# POLYAMINES AS A POTENTIAL CHEMOTAXONS OF RESISTANT AND SUSCEPTIBILE BIOTYPES OF *CHENOPODIUM ALBUM* TO ATRAZINE

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Abstract. The level of free (PAs) and conjugated (CPAs) soluble polyamines in leaves of *Chenopodium album* was determined using the fluorometric method for dansylated derivatives. The tests made at the 4-6 leaf growth stage showed that the concentration of PAs in susceptible (S) and resistant (R) biotypes ranged between 32-43  $\mu$ g and 45-56  $\mu$ g per 100  $\mu$ l of cell sap, respectively. The analyses performed later, namely at the beginning of flowering showed that the difference in the level of polyamines between S and R biotypes was greater. In this case the amount of PAs in biotype S ranged between 15-34  $\mu$ g and in biotype R between 51-73  $\mu$ g per 100  $\mu$ l of cell sap. Therefore, the level of PAs cans act as an indicator for susceptibility or resistance of *C. album* biotypes to atrazine.

Key words: Chenopodium album, atrazine-resistance, polyamines

### I. INTRODUCTION

Atrazine is commonly used in Poland for many years to weed control in maize. This herbicide kills broad spectrum of weed species including common lambsquarter (*Chenopo-dium album*), which is a serious problem in our country. However, in recent years we found in some regions the population resistant to atrazine. This resistance have been confirmed in biological tests made under glasshouse conditions.

During our studies on the effect of atrazine on *C. album* metabolism we put our attention particularly to the polyamines. These compounds can affect many processes in plant physiology, among others, changes in cell membrane fluidity, senescence and mitotic activity (Slocum et al. 1984). Unexpectedly we found that the level of polyamines in cell sap of the susceptible biotype of. *C. album* distinctly differed from that of the resistant one. Hence, it occurred to us that the level of polyamines in plants could be used as susceptibility / resistance indicator.

#### II. MATERIALS AND METHODS

We tested *C. album* plants of selected and homogeneous biotypes susceptible (S) and resistant (R) to atrazine, growing in greenhouse conditions  $(22/18 \pm °C \text{ day/night respectively})$ . Polyamines were detected in cell sap of the leaves sampled at the 4-6 leaf growth stage and then at the beginning of flowering. We expected a large specimen variability. Therefore ten samples each contained leaves from ten plants of both biotypes and growth stage were used.

For detection of PAs and CPAs, the method described by Smith and Davies (1987) was modified as shown on Figure 1. Soluble polyamines conjugated to small molecules (CPAs) were determined in cell sap after its hydrolysis with HCl (Torrigiani at al. 1987). Hydrolysates contained initial PAs plus those liberated from various types of conjugated polyamines (CPAs). They were dansylated and determined as described in the scheme presented in Fig.1.

Dansyl-PAs were quantified by their relative fluorescence intensities (excitation at 365 nm, emission at 510 nm). using a fluorescence spectrophotometer (Perkin Elmer, model 650-10LC) and corrected using putrescin as an internal standard. Data are presented as an average of 3 replicates of each sample.

Cell sap + 0.2 N HClO<sub>4</sub> (1:1) I Centrifugation (20 min, 18,000g, 0° C). Supernatant contains PA and CPA I To 100 µl supernatant add 200 µl sat. Na<sub>2</sub>CO<sub>3</sub> + 400 µl 1% dansyl-Cl in aceton I Sonification 3h – add 100 µl 10% proline – sonification 3 min I Extraction of dans – PA with 500 µl of benzene I Fluorimetric quantification and TLC of dans-PA

Fig. 1. Scheme of analytical procedure for the detection of polyamines



Fig. 2. Soluble free polyamines (PAs) level in C. album

biotypes

III. RESULTS AND DISCUSSION

Our results show that the level of free polyamines (PAs) provide a good method for a chemotaxonomy of C. album biotypes (Fig. 2). Another approach could be the ratio of the sum of PAs and CPAs to PAs (Fig. 4). Here we can observe a large deviations in CPAs levels (Fig. 3). Probably, fluoromertric quantification of dansylated polyamines did not give such reliable results. We realize that the most sensitive method for detection of polyamines is their dansylation followed by spectrofotometry. Dansyl derivatives are highly fluorescent and detectable in small amounts but dansyl chloride used for derivatization in non specific due to its high reactivity with



Fig. 3. Solouble conjugated poliamines (CPAs) level in *C. album* biotypes

Fig. 4. The Ratio of the sum of (PAs + CPAs) to PAs in cell sap from *C. album* biotypes

amino groups of many compounds like some phenols and alcohols (Seiler and Wiechmann 1970).

The results of our investigation show that the level of polyamines in cell sap of the susceptible biotype of. *C. album* distinctly differed from that of the resistant one. Therefore, the level of free polyamines and the ratio of the sum of free and conjugated polyamines to free polyamines could be a potential chemotaxons for susceptible and resistant biotype of *C. album*.

#### **IV. LITERATURE**

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# POLIAMINY JAKO POTENCJALNE CHEMOTAKSONY DLA ODPORNYCH I WRAŻLIWYCH NA ATRAZYNĘ BIOTYPÓW KOMOSY BIAŁEJ (CHENOPODIUM ALBUM L.)

#### STRESZCZENIE

Poziom wolnych (PAs) i związanych (CPAs) rozpuszczalnych poliamin w liściach komosy białej oznaczono metodą fluorymetryczną.

Testy przeprowadzone w stadium 4-6 liści komosy wykazały u biotypu wrażliwego 32-43  $\mu$ g PAs w 100  $\mu$ l soku komorkowego, podczas gdy u biotypu odpornego 45-56  $\mu$ g. W stadium początku kwitnienia różnice w poziomie poliamin między biotypami były większe, to znaczy u biotypu wrażliwego znaleziono 15-34  $\mu$ g PAs, a u odpornego 51-73  $\mu$ g.

Na podstawie przeprowadzonych testow można wnioskować, iż poziom poliamin rozpuszczalnych (PAs) może być dobrym wskaźnikiem w ocenie wrażliwości lub odporności komosy białej na atrazynę.