Proteins in somatic hybrids of potatoes *Solanum tuberosum* and *S. commersonii* after ABA treatment

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Abscisic acid (ABA) is know to participate in the response to abiotic stress in plants. The electrophoretic analysis of the leaf soluble proteins in asymmetric somatic hybrids of *Solanum tuberosum* and frost resistance *S. commersonii* allowed to detect the synthesis of a new fraction of protein. This fraction was induced after ABA treatment in hybrids as well as in *S. commersonii*. Analysis and comparison of ABA response and frost-induced proteins in hybrids and both parents should allow understanding the regulatory mechanism of frost resistance.

Key words: *Solanum tuberosum*, ABA (abscisic acid), frost resistance, stress

INTRODUCTION

Plants have developed a variety of defense mechanisms that enable them to survive severe environmental stress. Rapid activation of stress-inducible genes and *de novo* synthesis of a large number of “stress proteins” have been proposed to be at least part of plant defense mechanism [1].

The ability of plants to withstand frost is acquired by cold acclimation. During acclimation the freezing tolerance of plants is increased by involving many biochemical and physiological changes [2]. The involvement of some phytohormones, especially of abscisic acid (ABA) is related to cold acclimation. ABA is generally considered as a stress hormone.

The role of ABA is not uniform in freezing tolerance. The endogenous content of ABA increases during cold acclimation of wheat, barley, wild potato *Solanum commersonii.* The freezing tolerance of plants in some cases is increased by ABA treatment *in vivo* and in cell culture as well as by acclimation. It confirms that ABA participates in the mechanism of freezing tolerance [3]. However, there are reports that ABA not always increases freezing tolerance of intact plants [4]. Currently more than 150 ABA responsible genes are detected [5, 6]. Several reports have been described genes that are induced by cold acclimation, but are not responsive to exogenous ABA treatments. These findings suggest the existence of both ABA-independent and ABA-dependent signal transduction cascades between the initial signal of cold stress and expression of specific genes (COR-cold responsive). Perhaps ABA plays a significant role of temperature signal transduction in cold acclimation [7, 8].

The aim of the present study was to determine the involvement of acclimation and ABA in the induction of soluble proteins in somatic hybrids in frost-sensitive cultivated potato *Solanum tuberosum* and frost-resistant species of *S. commersonii*, allowing a better understanding of the regulatory mechanism of frost resistance.

MATERIALS AND METHODS

The wild species of potato *Solanum commersonii* Dun., cultivars of *Solanum tuberosum* L. *Matilda* and 8 somatic hybrids of *S. tuberosum* and *S. commersonii* were grown *in vitro* in MS medium at 20–25 °C and 14-h photoperiod. The control plants were grown at 20–25 °C [9]. The acclimation test was provided in two ways: 1) acclimation at low temperature was provided in chamber at +5 °C during 12 days, 2) with exogenous ABA application in MS medium (25 μM). The harvested leaves were examined in leaf extracts [10, 11].

Soluble proteins were separated by discontinuous 10% PAG without SDS, two-dimensional separation (SDS-PAG). The gels were stained with 0.1% Coomassie G-250 and distained with 7% acetic acid, 5% ethanol. The relative electrophoretic protein movement (Rf) was measured. Molecular mass standards were obtained from Sigma.

RESULTS AND DISCUSSION

The goal of this research was to compare how the spectrum of proteins changes in frost-resistant hybrid-478, frost-sensitive hybrids 480, 482, 485 and parental forms of *S. tuberosum* cv. 'Venta' and *S. commersonii* during acclimation and ABA treatment. The electrophoretical spectra of native proteins of all hybrids were identical for recipient partner *S. tuberosum* and donor *S. commersonii* when plants were grown at +25 °C (Fig. 1). Differences in proteins were detected after acclimation of plants at +4 °C or ABA treatment. The new fraction of protein with Rf 0.13 was induced in all hybrids and recipient partner *S. tuberosum* after ABA treatment but not after acclimation. It allows to predict that this fraction of protein or proteins is not directly related with frost resistance and is inherited from the frost-sensitive cv. 'Venta'. The proteins with Rf 0.45 were characteristic of the donor partner *S. commersonii* after acclimation and ABA treatment. This protein fraction was inherited in the frost-resistante hybrid-478 and induced only by ABA but not by acclimation. At present it is not clear why this protein in the frost-resistant hybrid-478 is not induced during acclimation as in the case of *S. commersonii*. However, it is possible to predict that induction of protein Rf 0.45 may be regulated in two different ways in *S. commersonii* and only in one way in hybrid-487 and likely is not directly related to frost resistance. It seems strange that no new proteins were induced during acclimation, at least in the frost-resistance hybrid. The electrophoretic pattern of native proteins extracted from all hybrids grown at +25 °C and at +4 °C were identical and resembled the pattern of the recipient partner cv. 'Venta'. It is known that in crosses of *S. commersonii* with the related frost-sensitive species *S. cardiophylum* frost resistance in the progeny is inherited as a non-polygenic trait. However, resistance to frost killing is accompanied by a complex of anatomic and biochemical changes that determine resistance [12]. It looks likely that in the species of the genus *Solanum* there exists a complex of genes that can be involved in frost-resistance, but in frost-sensitive species they are silent. It is very likely that the species of *S. commersonii* possess switching genes inducing genes determining frost resistance. It can be indirectly confirmed by the fact that the hybrids of *S. tuberosum* cv. 'Venta' and *S. commersonii* were highly asymmetric and some of them were selected as frost-resistant. These hybrids possess all chromosomes of the recipient partner *S. tuberosum* and no or few (1–2) chromosomes from the donor *S. commersonii*.

All the above facts imply induction of new proteins in frost-resistant hybrids. However, to detect them a different approach is necessary. For that purpose, two-dimensional electrophoresis (2DE) of proteins with SDS was started. 2DE separation of hybrid-485 proteins showed new polypeptides with the molecular mass in zones 50, 54, 56 kDa (Fig. 2.2).

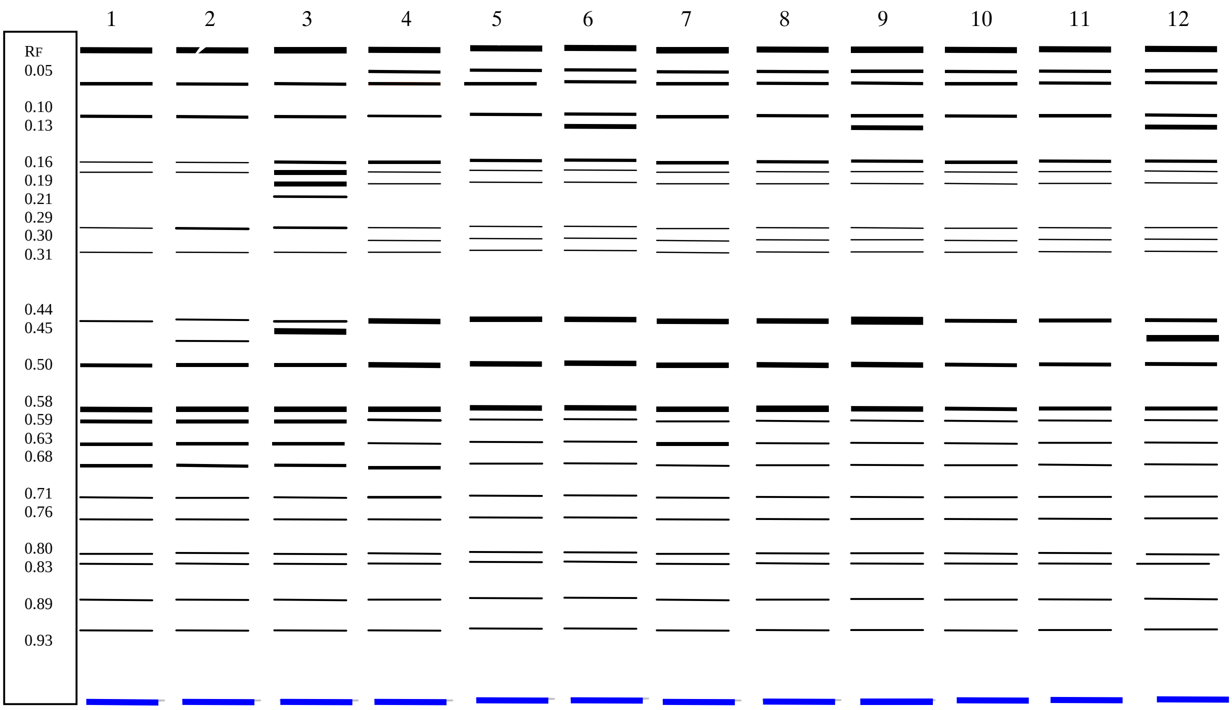

Fig. 1. The soluble proteins spectra of leaves of somatic hybrids of *S. tuberosum* cv. 'Venta' and *S. commersonii* after acclimation and after ABA treatment: 1, 2, 3 – *S. commersonii*; 4, 5, 6 – *S. tuberosum* cv. 'Venta'; 7, 8, 9 – frost sensitive hybrid-485; 10, 11, 12 – frost resistant hybrid-487; 1, 4, 7, 10 – proteins from plants grown at +25 °C (control); 2, 5, 8, 11 – proteins from plants after acclimation at +4 °C; 3, 6, 9, 12 – proteins from plants after ABA treatment (25 μM)

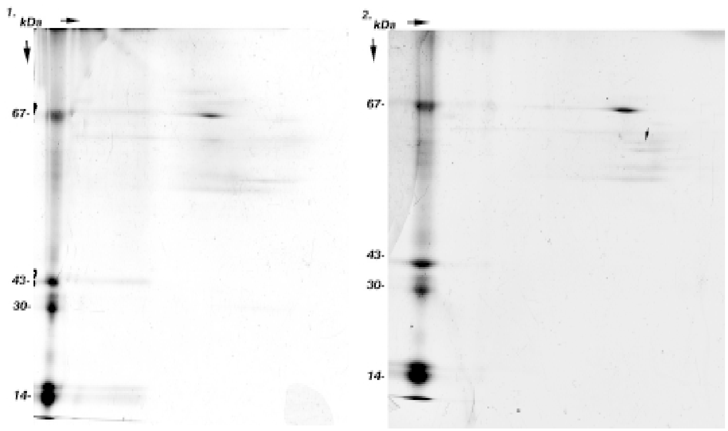

Fig. 2. Two-dimensional separations of hybrid-485 proteins fractionated by 10% PAG. Tick arrow – zone of three new polypeptides of molecular mass 50, 54, 56 kDa. 1 – Hybrid-485 (control), 2 – hybrid-485 after ABA treatment. (Markers: 67, 43, 30, 14 kDa)

Primary results allowed detecting greater differences between protein content in the frost resistante hybrid-478 after ABA treatment Analysis of denaturized proteins in hybrids and parental forms during 2DE is in progress. It should allow detecting proteins related to frost resistance.

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R. Vyšniauskienė, J. Proscevičius, Z. Jančys

BULVIŲ *SOLANUM TUBEROSUM* IR *S. COMMERSONII* SOMATINIŲ HIBRIDŲ BALTYMAI PAVEIKUS ABSCIZO RŪGŠTIMI

S a n t r a u k a

Žinoma, kad abscizo rūgštis dalyvauja augalų atsakuose į abiotinius stresus. Asimetrinių somatinių *Solanum tuberosum* ir šalnoms atsparių *S. commersonii* bulvių hibridų tirpių baltymų elektroforezine analize nustatytos naujos baltymų frakcijos. Hibriduose ir *S. commersonii* baltymai indukuojami ABA. Abscizo rūgšties ir šalnų indukuojamų baltymų analizė bei palyginimas leistų suprasti atsparumo šalnoms reguliavimo mechanizmą.