. Still on the 7th day of the infection, we could not detected significant differences between control and inoculated plants (data not shown).

Endogenous level of total SA were higher in lines carrying Lr33 and Lr19 genes than that of lines carrying Lr26 and Lr9 genes, and SA contents were not changed significantly during aging in control plant (Fig.1). Powdery mildew infection caused significant decrease in SA level after 3 days of infection, but on the 7th day slight increase was observed in line with Lr33 gene. Infection also increased significantly the SA content after 7 days in line carrying Lr19 gene, but the other genotypes could hardly affect.

The levels of free, conjugated and bound forms of the investigated polyamines, namely putrescine (PUT), cadaverine (CAD), spermidine (SPD) and spermine (SPN) showed little differences between control plants of the four lines. The level of CAD in free, conjugated and bound forms were 2-fold higher in lines with Lr33 and Lr9 genes than in lines with Lr26 and Lr19 genes. The free SPN content of line carrying Lr19 gene was 2-fold higher, than that of the other lines. Free, conjugated and bound forms were in the same magnitude, dominant with SPN in the free and with SPD in the conjugated and bound forms. Infection caused increases in the level of CAD, SPD and SPN in the free form, SPD and SPN in the conjugated form (data not shown). The highest accumulation induced by infection was observed in the case of free and conjugated SPD after 7 days, which was pronounced in lines carrying Lr33, Lr26 and Lr19 genes (Fig.2A, B). Infection could not affect either of the polyamines in the bound form.

Discussion

In order to obtain a better understanding of tolerance to powdery mildew in wheat, investigations were made on the protective mechanisms induced before pathogen causes per-

manent damage. It has been long known that powdery mildew infection causes inhibition in photosynthesis (Magyarosy et al. 1976; Prokopová et al. 2010), though under these conditions powdery mildew infection could not affect the quantum efficiency of PS 2. The present results however, indicate that the effect of infection can be detected as early as three days after inoculation, as demonstrated by changes especially in PAs contents. These results similar to others found in barley, where the levels of free PUT and SPN and conjugated forms of PUT, SPD and SPN were increased following inoculation with the powdery mildew (Cowley and Walters 2002). Remarkable increase no could be detected in the level of total SA after powdery mildew infection under these conditions, which is according to results of other authors. Powdery mildew could not cause any SA accumulation in different barley genotypes (Hückelhoven et al. 1999). Furthermore the levels of free and SA conjugates also remained low after infection with *Erysiphe graminis* f. sp, hordei or *E. graminis* f. sp. tritici in barley while they increased after inoculation with *Pseudomonas syringae* pv. syringae (Vallelian-Bindschedler et al. 1998). These result suggested that SA accumulation at least in barley is pathogen specific. Although there are some papers about existence of correlation between contents of endogenous SA and PAs and sensitivity or resistance to various biotic stresses (Talieva and Kondrat'eva 2002; Walters 2000b), in our case it could not be detected under these conditions. Bases on these results it was concluded that although salicylic acid and poliamines have important role in plant responses and defence mechanisms during biotic stress, there is no correlation between pathogen induced changes in the levels of them and the level of tolerance to powdery mildew of the investigated four wheat lines.

Acknowledgements

The authors are gratefully indebted to Zsuzsa Kóti and Edit Kövesdi for their technical assistance. This work was supported by grants from the Hungarian National Scientific Research Foundation (OTKA PD83840), which is gratefully acknowledged. A part of this study was presented on the 10th Congress of the Hungarian Society for Plant Biology, August 31 - September 2, 2011, Szeged, Hungary.

References

Catinot J, Buchala A, Abou-Mansour E, Métraux JP (2008) Salicylic acid production in response to biotic and abiotic stress depends on isochorismate in Nicotiana benthamiana. FEBS Letters 582:473-478.

Chaturvedi R, Shah J (2007) Salicylic acid in plant disease resistance. In Salicylic acid: A plant hormone. Hayat S and Ahmad A, eds., Springer, Dordrecht, The Netherlands, pp. 335-370.

Cowley T, Walters DR (2002) Polyamine metabolism in barley reacting hypersensitively to the powdery mildew fungus Blumeria graminis f. sp. Hordei. Plant Cell Environ 25:461-468.

Greenland AJ, Lewis DH (1984) Amines in barley leaves infected with brown rust and their possible relevance to formation of `green-islands'. New Phytologist 96:283-291.

Horváth E, Szalai G, Janda T (2007) Induction of abiotic atress tolerance by salicylic acid signalling. J Plant Growth Regul 26:290-300.

Hückelhoven R, Fodor J, Preis C, Kogel KH (1999) Hypersensitive cell death and papilla formation in barley attacked by the powdery mildew fungus are associated with hydrogen peroxide but not with salicylic acid accumulation. Plant Physiol 119:1251-1260.

Kotchoni SO, Gachomo EW (2006) The reactive oxygen species network pathways: an essential prerequisite for perception of pathogen attack and the acquired disease resistance in plants. J Biosci 31:389-404.

Loake G, Grant M (2007) Salicylic acid in plant defence - the players and protagonists. Current Opinion in Plant Biology 10:466-472.

Magyarosy AC, Schurmann P, Buchanan BB (1976) Effect of powdery mildew infection on photosynthesis by leaves and chloroplasts of sugar beets. Plant Physiol 57:486-489.

Meuwly P, Métraux JP (1993) Ortho-anisic acid as internal standard for the simultaneous quantitation of salicylic acid and its putative biosynthetic precursors in cucumber leaves. Anal Biochem 214:500-505.

Németh M, Janda T, Horváth E, Páldi E, Szalai G (2002) Exogenous salicylic acid increases polyamine content but may decrease drought tolerance in maize. Plant Sci 162:569-574.

Pál M, Horváth E, Janda T, Páldi E, Szalai G (2005) Cadmium stimulates the accumulation of salicylic acid and its putative precursors in maize (Zea mays L.) plants. Physiol Plant 125:356-364.

Prokopová J, Mieslerová B, Hlaváčková V, Hlavinka J, Lebeda A, Naušl J, Špundová M (2010) Changes in photosynthesis of Lycopersicon spp. plants induced by tomato powdery mildew infection in combination with heat shock pre-treatment. Physiol Mol Plant Pathol 74:205-213.

Radyukina NL, Shashukova AV, Mapelli S, Shevyakova NI, Kuznetsov VlV (2010) Proline controls the level of polyamines in common sage plants under normal conditions and at UV-B irradiation. Russ J Plant Physl 57:422-429.

Raskin I (1992) Role of salicylic acid in plants. Ann Rev Plant Physiol and Plant Mol Biol 43:439-463.

Talieva MN, Kondrat'eva VV (2002) Influence of exogenous salicylic acid on the level of phytohormones in tissues of Phlox paniculata and Phlox setacea leaves with special reference to resistance against the powdery mildew causative agent Erysiphe cichoracearum DC. f. phlogis Jacz. Biol Bull 29:551-554.

Vallelian-Bindschedler L, Metraux JP, Schweizer P (1998) Salicylic acid accumulation in barley is pathogen specific but not required for defense-gene activation. Mol Plant Microbe In 11:702-705.

Walters DR (2000a) Polyamines and plant disease. Phytochemistry 64:97-107.

Walters DR (2000b) Polyamines in plant-microbe interactions. Physiol Mol Plant Pathol 57:137-146.

Walters DR, Wilson PWF, Shuttleton MA (1985) Relative changes in levels of polyamines and activities of biosynthetic enzymes in barley infected with the powdery mildew fungus, Erysiphe graminis DC. Ex Merat f.sp. hordei Marchal. New Phytologist 101:695-705.