PHENYLALANINE AMMONIA-LYASE IN NORMAL AND BIOTIC STRESS CONDITIONS

ACTIVITATEA FENILALANIN AMONIA-LIAZEI ÎN CONDIȚII DE NORMALE ŞI DE STRES BIOTIC

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Abstract. *Phenylalanine ammonia-lyase (PAL) is a key enzyme that converts L-phenylalanine to trans-cinnamic acid, a precursor of various metabolites produced in response to environmental stress, including biotic factors. Artiffical infection of different sunflower genotypes with Orobanche cumana Wallr. collected from the South part of Republic of Moldova showed significant modification on PAL activity. O. cumana induced a significant increase of PAL activity in the root system at all sunflower genotypes resistant and tolerant to the broomrape (race E), from 20 - 30 days until the final investigated ontogenetic phase (60 days), which confirms the role of PAL in the expression of biochemical mechanisms of host-plant resistance to broomrape attack.*

Key words: sunflower, broomrape, phenylalanine ammonia-lyase, biotic stress

Rezumat. *Fenilalanin amonia-liaza (PAL) reprezintă o enzimă cheie care transformă L-fenilalanina în acid trans-cinamic, precursor al diferitor metaboliți produşi ca răspuns la stresul de mediu, inclusiv la factorii biotici. Infectarea artificială a diferitor genotipuri de floarea-soarelui cu Orobanche cumana Wallr. colectată din partea de sud a Republicii Moldova a demonstrat inducerea unor modificări semnificative ale activității PAL. Astfel, O. cumana induce majorarea activității PAL în sistemul radicular la toate genotipurilor de floarea-soarelui rezistente şi tolerante la rasa E de lupoaie, începând cu 20 - 30 zile până la ultima fază ontogenetică cercetată (60 zile), ceea ce confirmă rolul PAL în manifestarea mecanismelor biochimice de rezistență a plantei-gazdă la atacul lupoaiei.*

Cuvinte cheie: floarea-soarelui, lupoaia, fenilalanin amonia-liaza, stres biotic

INTRODUCTION

Plants have potential to mobilize biochemical response mechanisms against pathogenic attack including lignification (Köhle H. et al., 1985), suberization (Espelie K. E. et al., 1986), synthesis of phytoalexins (Kuc J., Ruch J. S., 1985), induction of hydrolytic enzymes (Broetto F., 1995) and activation of the antioxidative response system (Broetto F. et al., 2002). The regulation of enzymes involved in the biosynthesis of metabolites produced in response to environmental stress has been studied in cell cultures of different plant species (Messner B. et al., 1991). Schell & Parker (1990) suggested that the activation to the phenypropanoid

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metabolism can be easily detected by the variation in the activity of phenylalanine ammonia-lyase (PAL, E.C.4.3.1.5.).

PAL is a key enzyme that converts L-phenylalanine to trans-cinnamic acid, a precursor of various phenylpropanoids, such as lignins, coumarins and flavonoids (Birgid Schuster and Janos Rjetey, 1995 Phenylpropanoids play key roles in plant development and in protection against environmental stresses.

The downstream products of PAL activity in plants have been reported to influence the host response to pathogen invasion (Felton G. W. et al., 1999; Beno-Moualem and Prusky D., 2000), wounding reactions (Ismail M. A. and Brown G. E. 1979; Bucciarelli B. et. al., 1998), chilling susceptibility (Sanchez-Ballesta M. T. et al., 2000) and environmental stress (Lavola A. et al., 2000; Dixon R. A. and Paiva N. L., 1995; Byoung K. L. et. al. 2003). Numerous reports have demonstrated a positive correlation between increased enzyme activity, PAL protein accumulation and PAL gene expression (Kostenyuka I. A., Zonb J and Burnsa J. K., 2002).

The products of the phenylpropanoid pathway are important building blocks in several biosynthetic pathways (Bennet R. N. and Wallsgrove R. M., 1994). From cinnamic acid flavonoids and anthocyanins, lignin precursors (ferulic and pyrocatechol acid), coumarins, phytoalexins, and salicylic acid (SA) are derived. Salicylic acid is one of the precursors of catechols, tannins, saligenin, methyl salicylate and hydroxybenzoic acids (Bennet R. N. and Wallsgrove R. M., 1994). SA mediates the defence mechanisms of plants towards pathogens. The expression of pathogenesis related proteins and the establishment of systemic acquired resistance are results of SA (Loake G and Grant M., 2007). Plants respond similarly to aphid infestation by inducing SA and pathogenesis related proteins (Mohase L. and Van Der Westhuizen A. J., 2002; Berner J. M. and A.J. Van Der Westhuizen, 2010).

Since broomrape is a root parasite, his first contact with the host plant is the root system, so the purpose of this work included the PAL activity determination in roots of different genotypes of sunflower (*Helianthus annuus* L.) artificially infected with broomrape (*Orobanche cumana* Wallr .).

MATERIAL AND METHOD

Plant materials and growth conditions. The plant materials used for the study were 17 genotypes. Broomrape seeds were collected from the South of Moldova. The PAL activity was studied under artifficial infestation conditions. Sunflower plants were grown in a sand/compost mixture (1:1, V/v) in 10,0 kg plastic pots. The mixture of each pot was mixed with 50 mg of broomrape seeds per pot.

PAL assay. PAL activity was determined spectrophotometrically by following the absorption of the reaction product, cinnamate (Adamovskaia V. G. et al., 2007). Standard mixture for the PAL assay consisted of 100 μl enzymatic extract (0.1 M tampon borate, pH 8,8) and 400 μl 12 mM L-phenylalanine. The mixture was incubated at 37°C (16 hours) and absorbance measured at 290 nm. The absorbance of the reaction mixture contained L-Phe as a substrate. Control reaction mixture contained distillated water instead a substrate. The enzyme activity was expressed in optical density units to 1 mg of protein. The total protein concentration in soluble enzyme extracts was determined by the Bradford protocol (Bradford M. M., 1976).

RESULTS AND DISCUSSIONS

Like other higher plants, in sunflower were identified physiological and biochemical defense mechanisms that block broomrape development at different ontogenetic stages (Echevarría-Zomeño S. et al., 2006; Labrousse P. et al., 2001; Serghini K. et al., 2001). Broomrape shows a different parasitic activity, what manifested on the intensity of the disease, causing varying degrees disorders, sometimes reaching up to the total destruction of organs or the whole plant.

It is well known that an important role in providing of plants resistance to pathogens attack has different ontogenetic biochemical mechanisms (Dickinson M, 2003; Goldwasser Y. et al., 1999; Labrousse P. et al., 2001), and phenylalanine ammonia-lyase has the primary role in triggering the metabolic pathways of synthesis of key metabolites involved in providing host plant resistance to the action of biotic factors. Moreover, it was demonstrated that the increase in PAL activity is an index of resistance (Shadle, G. L. et al., 2003).

Spectrophotometric determination of PAL activity was performed on 17 sunflower genotypes, previously ranked (Duca M. et al., 2009) in three groups (sensitive, tolerant and resistant) depending on the response to the attack of phanerogam *Orobanche cumana*, collected in the south part of the Republic Moldova. Comparative analysis of PAL activity in these three groups of sunflower revealed significant differences dependent on the response to the broomrape attack. Thus, the four tolerant genotypes, showed the significant increase in enzymatic activity in all four periods (20, 30, 50 and 60 days post infection) of host plant development in the presence of phytopathogenic (fig. 1).

Sunflower resistant genotypes to the broomrape, race E showed the significant increase in PAL activity from 30 days ontogenetic phase. During the first 20 days, three resistant genotypes have been increased enzymatic activity, while the other three, showed a decrease of it, probably because of lack of contact between pathogen seeds and host plant. The same regularity was demonstrated at sensitive genotypes (fig. 1). Comparative analysis of phenylalanine ammonia-lyase activity at different ontogenetic stages of the host plant allowed us to determine the increase of enzyme activity referred to the period of 30 days, at which over 94% of the examined genotypes (except ASC1 line) had increased values, regardless of group (resistant, tolerant, sensitive). Probably, this ontogenetic phase is decisive in the manifestation of biochemical mechanisms of resistance where is involved key enzyme PAL. At later stages (50 and 60 days), already there is a strict dependency relation to groups of sunflower. Thus, both genotypes resistant and tolerant have maintained the increased enzyme activity, whereas susceptible genotypes, conversely, most have been lower values of PAL activity compared with control. This regularity may be explained by the fact that broomrape seeds that have the ability to germinate at any ontogenetic stage of host plant, determine the synthesis of PAL in an amount greater than the entire period, the host roots are in immediate proximity to phitoparasite seeds. In susceptible genotypes, which has already triggered the infection is not maintained the laws established.

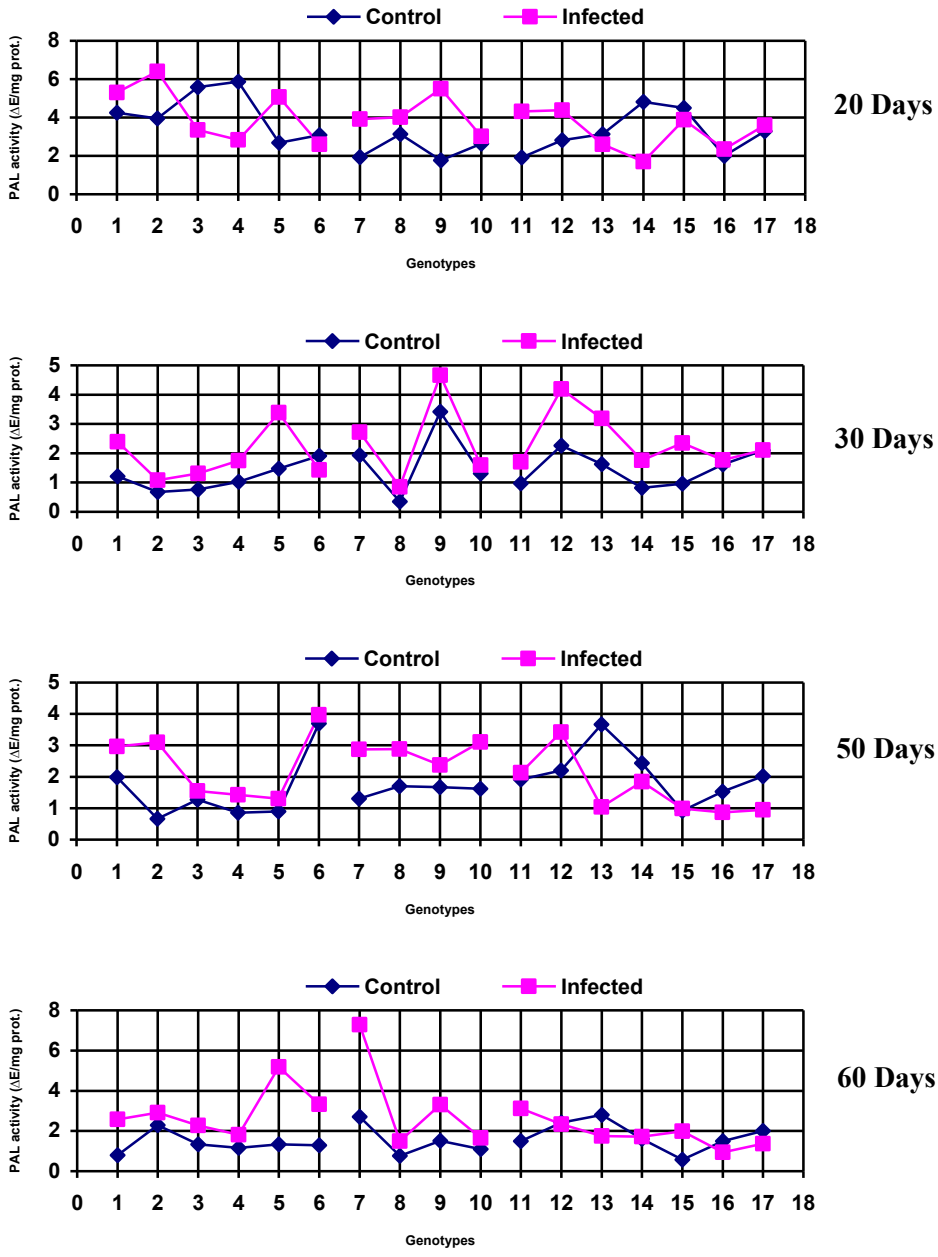

Fig. 1 – Phenialalanin ammonia-lyase activity in roots at different sunflower genotypes
1, 2, 3, 4, 5, 6 – resistant genotypes (FS9, FS12, FS17, FS21, FS26, ASC1 respectively);
7, 8, 9, 10 – tolerant genotypes (FS4, FS8, FS11, ASC2 respectively);
11, 12, 13, 14, 15, 16, 17 – sensitive genotypes (FS19, ASC10, Valentino ♀, Valentino ♂, Valentino F_1, Olea F_1, Performer F_1 respectively).

CONCLUSIONS

1. Artificial applying of *Orobanche cumana* Wallr. induces a significant increase of PAL activity in the root system at all sunflower genotypes resistant and tolerant to broomrape (race E), from 20 – 30 days to the final ontogenetic investigated phase (60 days), which confirms the role of PAL in the expression of biochemical resistance mechanisms of host plant to broomrape attack.

2. Ontogenetic development phase of the sunflower, of which more than 94% of genotypes have been significant increases in PAL activity, proved to be the 30 days probably be decisive in triggering mechanisms of resistance to *Orobanche cumana*.

3. Genotypes resistant to the broomrpe, race E maintain the increased PAL activity, which demonstrates the continuing manifestation of resistance mechanisms, whereas broomrape has the ability to germinate throughout the period of ontogenetic development of the host.

4. After 30 days, susceptible genotypes showed no increase in PAL activity, probably due to already trigger the infection process.

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