Final Scientific Report

Cover Page

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Project Title: Molecular genetic and anatomical characterization of potato tuber skin appearance

Investigators

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Collaborating Investigators: -

Institutions

ARO, the Volcani Center

Cornell University

Keywords *not* appearing in the title and in order of importance. Avoid abbreviations. Russeting, periderm, marker assist selection, heat stress, *Solanum tuberosum*, suberin

Abbreviations commonly used in the report, in alphabetical order:

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Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Final Scientific Report

Publication Summary (numbers)

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Submitted, in review, in preparation	2			2
Invited review papers				
Book chapters				
Books				
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Ph.D. theses				
Abstracts		2	3	5
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

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Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings		1		1
Longer Visits (Sabbaticals)				

Description Cooperation:

During the second year of the project the PIs met in Cornell University for exchange of data and gave oral presentations of their work in the PAA/Solanaceae 2006 meeting (Madison Wisconsin, USA, July, 23-27).

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Abstract

Potato (*Solanum tuberosum* L.) skin is composed of suberized phellem cells, the outer component of the tuber periderm. The focus of the proposed research was to apply genomic approaches to identify genes that control tuber skin appearance - smooth and shiny skin is highly preferred by the customers while russeted/netted skin potatoes are rejected. The breeding program (at Cornell University) seeks to develop smooth-skin varieties but has encountered frequent difficulties as inheritance of russetting involves complementary action by independently segregating genes, where a dominant allele at each locus is required for any degree of skin russetting. On the other hand, smooth-skin varieties frequently develop unsightly russetting in response to stress conditions, mainly high soil temperatures. Breeding programs in Israel aimed towards the improvement of heat tolerant varieties include skin quality as one of the desired characteristics. At the initiation of the present project it was unclear whether heat induced russetting and genetically inherited russetting share the same genes and biosynthesis pathways. Nevertheless, it has been suggested that russetting might result from increased periderm thickness, from strong cohesion between peridermal cells that prevents the outer layers from sloughing off, or from altered suberization processes in the skin. Hence, the original objectives were to conduct anatomical study of russet skin development, to isolate skin and russetting specific genes, to map the loci that determine the russet trait, and to compare with map locations the candidate russet specific genes, as well as to identify marker alleles that associated with russet loci.

Anatomical studies suggested that russet may evolve from cracking at the outer layers of the skin, probably when skin development doesn't meet the tuber expansion rate. Two-dimensional gel electrophoresis and transcript profiling (cDNA chip, *potato functional genomic project*) indicated that in comparison to the parenchyma tissue, the skin is enriched with proteins/genes that are involved in the plant's responses to biotic and abiotic stresses and further expand the concept of the skin as a protective tissue containing an array of plant-defense components.

The proteomes of skin from heat stressed tubers and native skin didn't differ significantly, while transcript profiling indicated heat-related increase in three major functional groups: transcription factors, stress response and protein degradation. Exceptional was ACC synthase isogene with 4.6 fold increased level in the heat stressed skin.

Russetting was mapped to two loci: *rusB* on chromosome 4 and *rusC* on chromosome 11; both required for russetting. No evidence was found for a third locus *rusA* that was previously proposed to be required for russetting. In an effort to find a link between the russetting character and the heat-induced russetting an attempt was made to map five genes that were found in the microarray experiment to be highly induced in the skin under heat stress in the segregating russet population. Only one gene was polymorphic; however it was localized to chromosome 2, so cannot correspond to *rusB* or *rusC*. Evaluation of AFLP markers tightly linked to *rusB* and *rusC* showed that these specific alleles are not associated with russetting in unrelated germplasm, and thus are not useful for MAS *per se*.

To develop markers useful in applied breeding, it will be necessary to screen alleles of additional tightly linked loci, as well as to identify additional russet (heat-induced and/or native) related genes.

Achievements

Significance of main scientific achievements

1. Characteristics of the skin/periderm as a protective tissue

Potato skin is composed of suberized phellem cells, the outer component of the tuber periderm. The periderm tissue consists of two additional cell types: a single-cell meristematic layer, the phellogen (cork cambium) that produces the phellem cells and is localized underneath them; and a parenchyma-like phelloderm that is derived from inward cell divisions of the phellogen. Periderm formation is a fundamental feature of the plant's response to disruption of its natural outer protective membranes (epidermis displacement or following injury). The protective characteristics of the periderm are thought to derive from the deposition of suberin on the walls of its skin cells, preventing microorganism invasion (mechanically and chemically) and desiccation of the plant tissue. The present work used two-dimensional gel electrophoresis (2-DE) and microarray techniques to identify genes that distinguish the skin from the tuber phelloderm and flesh parenchymatic tissues and the leaves. The skin tissue was found to be characterized by high expression of genes and increased level of proteins that are involved in plant responses to biotic and abiotic stresses. This result, together with the suggested role of suberin in resistance and our previous report on the phelloderm as the major producing tissue of tuber toxic steroidal glycoalkaloid (Krits et al., 2007, *Planta* 227:143-150), present the periderm as a "battery" of defense components, and further expand the concept of its protective role.

2. Modified gene expression in heat stressed skin

Protein and transcript profiling of heat-stressed skin detected relatively few significant changes (only 11 clones with expression differences > 1.5 fold) in the skin. It is possible that heat responsive elements are already up regulated in the native skin that, due to its protective function, is armed in advance against biotic and abiotic stresses. Negative results were obtained when five of them were tested for linkage to russet loci, which suggest that either there is no resemblance between heat-induced and inherited russetting or that the tested sequences are not involved in russet determination. Moreover, most of the sequences that exhibited altered expression in the heat-stressed skin were involved in regulatory functions, i.e., regulation of transcription, protein degradation and signaling. Thus, in an attempt to understand the phenotypic changes in the skin following exposure to high temperatures we

suggest focusing on analysis of transcription factors that may orchestrate the observed changes.

3. Mapping russet loci

In the present work we were able to show that genetically inherited russetting is determined by two russet loci instead of three that were suggested before. The loci were mapped on chromosome 4, *rusB*, and chromosome 11, *rusC*; both were required for russetting. No evidence was found of a third locus. This result implies that removing russetting alleles from germplasm should be easier than previously assumed. Unfortunately, AFLP markers that are tightly linked to *rusB* and *rusC* did not associated with russetting in unrelated germplasm, and thus are not useful for MAS *per se*. To develop markers useful in applied breeding, it will be necessary to screen alleles of additional tightly linked loci, to identify those (if any) that prove diagnostic in a wider range of potato germplasm.

Details of cooperation

During the first year of the project both PIs established the biological systems to fulfill the research objectives. The russeted and smooth isogenic lines of the Bake King variety were sent from Cornell to Israel for anatomical analyses. During the second year of the project the PIs met in Cornell University for exchange of data and gave oral presentations of their work in the PAA/Solanaceae 2006 meeting (Madison Wisconsin, USA, July, 23-27). Following analyses of microarray results that compared normal and heat-induced russeted skin samples (Ginzberg, I; third year of the project), sequences of candidate genes were tested for their linkage to the russeted loci (de Jong, W).

List of reviewed publications

Barel G, Ginzberg I (2008) Potato skin proteome is enriched with plant defence components.

J Exp Bot, Online Advance Access, published July 24, 2008.

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Brief summarization of unpublished data

1. Characteristics of the skin as a protective tissue

To identify skin specific genes that are involved in phellem/skin development a 2-DE approach was used to compare the skin and parenchymatic flesh proteomes of young developing tubers. At that developmental stage the tuber the skin has not yet set and can be separated easily from the underlying parenchymatic layers. As expected, the skin proteome was enriched in proteins whose activity is characteristic of actively dividing tissues. Interestingly, the major functional category (63%) consisted of proteins involved in plant-defense responses to biotic and abiotic stresses. This group included three isozymes of caffeoyl-CoA *O*-methyltransferase (CCoAOMT) and five isozymes of peroxidase that may play a role in suberization processes. The differential expression of these proteins in the skin was further verified by RT-PCR of their corresponding transcripts in skin and tuber flesh samples. This work was published, Barel and Ginzberg (2008), and is attached herein. Likewise, transcript profiling experiments (TIGR profiling service, *potato functional genomic project*) indicated of increased expression of stress and suberin related genes in the skin when compared to leaf and tuber-flesh tissues (Fig. 1).

Fig. 1. Functional categories of clones that exhibit the highest expression in the skin (tissue fold >16) compared to leaf and tuber phelloderm and flesh parenchymatic tissues.

2. Russet development

Smooth and russeted isogenic lines of Bake King potatoes and heat stressed tubers of the smooth-skinned variety Desiree were used to study russet development and to compare genetically inherited russetting with heat-induced russetting. Anatomical studies of Bake King lines suggested that russeted appearance evolves from cracking of the outer layers of skin (Fig. 2). Cracked skin is also seen in tubers that develop under high soil temperatures. Based on observations of skin from heat-treated tubers and *in vitro* generated microtubers of Desiree (Fig. 3), we hypothesize that during periods of high temperatures skin development is accelerated, resulting in increased numbers of suberized phellem cells. The characteristic morphology of well-organized cell columns is not kept, and skin layers collapse creating inflexible tissue with a rough surface. Upon tuber expansion the rigid skin is cracked, new layers develop underneath the fissures, and the skin loses its smoothness even further.

Fig. 2. Morphology of russeted skin. Samples of tuber surface were collected from smooth (A,B) and russeted (C,D) isogenic lines of Bake King cultivar at the end of growth, and were embedded in paraplast. Cross sections were stained with Safranin O/Fast Green and viewed with light (left panel) and UV (right panel) microscopes to examine tissue morphology (A,C) and autofluorescence (B,D) of suberized cells, respectively. Smooth skin is characterized by well organized columns of flattened cells, while russeted skin is fissured and less organized.

Fig. 3. Skin development under heat stress. Following tuberization at an optimal temperature of 24°C, *in vitro* generated synchronized cultures of 'Desiree' microtubers were transferred to 33°C and 15°C, or kept at 24°C for further growth. Samples of microtubers were taken for microscopic observation at different time points following the transfer, embedded in paraplast, stained with Safranin O/Fast Green and viewed with light (left panels) and UV (right panels) microscopes to examine tissue morphology and autofluorescence of suberized cells, respectively. Results indicate enhanced skin development at high temperatures and delayed development at low temperatures, compared to the optimal growth conditions.

3. Modified gene expression in heat stressed skin

The proteomes of heat-stressed and native skin were compared by 2-DE (2 replicates, 13-cm-long Immobiline DryStrip gels with a pH range interval of 4-7) to identify proteins with altered levels. Interestingly, protein patterns from both samples were similar. Gel image analyses were performed with Z3 software (Compugen Inc., Israel). The analyses were focused on proteins that were previously identified to accumulate differentially in the skin compared to the tuber flesh (Barel and Ginzberg, 2008). Results indicated moderate changes in protein levels: a decrease in stress proteins and an increase in peroxidase and CCoAOMT isogenes that may be related to suberization processes. RT-PCR of 5 peroxidase isozymes showed a slight increase in transcript level of three isozymes (Fig. 4), supporting the 2-DE results.

Fig. 4. Differential expression of peroxidase isogenes (POD5:CV504265, POD9:TA39454, POD18:TA25873, POD20:TA23216, POP_A:TA25019) in native (white) and heat-stressed (grey) skin. The expression levels were monitored relative to 18S rRNA levels in each sample. NAC (TA25576) was used as reference gene. Signal intensities were quantified by Image Gauge software.

RNA was extracted from the same native and heat-stressed skin samples as above and was subjected to microarray analyses (TIGR profiling service, *potato functional genomic project*). The experiment included samples of phelloderm tissue, the inner part of tuber periderm. Microarray results were analyzed by the MapMan program (<https://gabi.rzpd.de/projects/MapMan/>) and heat-induced sequences were sorted by functional categories (Fig 5).

Fig 5. Profiling of heat-stressed skin. Bars represent the percentage of EST clones that were up (black) or down (white) regulated upon heat stress in both skin and phelloderm (left chart), or mainly in the skin (right chart) tissues. ESTs were sorted to functional categories using the MapMan software. Number of total clones is indicated in brackets. Stars indicate relatively high expression differences for group members.

A high percentage of ESTs was of unknown function. Modified expression was observed mainly for clones involved in photorespiration, regulation of transcription and stress response (more than 15% of the clones in each group). As most of the clones could be classified as heat-response regulators (hormone, signaling, degradation, transcription factors) and did not point at a specific cellular function/s that may induce the changes that culminate with russeted skin, we decided to focus on the group of transcription factors (TF) that amounted to 45% of the total clones. Representatives of the various TF were analyzed by real-time PCR to verify the microarray results and to test their relative expression in the skin and in the phelloderm during heat stress (Fig. 6).

Fig 6. Real-time PCR profiling of selected TF in native and heat stressed periderm. Each EST was tested for expression in the skin and in the phelloderm layers of the periderm. *NAC* (TA25576) was used as reference gene. In the left column TF that were up-regulated; on the right, TF that were down regulated by heat treatment. Results represent an average of two independent biological replicates, each consisting of 5 plants. S- skin, Ph- phelloderm, [H] indicates heat treatment of these tissues.

Real-time PCR experiments supported the microarray results. Currently we are doing some literature mining in an effort to link the TF activity and the development of russetting.

4. Mapping russet loci

In the first two years of the project we genotyped 201 progeny of a diploid population that segregates for russetting, and mapped two loci, *rusB* on chromosome 4 and *rusC* on chromosome 11, that are both required for russetting. A third locus, *rusA*, has also been

proposed to be required for russeting, but it did not segregate in our initial cross. To map *rusA*, we analyzed a second population of 247 individuals, prepared by crossing two diploids that traced back to the classical genetic study where the three loci were first described. Based on pedigree, each of these two diploids was expected to be heterozygous at all three loci, i.e. to have genotype *AaBbCc*. Nevertheless, we found no evidence of a third locus; AFLP markers linked to *rusB* and *rusC* alone were sufficient to explain the phenotypes of the progeny in this second population (see figure below):

It appears, therefore, that only two loci are responsible for tuber russeting, and that earlier classical studies suggesting three loci were in error. Markers tightly linked to *rusB* were under-represented (compared to expectation) in both our diploid populations; this aberrant segregation may be what misled prior investigators.

Efforts were made to map five of the genes that were found in microarray experiments to be highly induced in the skin under heat stress. Of the five (corresponding to EST TCs with numbers 94265, 95796, 94822, 104599, 107970), the first could not be amplified, three were

not polymorphic, and only one (TC107970) could be mapped – and it was localized to chromosome 2, so cannot correspond to *rusB* or *rusC*.

Evaluation of russeted and non-russeted tetraploid potato cultivars with AFLP markers tightly linked to *rusB* and *rusC* showed that these specific alleles are not associated with russetting in unrelated germplasm, and thus are not useful for MAS *per se*. To develop markers useful in applied breeding, it will be necessary to screen alleles of additional tightly linked loci, to identify those (if any) that prove diagnostic in a wider range of potato germplasm.

Published papers

Attached