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Some evidence regarding chloroplast proteins of frost resistant hybrids of potato

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Abstract

Frost resistance is an important trait in plant breeding. The cultivated potato *Solanum tuberosum* L. is reputed to be a frost-sensitive crop. Frost resistance was incorporated into cultivated potato by protoplast fusion from a wild species *S. commersonii* Dun. The soluble proteins in leaves of asymmetric somatic hybrids of S*olanum tuberosum* and frost resistant *S. commersonii* were analysed. Differences of total soluble protein and chloroplast contents in frost resistant hybrids of potato were detected. These proteins were responsive to low temperature and may be play a role in frost resistance as representatives of the family of cold regulated proteins.

Key words: chloroplast, frost resistance, *Solanum commersonii* Dun., *Solanum tuberosum* L., soluble proteins.

Introduction

Plant resistance to environmental factors is an important property for consideration during the creation of new cultivars. It is possible to introduce valuable adaptive features, such as resistance to drought, temperature variations, ultraviolet radiation from wild plant species into cultivated species (Vayda 1994; Bujauskas 2001). The acquired tolerance to stress factors equips plants with a better degree of adaptation to varying environmental factors. Somatic hybridization *in vitro* is one of the ways to restore part of the genetic information in cultivated plants lost during selection, during cultivation of wild plants.

Solanum tuberosum plants grown in Lithuania are frost sensitive, as are the majority of potato cultivars. Cultivars of *S. tuberosum* can withstand a temperature of -3 °C, but the wild species *S. commersonii* and *S. acaule* in South America can survive at -4 °C, even to -11 °C after acclimation (Vayda 1994). Cold acclimation is associated with numerous biochemical changes including accumulation of special cryoprotective solutes, changes of pigment composition and the level of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco), as well as synthesis of cold-regulated (COR) proteins (Zhang et al. 2002; Spetea et al. 2004). The COR-15 protein induced in *Arabidopsis thaliana* participates in the mechanism of freezing tolerance (Steponkus et al.1998). The Rubisco protein and cold induced proteins are coded by the nucleus and chloroplast genomes. Both these proteins are related with chloroplasts and cold resistance (Zhang et al.2002). The protein COR-15 is encoded by the nucleus genome, synthesized in the cytoplasm and later localized in chloroplasts during cold acclimation (Steponkus et al. 1998). The subunits of Rubisco protein, coded by the nucleus and chloroplast genomes, can be used as specific markers

for determination of the origin of genetic information in hybrids, mutants and transgenic plants.

Frost resistant hybrids of potato have been created by incorporating the resistance into cultivated potato from wild species *S. commersonii*. The hybrids were created applying protoplast fusion by Dr. J. Proscevičius et al. (1998) of a wild potato species *S. commersoni*i (donor) and cultivated species *S. tuberosum* 'Matilda', 'Venta' (recipient). The irradiated *S. commersonii* (donor) genome is fragmented and asymmetric hybrids contain the complete genome of the recipient and a part of the donor genome. Phenotypes of frost resistant hybrids do not differ from the cultivar phenotype and they have acquired valuable characteristic from the donor i.e., frost resistance.

Our previous studies detected that species *S. commersonii* and *S. tuberosum* differed in four chloroplasts proteins, which were discriminated after fractionation by electrophoresis in denatured condition (Vyšniauskienė et al. 2003).

The goal of this study was to investigate the component spectrum of total protein and soluble chloroplast proteins of frost resistant hybrids, parental plants and to determine whether these changes in frost resistant hybrids were inherited from the wild species *S. commersonii*.

Materials and methods

Plants of wild species of potato *Solanum commersonii* Dun., cultivars of *S. tuberosum* L. 'Matilda' and frost resistant hybrids: H269, H515, H545, H188, H323, H487 were grown *in vitro* at 20 - 25 °C and under a 16-h photoperiod. The chloroplasts from leaves (1 g) were homogenized with 2 ml of cold buffer (0.35 M sucrose, 50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM $MgCl_2$, 5 mM DDT) with mortar and pestle at 4 °C (Ishida et al. 2002). Following extraction, the samples were centrifuged at 1000 g to remove insoluble debris. The supernatants were centrifuged at 4000 g and the debris was homogenized with buffer without sucrose. The soluble proteins from chloroplasts were separated on 10 % polyacrilamide gels (Laemmli 1970).

Results

Applying the Rubisco methods of protein extraction, the protein content of two frost resistant potato hybrids (H188 and H323) and parental forms – *S. tuberosum* (recipient) and *S. commersonii* (donor) – was analyzed by PAGE. It was found that the protein content of parental protein forms differ among themselves (Fig. 1A; 1, 2), but the profiles of the hybrids were the same as of the recipient *S. tuberosum*, but with changes in the amounts of some proteins (Fig. 1A; 2, 3, 4). SDS-PAGE showed that protein spectra of denatured proteins from the leaf extracts of hybrid H188 were discriminated from those of hybrid H323 and *S. tuberosum* 'Matilda'. The hybrid H188 had new band at 20 kDa. The protein spectra of *S. commersonii* differed from that of cultivated *S. tuberosum* 'Matilda' (Fig. 1B). It is difficult to detect whether the 20 kDa protein was inherited from *S. commersonii* as this protein was not found in the *S. commersonii* spectrum.

Employing PAGE, we further examined the component spectrum of the soluble chloroplast proteins of the frost resistant hybrids: H269, H515, H545, H188, H323. It was revealed that the wild species *S. commersonii* had four specific protein fractions: 1, 2, 3, 4

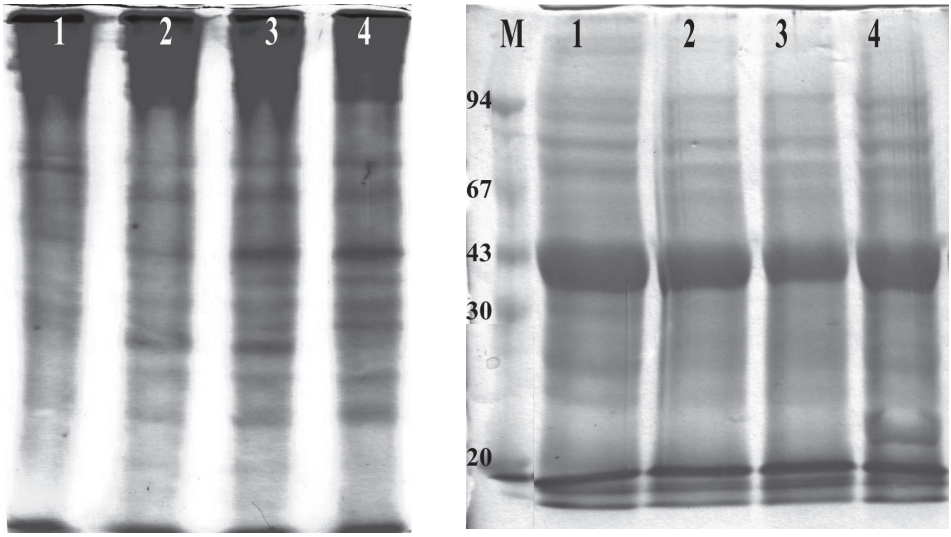

Fig. 1. The protein spectrum of frost resistant hybrids in 10 %-PAG. A, without 0.1 % SDS; B, with 0.1 % SDS. 1, *S. commersonii*, 2, *S. tuberosum* cultivar 'Matilda'; 3, frost resistant H323; 4, frost resistant H188.

not found in cultivated frost sensitive *S. tuberosum* 'Matilda'. Electrophoretical mobilities (Rf) of the fractions were 0.17, 0.20, 0.46, 0.62, respectively (Fig 2). The chloroplast protein spectrum of the hybrids H269, H188, H323 contained three proteins: 1, 2, 3, with Rf 0.17, 0.20, 0.46, also found in the *S. commersonii* spectrum. The hybrid H269 had one more protein (band 4) from the chloroplast protein spectrum of *S. commersonii*. Consequently, H269 had four additional protein fractions inherited from the wild species.

Thus, all frost resistant hybrids of the investigated potato inherited the chloroplast protein spectrum of *S. tuberosum* cultivars, but the hybrids H269, H188, and H323 together with the *S. tuberosum* protein spectrum also inherited some specific proteins from the parental wild *S. commersonii* species. The other frost resistant hybrids H515 and H545 had an identical chloroplast protein spectrum to *S. tuberosum* cultivar 'Matilda' and lacked a single protein from the wild species.

Discussion

The 20 kDa protein recorded in the hybrid H188 was not found in the protein spectra of *S. commersonii*, *S. tuberosum*, or hybrid H323. Supposedly, it is one of the Rubisco protein subunits coded by the nucleus genome (Zhang et al. 2002). Presently, the cold induced genes and Rubisco proteins of transgenic plants are being intensively studied in order to determine the gene expression in transgenic plants i.e., large subunit 55 kDa, coded by the genome of chloroplasts, and small subunit 14 kDa, coded by the nucleus genome. Each species is characterised by specificity of relative molecular mass of Rubisco subunits: *Coffea arabica* – 15 kDa, *Pisum sativum* L. – 14 kDa (Zhang et al. 2002). Both these subunits in the stroma of chloroplasts form an active functional enzymatic complex.

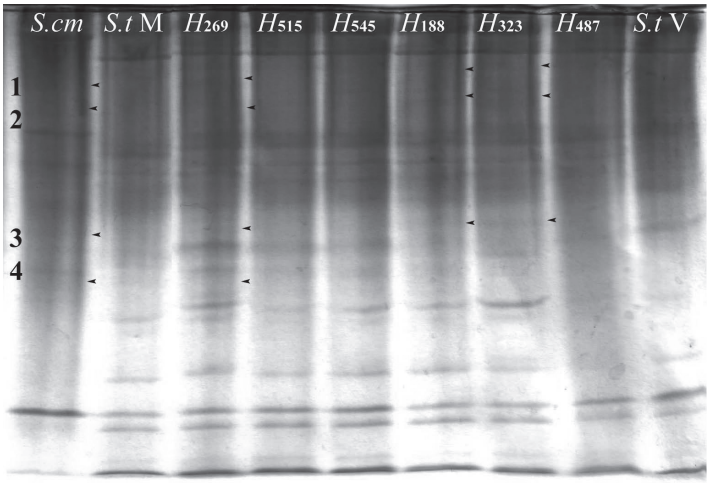

Fig. 2. The chloroplast protein spectrum in potatoe cultivars *S. tuberosum* and *S. commersonii* Dun. and their frost resistant hybrids. S.cm, *S. commersonii*; S.t.M, *S. tuberosum* cv. 'Matilda'; S.tV S. tuberosum cv. 'Venta'. Frost resistant hybrids: H269, H515, H545, H188, H323, H487.

Further investigations will be aimed at identification of this 20 kDa protein detected in the hybrid H188.

Determination of donor-specific *S. commersonii* proteins in the chloroplast protein spectrum of the hybrids H188 and H323 show that the hybrids inherited specific chloroplast proteins from the wild species. However, in the chloroplast protein spectrum of the hybrids H515 and H545, not a single specific protein characteristic of the wild donor was found, while their frost resistance had been confirmed. Therefore, specific proteins of the wild donor cannot be fully related with their participation in the processes of frost resistance. Still, the results of earlier investigations revealed that only hybrids H188 and H323 inherited two systems of the resistance to freezing from the wild species *S. commersonii*: permanent and inducible, while the other hybrids inherited only one of them (Proscevičius et al. 1999). Perhaps the inheritance of two systems predetermines a higher degree of resistance. However, the difference in chloroplast proteins between cultivars of *S. tuberosum* 'Matilda', 'Venta' and species *S. commersonii* Dun. can be used as a sampling method of hybrids after protoplast fusion. Resistance is a polygenic feature determined by the expression of many genes and the interaction of resistance systems (Vayda 1994). Further experiments should be performed to identify other defense mechanisms participating in frost resistance. These proteins are responsive to low temperature, and, may play a role in frost resistance as representatives of the family of COR (cold regulated) proteins. The analysis and comparison of new proteins by acclimation with low temperature should allow to understand better the frost resistance mechanism.

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