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BRIEF COMMUNICATION

# WHITE-FRUITED *DUCHESNEA INDICA* (ROSACEAE) IS IMPAIRED IN *ANS* GENE EXPRESSION[1]

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- *Premise of the study: Duchesnea indica* is a wild strawberry-like species that has red fruits. In a recent survey in the highlands of Tucumán (Argentina), a plant of *D. indica* with white fruits was discovered. The aim of this study was to investigate whether the white-fruited character was due to a phenotypic or genotypic change. The stability and heritability of the character and the expression of genes involved in anthocyanins synthesis were studied and compared with red-fruited genotypes. This study contributes to understanding the molecular basis of some factors involved in fruit pigmentation, a horticulturally and taxonomically important trait.
- *Methods:* Stability and heritability of the white-fruited character were evaluated in plants obtained by asexual propagation or by sexual crosses between the white- and red-fruited genotypes. Asexual multiplications were carried out by stolon rooting and sexual multiplications by germination of achenes obtained from crosses. The expression level of the genes involved in the synthesis and regulation of the anthocyanins pathway (*CHS*, *F3H*, *DFR*, *ANS*, and *MYB10*) were evaluated by RT-PCR using specific primers.
- *Key results:* Plants with the white-fruited character always yielded white-fruited progeny when propagated asexually, whereas in sexually propagated plants fruit color depended on the mother. Red-fruited mothers yielded red-fruited progeny, and white-fruited mothers yielded fruits ranging from dark pink to white. Molecular analysis suggested that the white-fruited character was due to the low expression of the *ANS* gene.
- *Conclusions:* Results obtained indicate that the white-fruited character was stable. Mother progenitors exert a strong influence on the expression of the white-fruited character. The white-fruited phenotype is due to the impairment or downregulation of the *ANS* gene.

**Key words:** *ANS*; anthocyanin; gene expression; intra-specific cross; *MYB10*; Rosaceae; white-fruited *Duchesnea indica*.

The genus *Duchesnea* Sm. (Rosaceae) includes only two species, which originated from India: *Duchesnea indica* (Andrews) Focke ($2n = 84$) and *Duchesnea chrysantha* (Zoll. & Moritzi) Miq ($2n = 14$). Whereas *D. indica* is ubiquitous and grows in different regions of the world (Zardini, 1973, 1999; Arias, 2007), *D. chrysantha* has been reported in Japan, China, India, Korea, Taiwan, the Philippines, and Indonesia (Kalkman, 1968; Sugimoto and Naruhashi, 1981; Kume et al., 1987; Sugimoto et al., 1991; Naruhashi, 1992, 2001). Morphologically, the two species are very similar, with yellow flowers and nonedible fruits, except that fruits of *D. indica* are red-scarlet and larger than the pink fruits presented by *D. chrysantha* (Naruhashi and Sugimoto, 1996). Naruhashi and Iwatsubo (1991) reported the occurrence of a white-fruited genotype called *Duchesnea chrysantha* f. *leucocephala* Hara ($2n = 14$), and Naruhashi (1992) reported a new botanical form of *D. indica* with white fruit, which was called *D. indica* f. *albocaput* Naruh ($2n = 84$). The latter, was cited as very rare, being endemic in Honshu (Fukui Prefecture), Japan (Naruhashi, 2001). However, no attempt was made to elucidate the nature of the white-fruited character. If this character is only an environmentally induced phenotypic change, then it should be possible to obtain red-fruited offspring from vegetatively propagating white-fruited specimens, and a change of fruit color should be expected from plants grown under different conditions.

The red color of flowers and fruits is mainly produced by two flavonoid-derived pigments: anthocyanins and betacyanins. In the biosynthesis of anthocyanins, many enzymes are involved;

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among them, chalcone synthase (CHS), chalcone isomerase (CHI), flavonone-3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), and anthocyanidin synthase (ANS) are the most important ones due to their roles in the synthesis of other important precursors (Deng and Davis, 2001; Shimada et al., 2004, 2005). In fact, it was demonstrated that the expression of the *CHS* gene is tightly associated with fruit color (Manning, 1998; Wesley et al., 2001; Aharoni and O'Connell, 2002; Goto-Yamamoto et al., 2002; Honda et al., 2002; Jaakola et al., 2002; Kumar and Ellis, 2003). It should be expected therefore that an inhibition or suppression of any of these enzymes would prevent the synthesis of the corresponding pigments.

The biochemical basis of anthocyanin synthesis and regulation was reported in strawberries by Li et al. (2001). Hoffmann et al. (2006) demonstrated that silencing a ripening related *CHS* gene in strawberry fruits caused: (1) reduction of the *CHS* mRNA level, (2) reduction of the activity of the enzyme CHS, (3) reduction of the content of anthocyanin and 4) the appearance of white patches of unpigmented fruit tissues.

Aharoni et al. (2001) have shown that the expression of the transcription factor R2R3 *FaMYB1* in ripening strawberry played a key role in the biosynthesis of anthocyanins as it repressed the transcription of anthocyanin-related genes. Later, the participation of MYB transcription factors in the regulation of anthocyanin biosynthesis in many plant species has been reviewed by Allan et al. (2008), suggesting that a protein with a basic helix-loop-helix motif (bHLH) would be additionally involved in the regulation of the pathway. Further studies suggested that small changes in the content of the MYB10 proteins can have a marked effect on fruit and flower anthocyanin level (Schwinn et al., 2006; Lin-Wang, et al., 2010).

In a survey of the wild strawberry-like species growing in highland forests of the Sierras de San Javier park, in Tucumán (Argentina), a white-fruited *D. indica* was discovered (M.E. Arias et al., Universidad Nacional de Tucumán, unpublished manuscript). In this work, we evaluate the stability, heritability, and molecular basis of the white-fruited character of this genotype. Because fruit color is a commercially and taxonomically important trait, these results should be of considerable interest to a range of botanists, horticulturists, and plant breeders.

## MATERIALS AND METHODS

For intraspecific crosses, plants of white- and red-fruited *D. indica* were used. In the molecular analysis, fruits and leaves of *Fragaria* × *ananassa* cv. Pájaro, *Fragaria vesca*, and *D. chrysantha* f. *leucocephala* were additionally used. Plants were vegetatively multiplied and maintained in a nursery under controlled conditions. Stolons were rooted in humus/perlome substrate (1 : 1) and daughter plantlets separated from mother plants every 2 mo.

***Intraspecific crosses*—**Numerous crosses between five accessions of the red-fruited and the only accession of white-fruited *D. indica* (in both directions) were carried out for the evaluation of the heritability of white-fruited character. Red- × red-fruited, white- × white-fruited crosses and self-pollination experiments were also performed (Table 1).

Closed blossoms of female progenitors and mature, fully expanded flowers of male progenitors were used. Flowers selected as mothers were emasculated prior to pollination and covered with a cotton plug to avoid accidental self- and outcrossing. Pollen viability was evaluated according to Marks (1954) before crosses. The pollen–pistil compatibility was analyzed. The styles were removed 48 h after pollination, fixed in FAA (1 : 1 : 8, v/v/v, formalin, glacial acetic acid, 80% ethanol) and stored at 4°C. Ovaries were left on the plant for fruit and seed formation and ectopically treated with an aqueous solution of 2,4-dichlorophenoxyacetic acid (2-4 D) (4 ppm) after removing the pistils (Dionne, 1958). Fixed styles were rinsed with tap water, treated according to Martin (1958) and observed with a Zeiss light microscope.

For F1 progeny analysis, fruits were harvested at maturity, ca. 21 d after pollinating. Achenes were harvested from fruiting receptacles, treated with NaClO (5%, v/v) and washed with distilled water. For each crossing experiment, 100 achenes were chosen at random and sown in sterile substrate for germination. Plantlets were grown under controlled conditions (23°C and 16 h light/8 h dark). Vegetative and reproductive traits of offspring were evaluated in laboratory and greenhouse conditions.

***Molecular analysis*—**Total RNA from young leaves and mature fruits of red-fruited and white-fruited *D. indica* (progenitors) and *F. vesca* was extracted to evaluate the expression level of the following genes: *CHS*, *F3H*, *DFR*, *ANS*, and *MYB10*. RNA from fruits of the species *F. ananassa* and *D. chrysantha* f. *leucocephala* were also extracted and used as additional controls of gene expression. For RNA extraction, leaves and fruits were frozen, ground in liquid nitrogen, and purified according to Iandolino et al. (2004). RNA extracts were first treated with Rnase-Free Dnase (Qiagen, Valencia, California, USA) and then with phenol-chloroform (1 : 1). RNA quality and concentration was evaluated by spectrophotometry at 230, 260, and 280 nm (Beckman DU 7500 spectrophotometer; Duarte, California, USA). For single-strand cDNA synthesis, 5 μg of total RNA was reverse-transcribed using Superscript II reverse transcriptase (Invitrogen, Carlsbad, California, USA). DNA from *D. indica* and *F. vesca* was extracted from young not expanded leaves using the Genelute plant genomic DNA kit (Sigma, St. Louis, Missouri, USA).

The cDNA obtained was used as template to perform PCR using specific primers to evaluate the expression of chalcone synthase (*CHS*), flavonone-3-hydroxylase (*F3H*), dihydroflavonol 4-reductase (*DFR*), anthocyanidin synthase (*ANS*), MYB transcription factor (*MYB10*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The latter was used as the internal control resident gene to verify fidelity of cDNA synthesis. Specific primers were designed from the conserved regions of the corresponding gene of different Rosaceae species included in GenBank. Sequences were obtained from the following annotated gene accessions: *CHS* (AY997297, AB201756, AB250913) *F3H* (AB201760, AY691918, AY679608), *DFR* (AF029685), *ANS* (13919608, 81295651, 73656899), and *GAPDH* (AB363963). Specific primers used were *CHS* (forward) 5′-AAC TCA CTT TTC TGG ATT GC-3′, *CHS* (reverse) 5′-CCC AAA CCC AAA TAG AAC AC-3′; *F3H* (forward) 5′-AAC CTG TGG AAG GAG CTT T-3′, *F3H* (reverse) 5′-GCC TCAA GTT TGG TTT TCT CT-3′; *DFR* (forward) 5′-GCT CGT CAT GAG ACT CCT-3′, *DFR* (reverse) 5′-CAT CCA ACC AGT CAT CTT-3′; *ANS* (forward) 5′-TGG CAA CAA GTG AGT ATG C-3, *ANS* (reverse) 5′-TGC ACT TAT GTC GGT ATG A-3′; *GAPDH* (forward) 5′-AAG GGA GGT GCC AAG AAG GTT −3′, *GAPDH* (reverse) 5′-TTA ATC TGG TCA TAG GTG GC −3′. Specific primers for *MYB10* were also used according to Lin-Wang et al. (2010). *MYB10* (forward) 5′- TCA AAT CAG GCT TAA ACA GA −3′, and *MYB10* (reverse) 5′- TTA AAG ACC ACC TGT TTC CT −3′.

All PCR reactions were performed using the following program: 10 min at 95°C followed by a number of cycles that was optimized for each specific amplified gene consisting of 30 s at 95°C, 1 min at 55°C, 1 min at 72°C; and a final extension of 5 min at 72°C. Amplification products were visualized in a 2% agarose gel (10 volts/cm) stained with ethidium bromide (0.5 μg/ml).

## RESULTS

Comparative morphological analysis between the red- and white-fruited *D. indica* did not reveal any conspicuous differences other than the color of the fruit. In both cases, the flowers were yellow (Fig. 1A). In contrast, whereas the red-fruited *D. indica* had scarlet-red fruiting receptacles and achenes (Fig. 1B), the white-fruited *D. indica* had white fruiting receptacles with ochre to creamy achenes (Fig. 1C). To test the stability of the white-fruited character over the time, we maintained the white-fruited specimens in a nursery for 5 yr, and the fruit never changed color. Also, with the aim to test whether the white-fruited character could be transferred to vegetatively multiplied progeny, white-fruited *D. indica* was regularly multiplied by stolon rooting during the 5 yr, and the fruits of new plants were always white. Similar experiments with the red-fruited *D. indica*

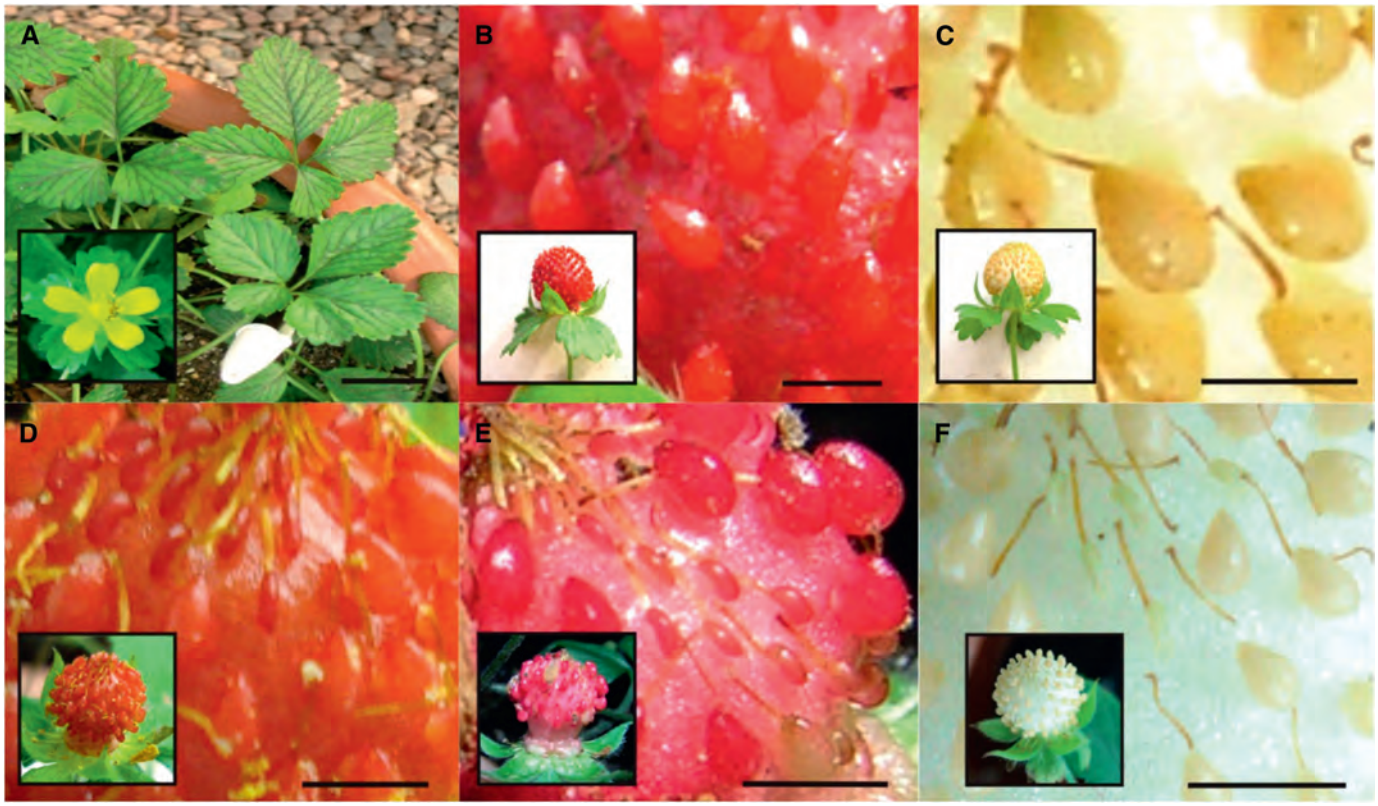


Fig. 1. Leaves, flower, and fruits of red-fruited and white-fruited *Duchesnea indica* and their putative hybrids. (A) Leaves; insert: yellow flower; scale bar = 1 cm. (B) Red fruiting receptacle with scarlet-red achenes; insert: whole fruit; scale bar = 0.125 cm. (C) White fruiting receptacle with creamy-ochre achenes; insert: whole fruit; scale bar = 0.25 cm. (D–F) Putative hybrid fruits obtained from white- × red-fruited *D. indica* crosses; inserts: whole fruit; scale bars = 1 cm (D), 0.5 cm (E) and 0.5 cm (F).

showed that the red fruit character was also stable, as expected. These results showed that the white-fruited character was stable and could be transferred to the clonal progeny.

***Intraspecific crosses*—**All combinations of crosses showed complete prezygotic compatibility, with pollen grains germinating at the stigma and pollen tubes growing down to the base of the style as previously reported (Arias et al., 2004). Crosses between red- and white-fruited *D. indica* in both directions (Table 1), including self-pollination, showed that the fruiting receptacle and achenes of newly formed fruits were always the same color as the mother plant. However, the fruit color of new plants (obtained from achenes produced by crosses) also depended on the mother but in a more complicated manner. All possible crosses between two red-fruited *D. indica* were also made, and the fruit color of new the plants was always red (in Table 1 only one cross is shown). Evaluation of the progeny obtained from each cross showed that when mothers were red-fruited, *D. indica* 100% of the fruits of the daughter plants ("putative hybrid") were red (Fig. 1B). In contrast, when mothers were white-fruited and fathers were red-fruited, 20% of the fruiting receptacle and achenes obtained were similar to the male progenitor (Fig. 1B) or were different shades of red (Fig. 1D), 40% had a dark to light pink-fruiting receptacle with red or ochre achenes (Fig. 1E), and 20% were completely white (as the mother progenitors) with ochre achenes (Fig. 1C) or with white achenes (Fig. 1F). Fruits of plants coming from achenes obtained from red- × red-fruited, white- × white-fruited crosses and self-pollinations experiments yielded red or white fruits matching those of the progenitors (Fig. 1B, 1C).

***Molecular basis of white fruit character*—**Results of the expression of selected genes involved in the anthocyanin pathway (Fig. 2A) are presented in Fig. 2B for genes expressed in fruits and in Fig. 2C for genes expressed in leaves. Estimation of gene expression by semiquantitative RT-PCR showed that genes *CHS*, *F3H*, *DFR*, and *GAPDH* (control) are expressed at roughly similar levels in fruits of all the genotypes analyzed (Fig. 2B). In contrast, the white fruit of *D. indica* exhibited a very low expression of the *ANS* gene as compared to the red-fruited plants. In control experiments with fruit cDNA of *D. chrysantha* f. *leucocephala* (the other white-fruited species used as control), the *ANS* gene was not amplified, indicating that this enzyme is also absent or underexpressed in this species (Fig. 2B).

When the expression of these genes was analyzed in leaves, different patterns were observed. Whereas *F. vesca* (control) expressed the four genes studied, both *Duchesnea* genotypes (e.g., Dir and Dc in Fig. 2B) showed a lower expression of the gene *DFR* and no expression of the gene *ANS* in (Fig. 2C). Since this result may be also due to slight sequence differences of the targeted templates, further experiments were conducted to investigate this effect by using genomic DNA (see below), higher concentration (×10) of cDNA template, and 35 amplification cycles. Results confirmed the previous observation. Since the expression of anthocyanin pathways is regulated by

TABLE 1. Genotypes of *Duchesnea indica* and their fruit color used in crosses and color of fruit characters of new plants obtained.

| | Color of parents | Color of F1 plants | |
|---|---|---|---|
| Genotype crosses | Fruit | Fruiting receptacle | Achenes |
| 5 × 59 | red × white | red | red |
| 8 × 59 | red × white | red or reddish | red |
| 55 × 59 | rxed × white | red | red |
| 56 × 59 | red × white | red or reddish | red and reddish |
| 64 × 59 | red × white | red or reddish | red |
| 79 × 59 | red × white | red | red |
| 59 × 5 | white × red | white, red or reddish | ochre |
| 59 × 8 | white × red | white, red or reddish | ochre |
| 59 × 55 | white × red | white, red or reddish | ochre |
| 59 × 56 | white × red | white, red or reddish | ochre |
| 59 × 64 | white × red | white, red or reddish | ochre |
| 59 × 79 | white × red | white, red or reddish | ochre |
| 56 × 55 | red × red (red control) | red | red |
| 56 selfed | red (control) | red | red |
| 59 × 59 d | white × white (white control) | white, white with pinkish spots and pinkish | ochre and/ or green |
| 59 selfed | White (control) | white, white with pink dots | ochre |

*Notes:* Numbers for genotype crosses correspond to different accessions of red-fruited *D. indica* except 59 that corresponds to the single white-fruited accession available at the BGA-UNT; 59 d = 59 daughter.

transcription factors such as MYB10, specific primers were used to detect the expression of the *MYB10* gene. Results presented in Fig. 3 show that the gene *MYB10* is expressed in all the genotypes analyzed regardless of the tissue source for the cDNA. Also, with the aim to rule out possible amplification artifacts, all primers were tested with DNA obtained from leaves of *F. vesca* and both genotypes of *D. indica*. Results presented in Fig. 3 show that amplification by the primers of *MYB10* and *ANS* was satisfactory with DNA from *F. vesca* and both *Duchesnea* genotypes.

## DISCUSSION

Experiments to test the stability and heritability of the white-fruited character showed that this character is maintained by the plant for a long time and is transmitted to progeny when plants are cloned vegetatively. These results led us to conclude that the white-fruited character is not the product of a phenotypic expression that can be modified in response to environmental changes, but rather is the result of a spontaneous modification at the genomic level. Thus, other spontaneous mutations may also be taking place which, although undetectable, could contribute to increasing the diversity of this species.

In contrast, in sexual crosses, newly formed fruits were different shades of red depending on the progenitor. Crosses between white-fruited progenitors (and in self-pollinations) always yielded daughter plants with white fruits, and when the female progenitor was red-fruited, fruits were always red (Table 1). However, when the female progenitor was white-fruited, plants that were obtained from achenes of the same fruiting receptacle (F1 progeny) yielded fruits with a wide range of colors from red to white, and this character was stable (Table 1). The latter was confirmed after several generations of vegetative propagations and 3 years of observation of the fruit color. This outcome suggests that the color character is defined at the fertilization moment and would explain why a single fruit receptacle can produce achenes that yield plants with different colors of fruits. We may also speculate that there is a "maternal effect" operating during achene formation that becomes evident when the mother is a white-fruited genotype. These outcomes expose that important factors are provided by the plant ovaries that contribute to determination of fruit color and may be related to cytoplasmic inheritance. This effect was observed by Mangelsdorf and East (1927) when they crossed *Fragaria hortensis alba* (a white-fruited subspecies of *F. vesca*) as the female progenitor with *Fragaria eflagelis* (a red-fruited subspecies) as the male progenitor.

Even though prezygotic analyses indicated that pollen tubes could grow to the base of the style (not shown), we cannot completely rule out that apomixis could have occurred, as reported by Darrow (1966) in strawberry. The hybrid nature of the progeny obtained can be assumed because the frequency of apomixes observed on either red- and white-fruited *D. indica* is well below the frequency of successful crosses obtained. Control emasculated and nonpollinated flowers (50 flowers) that were covered to avoid possible self- or outcrosses never yielded fruits.

When two varieties of the diploid *F. vesca* ($2n = 14$), one with pink flowers and red fruits, and the other with white flowers and white fruits, were crossed by East (1930), the progeny showed segregation of the two characters according to Mendelian factors (e.g., colored fruits and flowers were dominant over the colorless ones). Other authors also reported that when crossing the *Fragaria hortensis alba* with *Fragaria eflagelis* or with *F. semperflorens* (red-fruited subspecies), few white-fruited genotypes were obtained, and the character was inherited as a monogenic recessive one (Mangelsdorf and East, 1927). Accordingly, we may hypothesize that the white-fruited character of the newly discovered *D. indica* may also be a recessive monogenic character, although further studies should be performed to evaluate ploidy effects on the white-fruited character segregation and the number of alleles that might be involved.

Since the white-fruited character may be associated with the impairment of the production of natural pigments, we were interested to investigate whether any of the enzymes involved in the synthesis of anthocyanins would explain the lack of color in fruits. Results clearly showed an impairment or low expression of the *ANS* gene in fruits, suggesting that the expression of *ANS* is required for fruit pigmentation in this species. The latter was further confirmed with the result obtained with the white-fruited species *D. chrysantha* f. *leucocephala* used as an outgroup control.

Interestingly, similar results were reported by Kim et al. (2004, 2005) when analyzing participation of the *ANS* gene on anthocyanin accumulation. They reported that a natural mutation of the *ANS* gene was responsible for the pink character of a red onion cultivar (*Allium cepa*). Further analysis suggested that reduced transcription of the *ANS-p* allele may be caused by truncation of the 5′ end of the *ANS* gene promoter region (Kim et al., 2006).

The fact that many individuals of the progeny of *D. indica* from a cross between white- (mother) and red-fruited progenitors yielded white fruits indicates that the white-fruited character of the mothers prevails over the father (red-fruited), and most importantly, that the red pigmentation remained partially silenced regardless of the ploidy. This outcome is particularly interesting because the occurrence of white fruits in a heterozygous

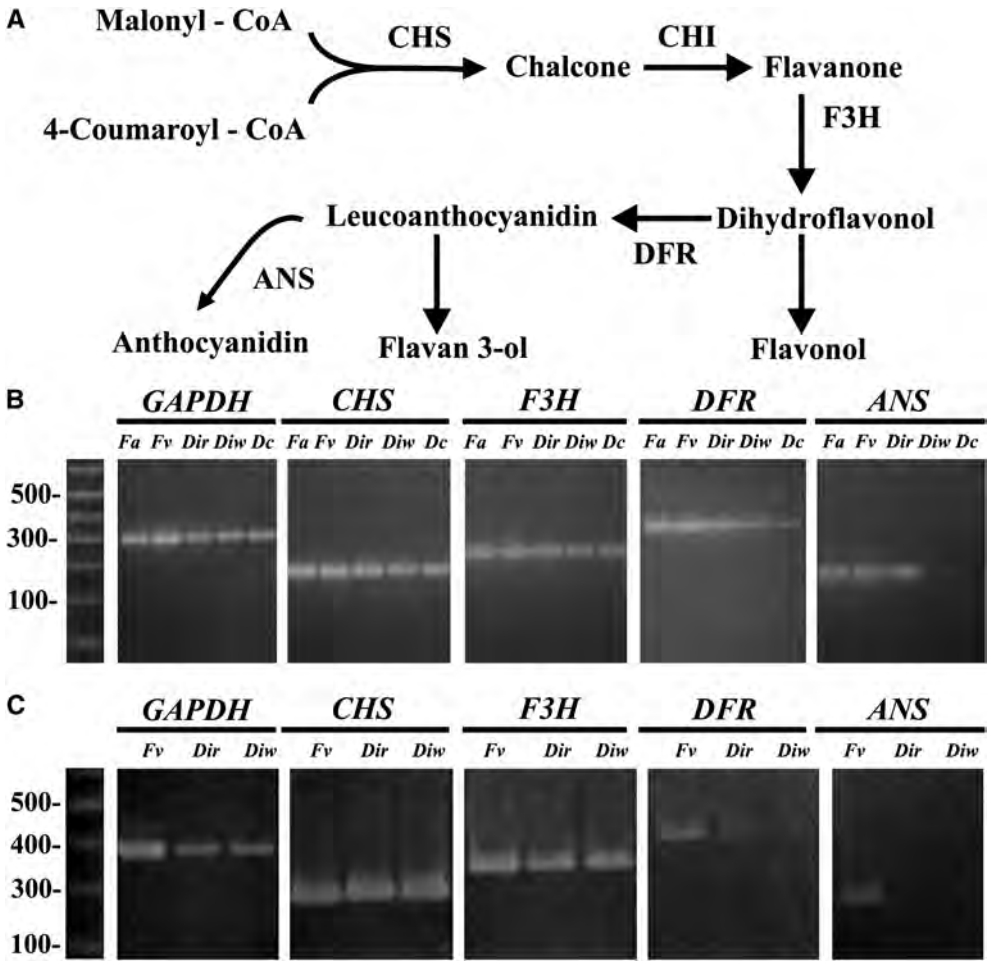


Fig. 2. (A) Simplified pathway of anthocyanidin biosynthesis showing the main enzymes involved. RT-PCR amplification bands of the genes *CHS*, *F3H*, *DFR*, *ANS*, and *GAPDH* (used as control) expressed in (B) fruits and (C) leaves of the species: *F.* × *ananassa* (*Fa*), *F. vesca* (*Fv*), red-fruited *D. indica* (*Dir*), white-fruited *D. indica* (*Diw*), and *D. chrysantha* f. *leucocephala* (*Dc*).

genetic background could only be understood if the expression of the genes involved in the synthesis of anthocyanins is repressed or downregulated by some factors present in the white-fruited individuals and is transmitted by mothers. Furthermore, taking into account the ploidy of the species ($2n = 84$) we may hypothesize that what is actually transmitted to white-fruited progeny is the ability to repress the expression of genes involved in the synthesis of pigments. The latter further suggests the participation of transcription factors that would regulate the anthocyanin pathways, as reported by Aharoni et al. (2001) and Allan et al. (2008), such as MYB and bHLH, respectively. To test this hypothesis, the expression of *CHS*, *F3H*, *DFR*, and *ANS* were also analyzed in leaves (Fig. 2C) and *MYB10* in fruits and leaves (Fig. 3). Whereas *CHS*, *F3H*, *DFR*, and *ANS* were expressed in leaves of *F. vesca* (as in fruits), in *D. indica* the situation was rather different. *CHS* and *F3H* were clearly

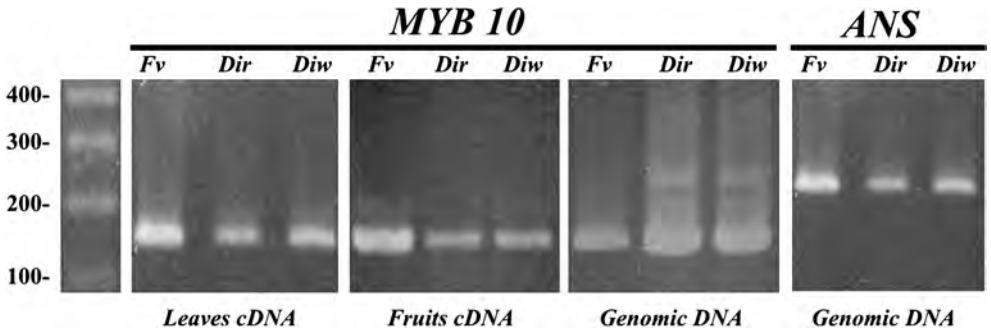


Fig. 3. Expression of the gene *MYB10* in leaves and fruits and presence of *MYB10* and *ANS* in genomic DNA of *F. vesca* (*Fv*), red-fruited *D. indica* (*Dir*), and white-fruited *D. indica* (*Diw*).

expressed in leaves (as in fruits), but *DFR* was underexpressed in leaves of the red-fruited and white-fruited *D. indica*, and *ANS* is completely silenced in leaves of both genotypes (Fig. 2C). However, the level of expression of *MYB10* observed in fruits and leaves of red- and white-fruited *D. indica* (Fig. 3) did not confirm that hypothesis and disagreed with results reported by Espley et al. (2009) for apples and by Lin-Wang et al. (2010) for fruits of *F.* × *ananassa* and *F. vesca*. They observed a higher expression level of *MYB10* in more pigmented fruits. By contrast, we observed a roughly similar level of expression of the *MYB10* gene. Therefore, the explanation of such an expression pattern would require the consideration of other factors such as other regulatory and transcription factors, namely MYB1, MYC, and WD40, that also regulate the anthocyanin pathway (Niu et al., 2010; Lightbourn, et al., 2007) or the evaluation of the expression of these genes at different ontological stages in different plant tissues as reported by Nakatsuka et al. (2003) and Lightbourn et al. (2007). These authors demonstrated that the expression of *CHS*, *F3H*, *DFR*, and *ANS* genes are complexly regulated by many factors that cause different expression patterns depending on the tissue and stage of development of the plant. The fact that we did not detect expression of *ANS* in leaves of either of the genotypes of *D. indica*, although expression was clearly detected for fruits and genomic DNA, may be attributed to the state of development of leaves. The latter explanation may be reasonable because, to avoid contamination and increase the RNA yield, total RNA was extracted from young not fully expanded leaves that may have not completely developed the machinery of pigment synthesis. Another interesting observation is that, although the RNA of *F. vesca* was also obtained from incompletely expanded leaves, the pattern of expression was different, confirming that the regulation of the anthocyanin pathway depends not only on the tissue studied but also on the species, as demonstrated by Nakatsuka et al. (2003) and Lightbourn et al. (2007). In our laboratory, we are working to untangle this situation.

In conclusion, although the lack of color in fruit of the white-fruited *D. indica* may be attributed to the low expression of the *ANS* gene, the reason for the persistence of the white-fruited character in some progeny from white- × red-fruited crosses remains elusive and requires more investigation.

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