Theor Appl Genet (2005) 110: 269–275
DOI 10.1007/s00122-004-1829-z

ORIGINAL PAPER

**Chun Suk Jung · Helen M. Griffiths
Darlene M. De Jong · Shuping Cheng · Mary Bodis
Walter S. De Jong**

The potato *P* locus codes for flavonoid 3′,5′-hydroxylase

Received: 6 July 2004 / Accepted: 27 September 2004 / Published online: 24 November 2004

Abstract The potato *P* locus is required for the production of blue/purple anthocyanin pigments in any tissue of the potato plant such as tubers, flowers, or stems. We have previously reported, based on RFLP mapping in tomato, that the gene coding for the anthocyanin biosynthetic enzyme flavonoid 3′,5′-hydroxylase (*f3′5′h*) maps to the same region of the tomato genome as *P* maps in potato. To further evaluate this association a *Petunia f3′5′h* gene was used to screen a potato cDNA library prepared from purple-colored flowers and stems. Six positively hybridizing cDNA clones were sequenced and all appeared to be derived from a single gene that shares 85% sequence identity at the amino acid level with *Petunia f3′5′h*. The potato gene cosegregated with purple tuber color in a diploid F_1 subpopulation of 37 purple and 25 red individuals and was found to be expressed in tuber skin only in the presence of the anthocyanin regulatory locus *I*. A potato *f3′5′h* cDNA clone was placed under the control of a doubled CaMV 35S promoter and introduced into the red-skinned cultivar 'Désirée'. Tuber and stem tissues that are colored red in Désirée were purple in nine of 17 independently transformed lines.

Introduction

Anthocyanin coloration in plants can be influenced by many factors such as pigment composition (Lewis et al. 1998), the presence of ions (Naumann and Horst 2003), the presence of co-pigments (Mol et al. 1998), vacuolar pH (Yoshida et al. 1995), and cell shape (Noda et al. 1994).

In diploid potato three classical loci—*I*, *R*, and *P*—are known to be involved in coloration of potato tuber skin (Dodds and Long 1955, 1956). *R* and *P* encode basic factors required for the production of red pelargonidin or blue/purple petunidin-based anthocyanin pigments, respectively, while *I* is required for the tissue-specific expression of anthocyanin pigments in tuber skin (Dodds and Long 1955, 1956). *P* is known to be epistatic to *R* (Dodds and Long 1955). Thus tubers with genotype *I- Rr Pp* or *Ii rr P* have purple skin while those with genotype *I- R- pp* are red. Potatoes lacking a functional allele at *I* produce white tubers, irrespective of the alleles present at *R* and *P*. The diploid *I*, *R*, and *P* loci functionally mimic, and are likely to be allelic to, the tetraploid *D*, *R* and *P* loci, respectively (De Jong 1991), originally described by Salaman (1910).

R, *I*, and *P* have previously been localized to chromosomes 2, 10, and 11, respectively (Van Eck et al. 1993, 1994). De Jong et al. (2004) recently mapped 13 anthocyanin-related genes in tomato, a crop that shares extensive conservation of marker order with potato. Comparing map locations across these two species led to the hypotheses that *I*, *R*, and *P* encode a MYB transcription factor, dihydroflavonol 4-reductase (*dfr*), and flavonoid 3′,5′-hydroxylase (*f3′5′h*), respectively.

In this research we test the latter hypothesis more thoroughly, and now report that the potato *f3′5′h* gene cosegregates with *P*, is expressed in tuber skin only in the presence of *I*, and when expressed as a transgene in the red-skinned cultivar Désirée changes tuber skin color from red to purple.

Communicated by C. Möllers

C. S. Jung · H. M. Griffiths · D. M. De Jong
S. Cheng · M. Bodis · W. S. De Jong (✉)
Department of Plant Breeding, Cornell University,
Ithaca, NY, 14853-1901 USA
E-mail: wsd2@cornell.edu
Tel.: +1-607-254-5384
Fax: +1-607-255-6683

Materials and methods

Plant material

Diploid potato clones W5281.2 (female parent/purple tuber skin/genotype *Ii RR Pp*) (De Jong and Burns 1993)

and 07506-01 (male parent/white tuber skin/genotype *ii rr pp*) (De Jong 1987; De Jong and Burns 1993) were crossed to generate an F_1 population that segregates for tuber skin color (approximately 2 white:1 red:1 purple). The cultivar Désirée was obtained from the New York State Foundation Seed Farm, Lake Placid, N.Y.

cDNA library screening

A cDNA library from pigmented flowers and stems of W5281.2 has previously been constructed (De Jong et al. 2003b). A fragment of the *Petunia f3′5′h* gene, corresponding to the majority of the third exon, was amplified with primers 3Hf1-F (5′-TTTGTTCACAGC-TGGTACGG) and 3Hf1-R (5′-AGAGGGACAGCT-TTCTGCAA), radioactively labeled, and then used as a hybridization probe to screen the cDNA library. Positive clones were plaque-purified and the inserts then excised to permit propagation in a pBlueScript SK− phagemid vector, following the manufacturer's instructions. Inserts were sequenced using universal M13 vector primers.

Cloning and analysis of genomic DNA

One of the cDNA clones isolated from the potato library appeared to contain the entire open reading frame (ORF) of a gene with high sequence similarity (82% nucleotide identity) to *Petunia f3′5′h*. Primers 1F (5′-CGCGGCAATAATATACATCATAGT) and 1R (5′-GCAAGGGCCCATTCTATTACA) were designed to amplify the first three-quarters of the corresponding gene from genomic DNA. The resulting PCR products were cloned into the vector pGEM-T (Promega), sequenced, and examined for polymorphism. Two haplotypes were observed. To determine whether either cosegregated with purple skin color, primers 3F (5′-ATGAACGTGTGCAAAAGAAAACTC) and 3R (5′-AATTTATAGTACTTTTCGTATAGTTTTTGA) were designed to flank a polymorphic *Taq* I restriction site. Products were digested with *Taq* I after amplification, separated on a 1% agarose gel, and visualized by staining with ethidium bromide. Primers 2F (5′-GTCTGCTCTCTTAAGATATTGTTGTG) and 2R (5′-AATTACAAAACAAATCCCTTCAAG) were used to amplify the 3′ third of the potato gene, which was also cloned into pGEM-T and sequenced.

RT-PCR analysis of gene expression

Primers 4F (5′-GTTGTTGCCTCTACCCCTAAT) and 4R (5′-AACTTTTCATCCCTTTTTCAAT) flank a polymorphic *Cla* I restriction site and were used to study expression of potato *f3′5′h*. These primers flank an intron so that amplification products generated from genomic and cDNA templates can readily be distinguished. Products were digested with *Cla* I after amplification, separated on a 1% agarose gel, and visualized by staining with ethidium bromide. For each independent experiment evaluating *f3′5′h* gene expression total RNA was isolated with an RNeasy Plant Mini Kit (Qiagen) from the tuber skin of each parent (W5281.2 and 07506-01), two progeny with red tuber skin, two progeny with white skin, and two progeny with purple skin. The experiment was performed four times so that a total of eight red, eight white, and eight purple progeny were examined. RNA was isolated from young tubers, 1–3 cm in diameter. Total RNA from each clone was treated with RQ1 RNase-free DNase (Promega) to eliminate genomic DNA prior to reverse transcription. Treated total RNA was then converted into cDNA using random primers and a Reverse Transcription System kit (Promega). Approximately 70 ng of cDNA was then used as a template for PCR, employing the following thermal profile: 1 min at 94°C, followed by 35 cycles of 15 s at 94°C, 15 s at 53°C, and 1 min at 72°C, and a final 10 min incubation at 72°C. The same procedure was used to examine *f3′5′h* expression in leaves of transgenic plants, except that products were not digested with *Cla* I prior to electrophoresis.

Transformation construct

The complete potato *f3′5′h* ORF was amplified from a cDNA clone using a low error-rate polymerase (Platinum *Pfx* DNA polymerase, GIBCO-BRL) and primers F35HORF-XbaI-F (5′-GGG TCTAGAATGACGTT-ACGTATTAGTGAGTTGTTTGC) and F35HORF-SacI-R (5′-CCC GAGCTCTCAGCAACAATAAAC-GTCCAAAGATAG). The 5′ portions of the primers were extended to include *Xba* I and *Sac* I restriction sites, respectively (underlined). The products were digested with both enzymes and ligated into the *Xba* I and *Sac* I sites of transformation vector pPS1 (Huang and Mason 2004). This vector contains a doubled CaMV promoter and tobacco etch virus translational enhancer sequence to enhance transgene expression. The insert was sequenced to ensure that no mutations had been introduced during PCR and then the transformation construct was introduced into *Agrobacterium tumefaciens* strain LBA4404.

Potato transformation

Potato internode segments from 8-week-old in vitro-grown cultivar Désirée were cut into 0.5–1.0 cm explants, and then placed on callus induction medium (CIM) [4.3 $g.l^{-1}$ Murashige and Skoog salts, 26.64 μM glycine, 4.06 μM nicotinic acid, 2.43 μM pyridoxine HCl, 1.19 μM thiamine HCl, 0.57 μM folic acid, 0.20 μM biotin, 0.555 mM myo-inositol, 87.64 mM sucrose, 4.44 μM 6-benzylamino purine (BAP), 10.74 μM 1-naphthaleneacetic acid (NAA), and 2.5 $g.l^{-1}$ Phytagel (Sigma,

catalog no. P8169), with pH adjusted to 5.6] in plates. The explants were incubated in *Agrobacterium* inoculum for 10 min, agitating occasionally, removed from the inoculum, and blotted on sterile filter paper. The explants were then plated on CIM and incubated for 48 h in the dark at 19°C. These explants were then transferred to 3C5ZR medium [4.3 g.l^{-1} Murashige and Skoog salts, 2.96 μM thiamine HCl, 4.06 μM nicotinic acid, 2.43 μM pyridoxine HCl, 0.555 mM myo-inositol, 87.6 mM sucrose, 2.85 μM 3-indoleacetic acid (IAA), 8.54 μM trans-zeatin-riboside, and 2.5 g.l^{-1} Phytagel (Sigma), with pH adjusted to 5.9] containing 75 mg.l^{-1} kanamycin monosulfate and 200 mg.l^{-1} timentin (ticarcillin disodium and clavulanic acid) (GlaxoSmithKline, Research Triangle Park, N.C., USA), and then transferred to fresh 3C5ZR medium weekly for a month and every 10–14 days thereafter. After approximately 8 weeks regenerated shoots (1.5 cm long) were excised and transferred into shoot propagation/rooting medium (CM) (4.3 g.l^{-1} Murashige and Skoog salts, 1.19 μM thiamine HCl, 0.555 mM myo-inositol, 58.43 mM sucrose, and 2.5 g.l^{-1} Phytagel, with pH adjusted to 5.7) containing kanamycin and timentin. After 1 month, shoots were transferred to fresh CM containing timentin and no kanamycin. After another month they were transferred to fresh CM without timentin and then monitored for growth of *Agrobacterium* to ensure that the plants were free of bacteria.

Thin layer chromatography

Approximately 0.1 g of tuber skin was macerated in a 1.5 ml tube containing 200 µl of 99% ethanol/1% HCl (v:v) and then centrifuged to pellet insoluble material. Repeated applications of 5 µl aliquots were spotted onto cellulose thin layer chromatography plates (Eastman Kodak 6064), allowed to dry, and then developed with butanol/2 N HCl (1:1 v:v) (Dodds and Long 1955).

Characterization of *f3′5′h* cDNA in a red-skinned cultivar

Primers F35Hexon1F (5′- ATGACGTTACGTATTAGTGAGTTG) and F35Hexon3R (5′- TCAGCAACAATAAACGTCCAAA) anneal to the beginning and end of the *f3′5′h* ORF, respectively; bases corresponding to the start and stop codons are underlined. These primers were used to amplify cDNA prepared as described above from tuber skin of the cultivar 'Rideau'. The PCR products were sequenced directly without cloning.

Results

Isolation of a candidate gene for *P*

A potato cDNA library, prepared from purple flowers and stems of the diploid potato clone W5281.2 (genotype *Ii RR Pp;* De Jong and Burns 1993), was screened using a PCR product corresponding to most of the third exon of *Petunia f3′5′h* as a hybridization probe. Six independent cDNA clones were isolated and partially sequenced. All appeared to be derived from the same gene, and likely the same allele, as no sequence polymorphism was observed. One cDNA clone contained the entire ORF and was sequenced in its entirety (GenBank accession AY675558). Potato *f3′5′h* is predicted to encode a protein of 509 amino acids and share 85% amino acid sequence identity with *Petunia f3′5′h* (GenBank accession P48419) (Fig. 1). The gene product contains a cytochrome P450 domain with the conserved group A signature motif PFGXGRRXCXG (Durst and Nelson 1995) at amino acids 438 through 448.

Corresponding genomic DNA of W5281.2 was characterized by amplifying, cloning, and sequencing two overlapping fragments of the gene, using the cDNA sequence as a guide for primer design. The first

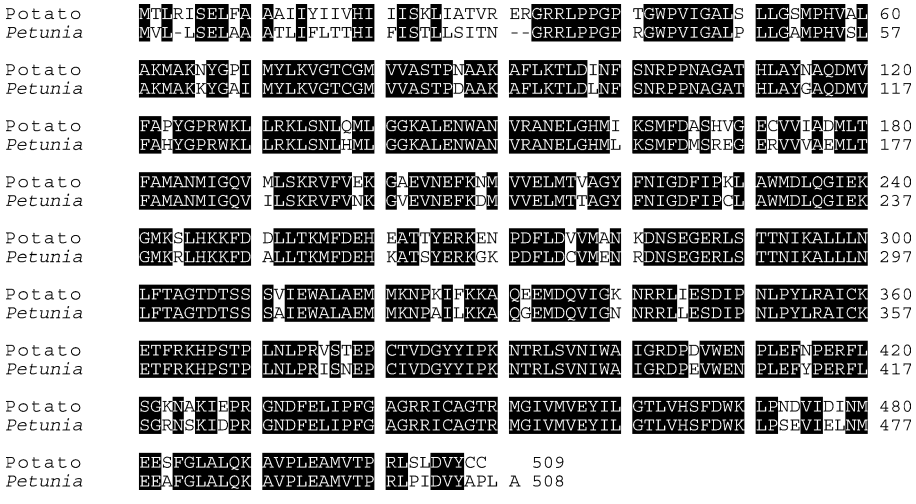

Fig. 1 Alignment of potato and *Petunia f3′5′h* gene products. Identical amino acids are shown with *white type* and *black highlighting*, while non-conserved amino acids are shown with *black type on a white background*

fragment evaluated corresponded to the first three-quarters of the gene. Cloning and sequencing this region revealed two alleles that shared 96% sequence identity, indicating that W5281.2 is heterozygous for *f3′5′h*. The predicted exons of one of these two alleles matched the original cDNA sequence perfectly. When amplification products corresponding to the 3′ third of the gene were cloned and sequenced only one allele—that corresponding to the original cDNA sequence—was observed. The same result occurred when several alternative primers in the 3′ untranslated region and 3′ end of the third exon were used, suggesting considerable sequence polymorphism between the two alleles in this region. The structure of the allele corresponding to the original cDNA sequence is shown in Fig. 2a. Like *Petunia* the potato *f3′5′h* gene contains three exons and two introns.

Cosegregation and appropriate tissue expression

To investigate the relationship between potato *f3′5′h* and *P* we first asked whether either allele of *f3′5′h* cosegregated with purple skin color. An F_1 population that segregated 2:1:1 for white:purple:red tuber skin color was constructed by crossing W5281.2 with the white-skinned clone 07506-01 (*ii rr pp*). Using a polymorphic *Taq* I restriction site in the second intron (Fig. 2a) to differentiate haplotypes revealed that the allele corresponding to the original cDNA sequence absolutely cosegregated with purple skin color among the 37 purple-skinned and 25 red-skinned progeny tested (Fig. 2b).

The expression of potato *f3′5′h* was then examined in tuber skin of W5281.2 and 07506-01, as well as in selected progeny, by RT-PCR (Fig. 3). Both alleles of the gene were found to be expressed in W5281.2, and at least one allele was expressed in all purple and red-skinned progeny, i.e., all progeny that carry a dominant allele at the regulatory locus *I*. The gene was not expressed in 07506-01 or in any white progeny. This result is in accordance with our recent finding (C.S. Jung et al. unpublished) that *I* is a transcription factor that coordinately regulates the expression of multiple anthocyanin pathway genes. In addition to the polymorphic *Taq* I site described above, a polymorphic *Cla* I restriction site within the second exon also differentiates the two alleles of *f3′5′h* in W5281.2 (Fig. 2a). The *Cla* I site is absent in the allele linked in coupling with *P*. RT-PCR followed by *Cla* I digestion revealed that this allele was expressed in progeny clones with purple tuber skin (Fig. 3).

Complementation

The ORF of the potato *f3′5′h* allele linked in coupling with *P* was placed into an *Agrobacterium* binary vector under the control of a doubled CaMV 35S promoter and introduced into the red-skinned cultivar Désirée. Nine of 17 plants transformed with this construct displayed purple stems and purple tubers, while the remaining eight plants displayed only pale red tubers and stems that were indistinguishable in color from untransformed Désirée as well as control plants transformed with empty vector (Fig. 4 and data not shown). Except for the difference in color, transformed and

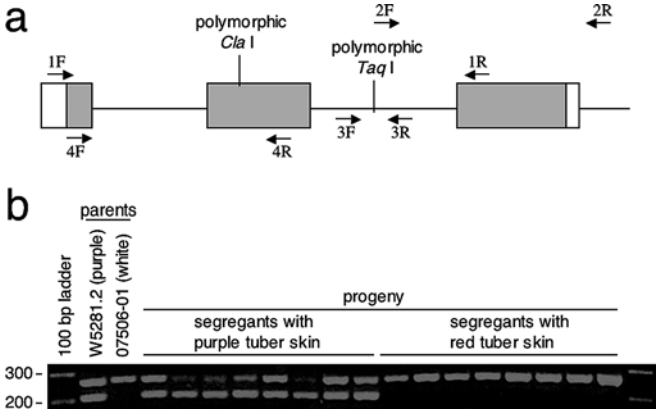

Fig. 2 Structure of the potato *f3′5′h* gene and cosegregation of this gene with purple tuber skin color. **a** The potato *f3′5′h* gene contains three exons (indicated with *open boxes*) and two introns (indicated with *solid lines*). Regions with sequence similarity to a domain conserved in cytochrome P450 proteins (Marchler-Bauer et al. 2003) are highlighted in *gray*. Primer binding sites are indicated with *arrows*. Primer pairs 1F + 1R and 2F + 2R were used to clone and sequence the gene (GenBank accessions AY675559, AY675560). Primer pairs 3F + 3R and 4F + 4R flank polymorphic *Taq* I and *Cla* I restriction sites, respectively, and were used to differentiate the two alleles of this gene in diploid W5281.2. One W5281.2 allele possesses a *Taq* I restriction site but lacks a *Cla* I restriction site, while the other lacks the *Taq* I site but can be cleaved with *Cla* I. **b** Products generated with primers 3F + 3R were digested with *Taq* I and then electrophoresed on a 1% agarose gel. The W5281.2 *f3′5′h* haplotype defined by the presence of the *Taq* I restriction site yields a 210 bp fragment with this assay; this fragment was observed to cosegregate with purple tuber color. The *left lane* contains a 100 bp size ladder; the length of two marker bands is indicated at *left*

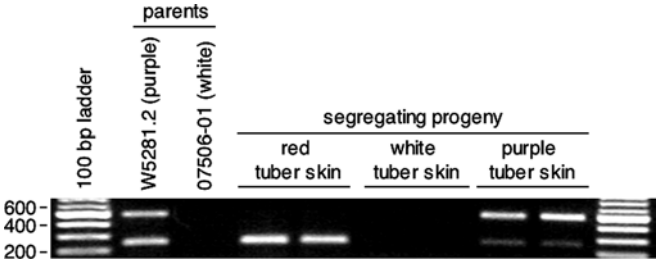

Fig. 3 Expression of potato *f3′5′h* in tuber skin. Primers 4F and 4R (Fig. 2a) were used to amplify cDNA prepared from tuber skin of parents W5281.2 and 07506-01 as well as from two progeny with red skin, two progeny with white skin, and two progeny with purple skin. PCR products were digested with *Cla* I and electrophoresed on a 1% agarose gel to discriminate the alleles expressed

untransformed plants were otherwise phenotypically indistinguishable.

Thin layer chromatography showed that the anthocyanin pigment profile in transformed Désirée tubers with the purple phenotype was indistinguishable from that of W5281.2, but clearly distinct from that of untransformed Désirée (Fig. 4). RT-PCR analyses indicated that at least one allele of *f3′5′h* is expressed in Désirée leaf tissue (Fig. 5). Accumulation of *f3′5′h* transcripts was noticeably higher in transgenic plants with purple tubers and stems, while *f3′5′h* levels in transformed plants that showed no purple color were comparable to untransformed Désirée (Fig. 5).

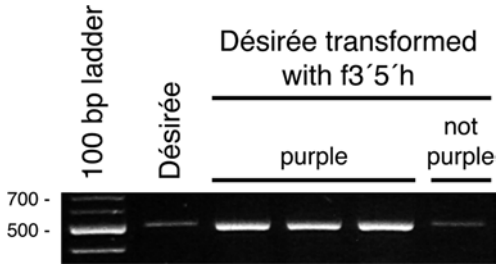

Fig. 5 RT-PCR expression analysis of the *f3′5′h* transgene. Total RNA was isolated from leaves of Désirée and of Désirée transformed with *f3′5′h*. RNA was treated with DNase to eliminate genomic DNA, converted into cDNA, and then subjected to PCR with primers 4F and 4R (Fig. 2a). Amplification products were separated on a 2% agarose gel. Transformed Désirée plants included those with a purple tuber phenotype, as well as those indistinguishable from wild type (*not purple*)

Sequence of a transcribed, non-functional allele of *f3′5′h*

The RT-PCR analyses of Figs. 3 and 5 revealed that *f3′5′h* is transcribed in at least some plants with red tuber skin. Any *f3′5′h* alleles expressed in tissue with red anthocyanin presumably carry one or more mutations that interfere with protein function. To determine whether this was indeed the case we characterized an *f3′5′h* allele expressed in the red-skinned cultivar 'Rideau'. Primers designed to amplify the entire ORF of the functional allele from W5281.2 directed amplification of a 1,530 bp product from Rideau tuber skin cDNA. The sequence trace of the uncloned Rideau PCR product (GenBank accession AY675561) showed no mixed bases indicating that only one allele had been amplified. The predicted amino acid sequence of this allele differed at ten residues from the functional allele of W5281.2 (data not shown). One of the ten changes was within the

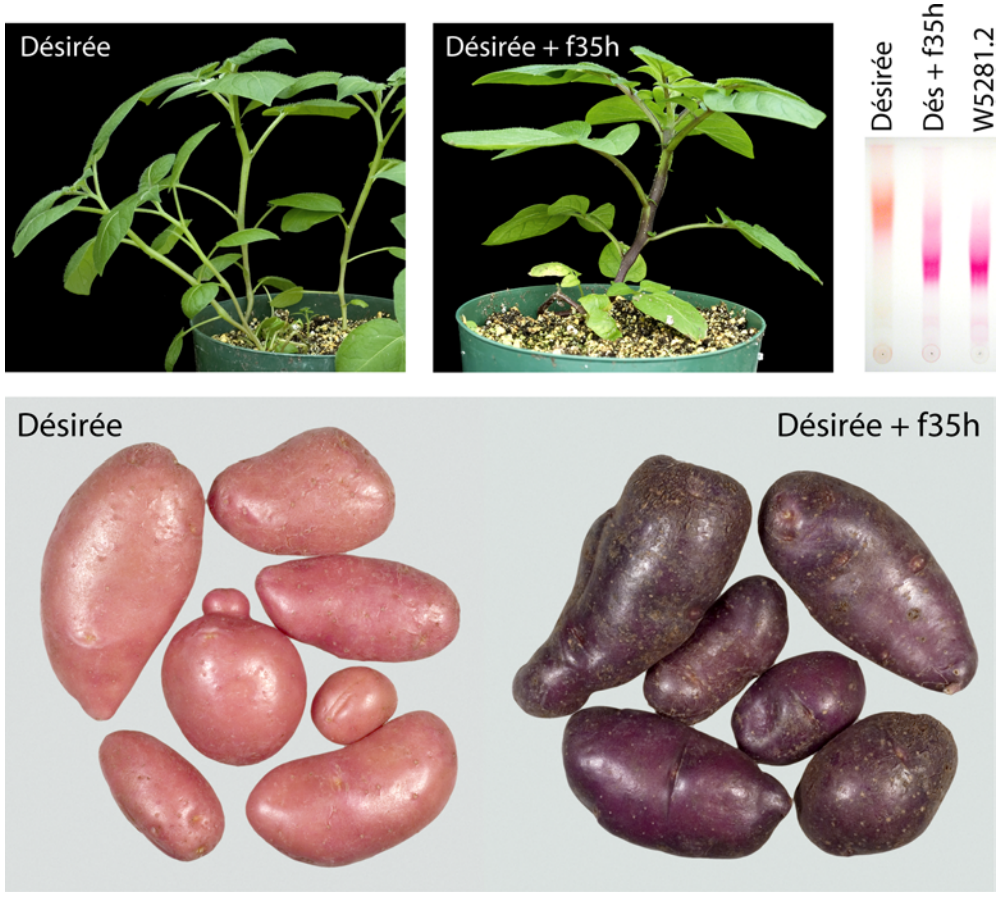

Fig. 4 Transformation of the red-skinned cultivar Désirée with potato *f3′5′h*. Foliage and tubers of Désirée and a representative Désirée line transformed with *f3′5′h* are shown. *Top right*: thin layer chromatography of anthocyanin pigments isolated from tuber skin of untransformed Désirée, Désirée transformed with *f3′5′h*, and W5281.2. Pigment samples were applied near the bottom of the TLC plate shown and developed with a solvent comprising butanol/2 N HCl. The orange color and faster migration of the sample prepared from Désirée is characteristic of derivatives of pelargonidin, while the purple color and slower migration in the remaining two lanes is characteristic of derivatives of petunidin (Dodds and Long 1955)

conserved group A P450 signature motif PFGXGRRXCXG (Durst and Nelson 1995). The sequence was PFGAGRRICAG in W5281.2 and PFWAGRRICAG in Rideau.

Discussion

The *P* locus has long been known to be required for the production of blue/purple anthocyanins in potato (Dodds and Long 1955, 1956). The observations that *f3′5′h* is required for blue/purple pigment production in *Petunia* (Holton et al. 1993) and that *f3′5′h* and *P* map to similar regions of the genome (De Jong et al. 2004) led us to hypothesize that the potato homolog of *f3′5′h* would correspond to *P*. To test this we isolated an *f3′5′h* cDNA clone from a purple-skinned potato using the *Petunia* gene as a hybridization probe. The corresponding gene was found to cosegregate with purple tuber skin color and was expressed in tuber skin only in the presence of the anthocyanin regulatory locus *I*. When the potato cDNA clone was transformed into the red-skinned cultivar Désirée, tissues that were normally red in color were now purple. The genetic cosegregation, biologically relevant transcriptional regulation, and complementation data collectively provide strong evidence that the potato *f3′5′h* gene corresponds to *P*.

Identification of the gene underlying *P* provides opportunities to more efficiently manipulate color in potato breeding programs through marker assisted selection or transgenic approaches. It is often possible to determine whether a given potato clone has a functional allele of *P* simply by looking for the presence of purple pigment in tubers, sprouts, stems, or flowers. Visual inspection cannot, however, reveal dosage (*Pppp*, *PPpp*, *PPPp*, *PPPP*) of the functional allele. Parental clones with higher dosage transmit the corresponding trait to a higher proportion of progeny, and are thus preferred when breeding. It may now be possible to develop an assay to measure dosage of desirable alleles at this locus, e.g., by utilizing a 5′ fluorogenic nuclease approach, as has been done with *dfr* (De Jong et al. 2003a). As demonstrated in this work it is also possible to convert a red-skinned cultivar into a purple-skinned derivative by transformation with a functional allele. The same approach would likely convert a red-fleshed cultivar into a purple-fleshed derivative. Converting white-skinned clones into purple-skinned clones will presumably require co-transformation with a functional allele of *I*. With appropriate promoters driving tissue-specific expression (or silencing) of *P* it may also be possible to create novel bi-color patterns as well, e.g., a red-skinned tuber with purple flesh.

Lewis et al. (1998) have previously shown that anthocyanin pigments in tubers of red cultivars are derived primarily from pelargonidin, while in purple clones they are derived mostly from petunidin. Dodds and Long (1955) reported that *P* is epistatic to *R*; the pelargonidin pigments present in tubers with genotype *I- R- pp* are to a large extent replaced with petunidins in tubers with genotype *I- R- P-*. Dodds and Long (1955) also observed in plants with both *R* and *P* that as the concentration of pelargonidin went up, that of petunidin was reduced, and vice versa. Epistasy of *P* to *R* was thus suspected to occur as a result of competition for a common precursor (Dodds and Long 1955). De Jong et al. (2003b) have recently presented evidence that the *R* locus codes for dihydroflavonol 4-reductase (*dfr*). Consistent with the early inference by Dodds and Long (1955), the gene products of *dfr* and *f3′5′h* are thought to compete for the common precursor dihydrokaempferol (Holton and Cornish 1995).

At least one allele of *f3′5′h* was found to be expressed in the red-skinned cultivars Désirée and Rideau, as well as in the red-skinned progeny of the cross between W5281.2 and 07506-01. In all these cases the difference between dominant (functional) and recessive (non-functional) alleles at *P* thus appears to result from mutations that affect protein function rather than mutations that affect gene transcription. Sequencing the allele expressed in Rideau revealed ten amino acid differences compared to the functional allele of W5281.2, including one within a highly conserved motif. The latter mutation is a promising candidate for explaining why the Rideau allele is non-functional, although a role for one or more of the other mutations cannot be ruled out.

Acknowledgements We thank J. Van Eck for the transformation protocol, K. Paddock and M. Compton for maintenance of field and greenhouse plants, K. Loeffler for photography, and H. De Jong for reviewing the manuscript. This work was supported in part by Federal Hatch funds provided to W.D.

References

De Jong H (1987) Inheritance of pigmented tuber flesh in cultivated diploid potatoes. Am Potato J 64:337–343

De Jong H (1991) Inheritance of anthocyanin pigmentation in the cultivated potato: a critical review. Am Potato J 68:585–593

De Jong H, Burns VJ (1993) Inheritance of tuber shape in cultivated diploid potatoes. Am Potato J 70:267–283

De Jong WS, De Jong DM, Bodis M (2003a) A fluorogenic 5′ nuclease (TaqMan) assay to assess dosage of a marker tightly linked to red skin color in autotetraploid potato. Theor Appl Genet 107:1384–1390

De Jong WS, De Jong DM, De Jong H, Kalazich J, Bodis M (2003b) An allele of dihydroflavonol 4-reductase associated with the ability to produce red anthocyanin pigments in potato (*Solanum tuberosum* L.). Theor Appl Genet 107:1375–1383

De Jong WS, Eannetta NT, De Jong DM, Bodis M (2004) Candidate gene analysis of anthocyanin pigmentation loci in the *Solanaceae*. Theor Appl Genet 108:423–432

Dodds KS, Long DH (1955) The inheritance of colour in diploid potatoes. I. Types of anthocyanidins and their genetic loci. J Genet 53:136–149

Dodds KS, Long DH (1956) The inheritance of colour in diploid potatoes. II. A three-factor linkage group. J Genet 54:27–41

Durst F, Nelson DR (1995) Diversity and evolution of plant P450 and P450-reductases. Drug Metabol Drug Interact 12:189–206

Holton TA, Cornish EC (1995) Genetics and biochemistry of anthocyanin biosynthesis. Plant Cell 7:1071–1083

Holton TA, Brugliera F, Lester DR, Tanaka Y, Hyland CD, Menting JGT, Lu CY, Farcy E, Stevensen TW, Cornish EC (1993) Cloning and expression of cytochrome P450 genes controlling flower colour. Nature 366:276–279

Huang Z, Mason HS (2004) Conformational analysis of hepatitis B surface antigen fusions in an *Agrobacterium*-mediated transient expression system. Plant Biotechnol J 2:241–249

Lewis CE, Walker JRL, Lancaster JE, Sutton KH (1998) Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: Coloured cultivars of *Solanum tuberosum* L. J Sci Food Agric 77:45–57

Marchler-Bauer A, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ, Liebert CA, Liu C, Madej T, Marchler GH, Mazumder R, Nikolskaya AN, Panchenko AR, Rao BS, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Vasudevan S, Wang Y, Yamashita RA, Yin JJ, Bryant SH (2003) CDD: a curated Entrez database of conserved domain alignments. Nucleic Acids Res 31:383–387

Mol J, Grotewold E, Koes R (1998) How genes paint flowers and seeds. Trends Plant Sci 3:212–217

Naumann A, Horst WJ (2003) Effect of aluminum supply on aluminum uptake, translocation, and blueing of *Hydrangea macrophylla* (Thunb.) Ser. cultivars in a peat clay substrate. J Hortic Sci Biotechnol 78:463–469

Noda K, Glover BJ, Linstead P, Martin C (1994) Flower colour intensity depends on specialized cell shape controlled by a Myb-related transcription factor. Nature 369:661–664.

Salaman RN (1910) The inheritance of colour and other characters in the potato. J Genet 1:7–46

Van Eck HJ, Jacobs JME, van Dijk J, Stiekema WJ, Jacobsen E (1993) Identification and mapping of three flower colour loci of potato (*S. tuberosum*) by RFLP analysis. Theor Appl Genet 86:295–300

Van Eck HJ, Jacobs JME, Van Den Berg PMMM, Stiekema WJ, Jacobsen E (1994) The inheritance of anthocyanin pigmentation in potato (*Solanum tuberosum* L.) and mapping of tuber skin colour loci using RFLPs. Heredity 73:410–421

Yoshida K, Kondo T, Okazaki Y, Katuo K (1995) Cause of blue petal colour. Nature 373:291