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BRIEF COMMUNICATION

Isolation and partial characterization of aluminium-induced cytoplasmic polypeptides from barley root

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Abstract

Using preparative isoelectric focusing, fast performance liquid chromatography, and polyacrylamide gel electrophoresis accumulation of several Al-induced cytoplasmic proteins was described in barley roots. Two of them, 27 and 28 kDa polypeptides were isolated by continuous-elution electrophoresis system in sufficient quantities for their further characterisation.

Additional key words: IEF, *Hordeum vulgare*, FPLC, SDS-PAGE.

In acid soil aluminium is the major factor limiting crop productivity (von Uexküll and Mutert 1995). Over the past decade considerable research has been directed to elucidate the biochemical and molecular basis of aluminium toxicity and resistance (or tolerance) in plants (see reviews Delhaize and Ryan 1995, Kochian 1995, Matsumoto 2000). The first evidence that proteins play crucial role in stress response to Al came from experiment, where low doses of aluminium in the nutrient solution induced resistance in wheat seedlings to lethal aluminium concentration (Aniol 1984). When the preincubation of wheat seedlings with sublethal doses of Al was performed in the presence of protein synthesis inhibitor, resistance to Al has not occurred. The results of Aniol (1984) as well as Basu *et al.* (1994) who identified two 51 kDa microsomal proteins in roots of Al-resistant wheat cultivars after Al exposure, demonstrate that some protein may play an important role in Al tolerance.

The aim of this paper was to determine Al-induced root cytoplasmic proteins and their isolation and partial characterisation in Al-resistant barley cultivar Bavaria.

Barley (*Hordeum vulgare* L. cv. Bavaria) caryopses after surface sterilization with 12 % H_2O_2 were germinated in dark at temperature of 25 °C and relative humidity of 98 %. Three-day-old barley seedlings were transferred into 3 dm^3 glass jars (60 seedlings per jar) containing 1 mM $CaCl_2$, pH 4.5, and cultivated in a growth chamber (*E 8 Conviron*, Winnipeg, Canada) at 20/16 °C (light/dark) temperature and irradiance of 70 μmol m^{-2} s^{-1} (400 - 700 nm) for 16-h photoperiod. After 4 d of cultivation plants were transferred into a fresh 1 mM $CaCl_2$ solution with pH 4.5 (control) and Al stress was triggered by application of 100 μM $AlCl_3 . 6 H_2O$ into 1 mM $CaCl_2$ solution, pH 4.5 (Al-treated). After 4-d treatment, whole roots (primary and adventitious seminal roots) were harvested, and immediately frozen in liquid nitrogen. Samples (200 g, with 3 repetitions) were ground to a fine powder in a cold mortar in liquid nitrogen and the resulting powder was re-homogenized in extraction buffer (100 mM Tris buffer, pH 8.0, 0.3 M sucrose, 1 mM EGTA, 2.5 mM potassium metabisulfite, 2 mM SHAM, 1 mM DTT; 3 % PVP) with homogenizator (*DIAX 900 Heidolph GmbH*, Keilheim, Germany). After filtration the homogenate was centrifuged, first at 1 500 *g* for 5 min, then the supernatant was re-centrifuged at 12 000 *g* for 15 min, then again at 150 000 *g* for 30 min

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Abbreviations: BSA - bovine serum albumine; DTT - dithiothreitol; EGTA - ethyleneglycol-bis-(β-amino-ethyl ether) N,N′-tetraacetic acid; FPLC - fast performance liquid chromatography; PVP - polyvinylpyrrolidone; SHAM - salicylhydroxamic acid.

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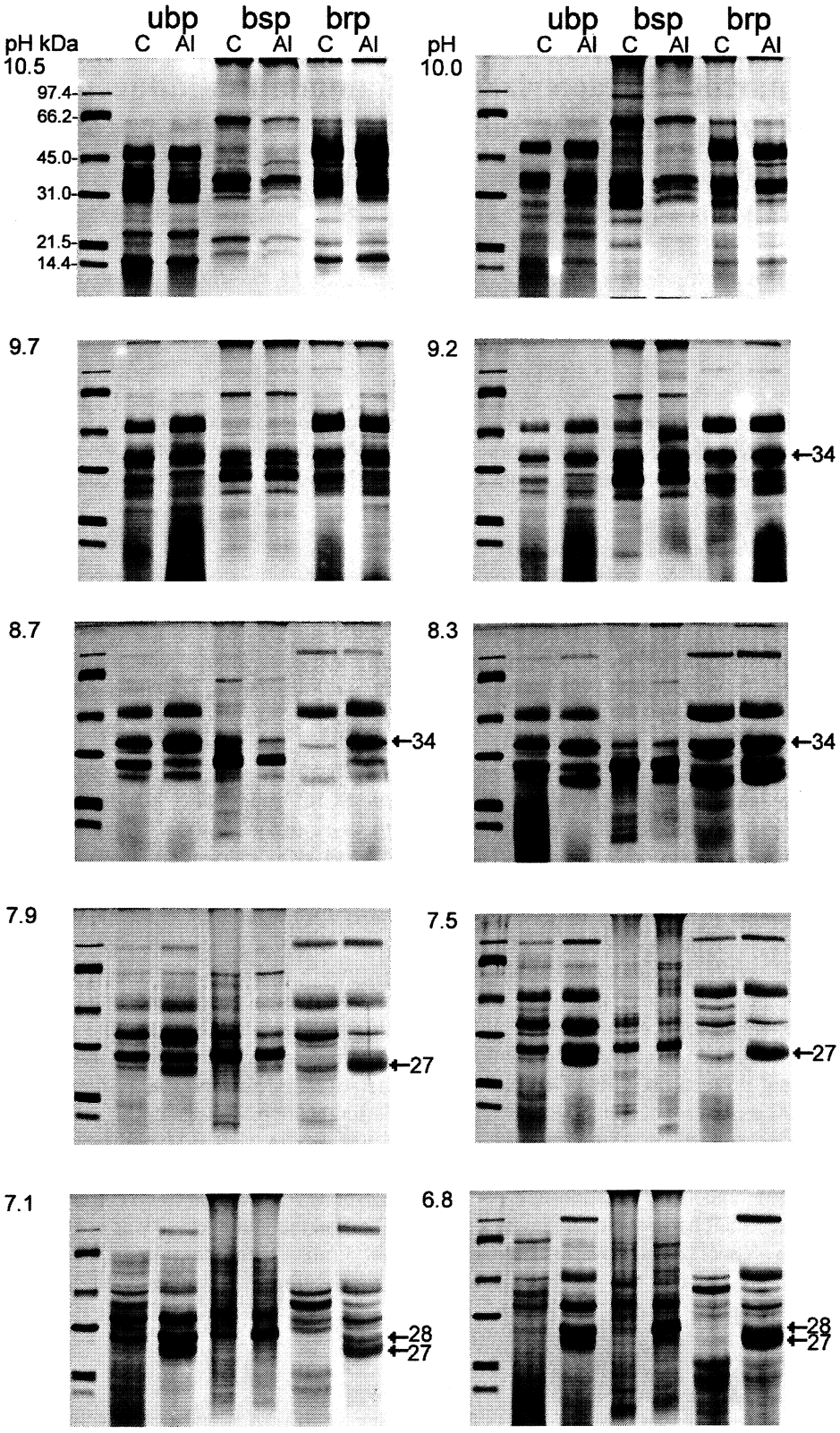

Fig. 1. SDS-PAGE analysis of twenty protein fractions (pH from 10.5 to 6.8) fractionated on preparative IEF system. C - control plants, Al - aluminium treated plants, ubp - unboiled proteins, bsp - boiling sensitive proteins, brp - boiling resistant proteins. *Arrows* indicate the Al-induced polypeptides with appropriate molecular mass.

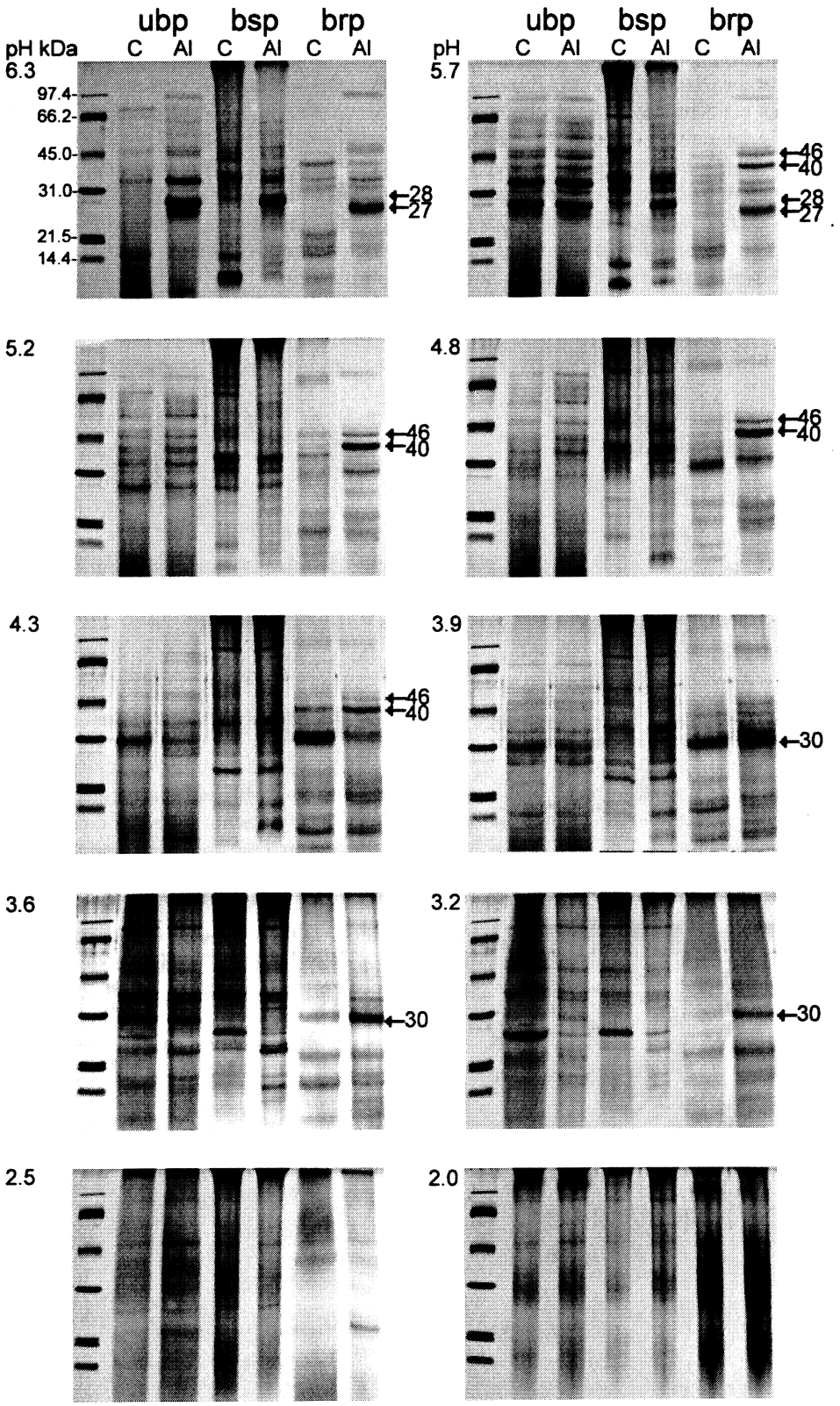

Fig. 2. SDS-PAGE analysis of twenty protein fractions (pH from 6.3 to 2.0) fractionated on preparative IEF system. C - control plants, Al - aluminium treated plants, ubp - unboiled proteins, bsp - boiling sensitive proteins, brp - boiling resistant proteins. *Arrows* indicate the Al-induced polypeptides with appropriate molecular mass.

(*L8-M, Beckman Instruments,* Palo Alto, USA). The resulting supernatant represented the cytoplasmic fraction. After purification (*Sephadex G-25, Pharmacia,* Uppsala, Sweden) and concentration, the proteins were fractionated using preparative isoelectric focusing (*Rotofor System, Bio Rad Lab.*, Hercules, USA) with ampholytes pH range 3/10 and anion exchange column (*Bio-Scale Q2, Bio Rad Lab.*) equilibrated with 25 mM Tris buffer, pH 8.0. The adsorbed proteins were eluted with a step 0 - 1.0 M NaCl gradient in the same buffer. Proteins from each fractions were divided into two subfractions (boiling resistant and sensitive proteins) by 10 min boiling and centrifugation at 12 000 *g* for 10 min. After centrifugation boiling resistant proteins were precipitated overnight at -20 °C with ice cold acetone. Proteins were solubilized and separated under denaturing conditions on 12 - 18 % gradient polyacryl-amide slab gels using the discontinuous buffer (Laemmli 1970), and silver stained. The apparent molecular masses of polypeptides were calculated based on the mobilities of protein standards obtained from *Bio Rad* with gel documentation system (*UVP 5000*, England). For final purification we have used continuous-elution electrophoresis system (*Mini Prep Cell, Bio Rad Lab.* Hercules, USA).

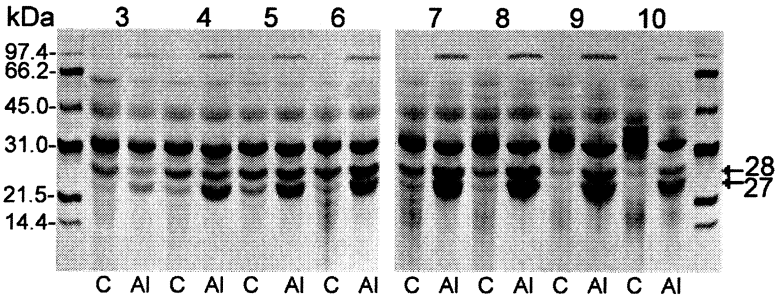

Fig. 3. SDS-PAGE analysis of fractions (3 - 10) containing 27 and 28 kDa polypeptides after refractionation on preparative IEF system. C - control plants, Al - aluminium treated plants.

The cytoplasmic proteins isolated from barley roots were fractionated using preparative isoelectric focusing into twenty fractions from pH 10.5 to 2.0. SDS-PAGE of these twenty fractions are demonstrated together with two subfractions containing the boiling sensitive and boiling resistant proteins of the original samples (Figs. 1, 2). *Arrows* indicate the Al-induced polypeptides with appropriate molecular mass in individual fractions (typically 3 - 5 fractions) such as 34 kDa boiling resistant polypeptide in fractions with pH 9.2, 8.7 and 8.3; 27 kDa boiling resistant polypeptide in fractions with pH from 7.9 to 5.7; 28 kDa boiling sensitive polypeptide in fractions with pH from 7.1 to 5.7; 46 and 40 kDa boiling

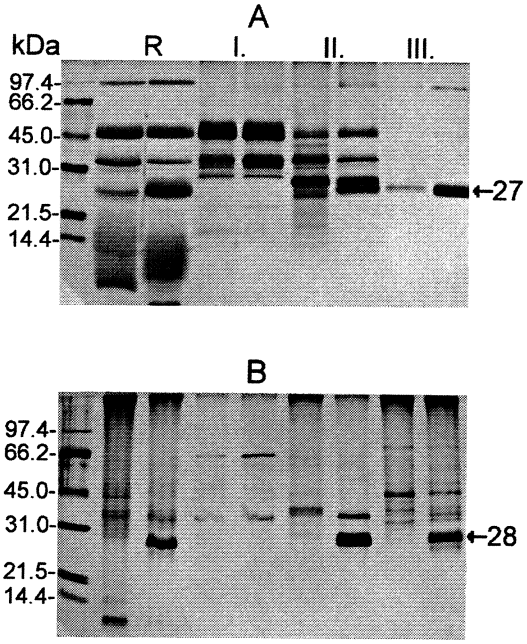

Fig. 4. SDS-PAGE analysis of pooled fractions (R) from rotofor containing 27 and 28 kDa polypeptide and fractions (I, II, III) after separating on anion exchange column. *A* - boiling resistant, and *B* - boiling sensitive fraction.

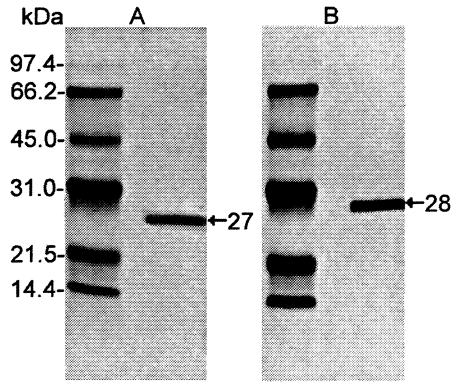

Fig. 5. SDS-PAGE analysis of 27 kDa (*A*) and 28 kDa (*B*) polypeptide isolated by continuous-elution electrophoresis system.

resistant polypeptides in fractions with pH from 5.7 to 4.3, and 30 kDa boiling resistant polypeptide in fractions with pH 5.2, 4.8 and 4.3. From these results it is obvious that except of 28 kDa polypeptide all Al-induced polypeptides were boiling resistant. The greatest change during Al stress were connected to the accumulation of 27 and 28 kDa polypeptide, therefore we continued their isolation by refocusing of fractions with pH 7.9 to 5.7. After these steps the proteins of our interest were situated in fractions 4 - 10 (Fig. 3). These pooled fractions after buffer exchange were separated on anion exchange column, and 27 and 28 kDa polypeptides were separated one from other by boiling (Fig. 4). 27 kDa boiling resistant polypeptide was eluted in fraction III (Fig. 4*A*) while 28 kDa boiling sensitive polypeptide in fraction II (Fig. 4*B*). Finally we have isolated 27 and 28 kDa polypeptides from Al-stressed barley roots in sufficient quantities for further characterisation by using continuous-elution electrophoresis system (Fig. 5).

Plants respond to stresses through activation of numerous genes. Basu *et al.* (1994) observed Al-induced exudation of several polypeptides by roots of Al-resistant wheat cultivars. Ownby and Hruschka (1991) noted four cytoplasmic proteins (18.5, 27, 33 and 55 kDa) which were Al-inducible in winter wheat. In our previous work (Tamás *et al.* 1999) besides several Al-induced cell wall and membrane polypeptides in barley roots we found only one Al-induced cytoplasmic polypeptide (28 kDa). In this study, due to the application of more precise and sensitive methods, we have described accumulation of several cytoplasmic polypeptides during Al stress. The most abundant Al-induced polypeptides were two polypeptides with molecular mass of 27 and 28 kDa, which in our previous work (Tamás *et al.* 1999) represented one large 28 kDa spot. The further characterisation of these two polypeptides by protein N-terminal sequencing is just in progress.

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