Biochemical Systematics and Ecology 65 (2016) 33–39

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Participation of the enzymes involved in the biosynthesis of biogenic amines in biochemical interactions between wheat (*Triticum aestivum*; Poaceae) and bird cherry-oat aphid (*Rhopalosiphum padi*; Aphididae)

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ARTICLE INFO

Article history:
Received 3 September 2015
Received in revised form 12 January 2016
Accepted 31 January 2016
Available online xxx

Keywords:
Triticum aestivum
Rhopalosiphum padi
Amino acid decarboxylation
Polyamines
Tyramine

ABSTRACT

The studies concerned changes in the activities of ornithine decarboxylase (ODC), lysine decarboxylase (LDC) and tyrosine decarboxylase (TyDC) in tissues of wheat (*Triticum aestivum* L.) infested with bird cherry-oat aphid (*Rhopalosiphum padi* L.).
Obtained results showed that the activities of the enzymes were stimulated in the less susceptible wheat Kontesa cv. infested by the aphids. In the case of the more susceptible Tonacja cv., on most occasions a decrease in the enzyme activities occurred. Such responses were especially clear for TyDC in both analysed cvs., and for LDC and ODC in the case of Kontesa cv. Thus it may be concluded that amino acid decarboxylation plays an important part in the biochemical defence developed in wheat tissues in response to *R. padi* infestation. The changes in the activities of the decarboxylases were dependent on the wheat genotype as well as the duration of the infestation.

1. Introduction

Biogenic amines are known biomolecules involved in plant responses to abiotic and biotic stresses (Feriduddin et al., 2013; Jiménez-Bremont et al., 2014). However, the participation of plant amines and their amide derivatives obtained by conjugation with hydroxycinnamic acids (HCAAs) in insect–plant interactions has so far been poorly described. Strong participation of polyamines (PAs) in molecular plant defence mechanisms may result from scavenging and/or generating free radicals, regulation of gene expression and formation of toxic products (Del Duca et al., 2014). In addition, these compounds may form ionic and/or covalent bonds with nucleic acids, proteins and phospholipids. As a result, PAs influence enzyme activities, the integrity of chromatin and biomembranes, and the functioning of receptors and transcriptional factors in signalling pathways. HCAA derivatives were involved in responses of different plant species against *Liriomyza trifolii* (Burgess), *Manduca sexta* (L.) and *Spodoptera littoralis* (Boisduval) (Tebayashi et al., 2007; Bassard et al., 2010). These

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http://dx.doi.org/10.1016/j.bse.2016.01.005
0305-1978/

compounds block synaptic transmission through binding to quisqualate-type glutamate receptors on exoskeletal muscles of arthropods (Klose et al., 2002; Fixon-Owoo et al., 2003). Our earlier studies showed that free exogenous PAs may also disturbed feeding, survival and settling behaviour of cereal aphids (Sempruch et al., 2010a, 2011). However, endogenous spermidine regulated the prokaryotic intracellular symbiotic system of the pea aphid (*Acyrthosiphon pisum* Harris) (Nakabachi and Ishikawa, 2001).

Many plant amines are synthesised from amino acids with the participation of amino acid decarboxylases. For example, ornithine decarboxylase (ODC; EC 4.1.1.17) is involved in ornithine decarboxylation, which is the first step in putrescine biosynthesis in plant and animal cells. Cadaverine is synthesised from lysine in Fabaceae, Poaceae and Solanaceae plants with the participation of lysine decarboxylase (LDC; EC 4.1.1.18) (Bagni and Tassoni, 2001; Fariduddin et al., 2013). Tyrosine decarboxylase (TyDC; EC 4.1.1.25) is needed for the biosynthesis of tyramine, which may be transformed into some classes of defencive plant compounds and hormones in the insect's nervous system (Lee et al., 2009; Varlinden et al., 2010).

Changes in activities of amino acid decarboxylases within tissues of host plants infested by aphids have been reported earlier. Important part of these responses stated induction of LDC activity in aphid-infested more resistant cvs. of triticale and maize, while susceptible plants were not demonstrated such changes (Sempruch et al., 2010b, 2013a; 2015). Increase of ODC activity was observed in *Pisum sativum* L. after *A. pisum* attack and in susceptible triticale cvs. infested by *Ropalosiphum padi* and *Sitobion avenae* (F.) (Sempruch et al., 2008, 2013b). TyDC was stimulated in triticale infested by *S. avenae*, in pea infested by *A. pisum* and in orchid after *Pseudococcus longispinus* (Targ. Tozz.) and *P. maritimus* (Ehrh.) attack (Sempruch et al., 2009, 2013b; 2014). Finally, a systemic effect of these changes was suggested, since they were not only restricted to aerial parts directly damaged by the aphids but also occurred in root tissues.

The presented data clearly proved that biogenic amines and key enzymes in their biosynthesis are involved in responses of plants towards aphid infestations. However, the majority of data to this phenomenon comes from studies of biochemical mechanisms of the response of the triticale to cereal aphids attack, and it participation in the biochemical defence of such important cereal species as the wheat was not studied. Thus the aim of the present paper is to report on changes in the activities of LDC, TyDC and ODC induced by *R. padi* infestation in tissues of wheat varied in susceptibility to the aphid infestation.

2. Material and methods

2.1. Plants

Wheat (*Triticum aestivum* L.) cvs. Kontesa and Tonacja were used in the experiments. These cvs. were earlier selected on account of the different susceptibility to *R. padi* with use of entomological tests conducted in field and laboratory conditions (unpublished data). Seeds of both wheat cvs. were descended from the Plant Breeding and Acclimatization Institute in Strzelce (Poland) and germinated in a climatic chamber at 24 °C during the daytime and 18 °C at night, 70.0% RH and a photoperiod of 16L:8D. Seedlings were grown in medium nutrient fine-structure compost with sand, in 8.0 × 9.5 cm plastic pots, and watered regularly.

2.2. Aphids

Parthenogenetic multiclonal generations of *R. padi* were reared on seedlings of the susceptible cultivar Tonacja in a climatic chamber at 24 °C during the day and 18 °C at night, 70.0% RH and a photoperiod of 16L:8D.

2.3. Influence of bird cherry-oat aphid infestation on enzyme activities within wheat tissues

Thirty of the 7-day-old seedlings of the wheat cultivars growing in three pots (ten seedlings per pot) were artificially infested with five adult wingless females of *R. padi* (8–12 − days-old), and control plants (without aphids) were prepared similarly. Each experiment was established in three independent replications for each higher described variant and studied cultivar. Infested and control seedlings were collected after one day (24 h), one week and two weeks from the beginning of the experiment. On each occasion the number of aphids was counted on ten seedlings (one pot), and next the insects were brushed out and the seedlings were harvested in three independent replications for each cultivar. Uninfested seedlings (control) were harvested in the same way. Aphid density on infested plants was calculated as the average number of individuals per seedling.

2.4. Enzyme assays

ODC was extracted by homogenization of freshly collected aerial parts of wheat seedlings with 0.2 mol phosphate buffer (pH 8.2) containing β-mercaptoethanol and ethylenediaminetetraacetic acid (EDTA). Tris–HCl buffer (0.2 mol; pH 5.6) and acetate buffer (0.5 mol; pH 5.6) were used for extraction of LDC and TyDC, respectively. The obtained extracts were filtered through two layers of cheesecloth and centrifuged at 18 000 × *g* at 5 °C.

The procedure described by Ngo et al. (1987) was used for ODC assays. Substrate solution (ornithine in phosphate buffer, pH 8.2, with added pirydoxal-5-phosphate − PLP) was added to the enzyme extract. The enzymatic reaction was developed at