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Red-flowered white clover (T. repens L.): insights from morphobiological and genetic analysis

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Abstract

A five year breeding program (2021-2025) successfully established white clover (*Trifolium repens* L.) lines with stable, bright pink inflorescences, achieving a frequency up to 94.4% in the F4 generation.

Molecular genetic analysis identified a polymorphism in the promoter region of the *F3’5’H* gene in red flowered (RF) plants. Bioinformatic analysis of these contrasting promoter regions revealed significant differences in the composition of cis-regulatory elements (CREs) including light-sensitive motifs (AE-box, TCT-motif), which apparently explains the light-dependent expression of anthocyanins.

In silico analysis ruled out that the studied plants belong to *T. occidentale* Combe and confirmed their hybrid nature. This conclusion was based on the presence of sequences from flavonoid biosynthesis genes characteristic of the modern form of *T. occidentale* along with the *T. pallescens* Schreb subgenome. The specific SCAR marker 865_*F3’5’H* can reliably identify RF plants and detect recent hybridization events between *Trifolium repens* and *T. occidentale*.

Introduction

White clover (*Trifolium repens* L.) is an important component of pastures in the majority of temperate regions of the world, and its inclusion in grasslands leads to increased dry matter yield, improved feed value, and prolonged pasture use (Privalova 2004).

Recent genomic studies have established that white clover is an allopolyploid species derived from hybridization between *T. pallescens* Schreb and *T. occidentale* Combe (Griffiths et al. 2019; Fechete et al. 2024). At the same time, most varieties of *T. repens* have white inflorescences, while *T. pallescens* and some *T. occidentale* plants have pink petals. There are several forms of white clover with pigmented inflorescences that are known in the literature. For example, such varieties as Dragon's Blood and Dark Dancer (Tashiro, 2009) have pink petals, while the plants described by Brubaker (Brewbaker 1962) and those found in Iowa in 1983 (Pederson and McLaughlin 1995) had highly pigmented flowers.

Plant pigmentation serves as a marker trait for botanical identification and classification by family, genus or species. Breeders analyze the color of flowers, leaves, stems and other plant parts to breed visually distinct varieties. Consequently, early botanical and agricultural studies in the 19th century naturally focused on plant pigments such as carotenoids and chlorophylls (Młodzińska et al. 2009).

Later, it was established that betalains and flavonoids were also responsible for pigmentation (Tanaka et al. 2010). Anthocyanins, a subclass of flavonoids, are widely studied in plant breeding programs targeting both ornamental and food crops due to their beneficial properties as secondary metabolites. Plants with high anthocyanin content have been shown to be less susceptible to ultraviolet radiation and more resistant to biotic and abiotic environmental factors (Guo et al. 2008; Winkel-Shirley 2001). Consuming foods rich in anthocyanins has a beneficial effect on heart diseases, hyperglycemia, and vision problems (Molina et al. 2023). A number of studies have also investigated their anticancer (Faria

et al. 2010; Hui et al. 2010; Jing et al. 2008), antioxidant (Molina et al. 2019; Gonçalves et al. 2018), and antimicrobial (Bendokas et al. 2018; Chen et al. 2018) effect.

Previous studies have established that anthocyanin biosynthesis involves a cascade of structural and regulatory genes (Sunil and Shetty 2022). Previously, a team from China (Zhang et al. 2018) analyzed the transcriptome of the white clover forms that produce brightly colored flowers upon light exposure and compared them to the same genotype grown in shaded conditions. They found these forms differ significantly in the expression of eight genes: *CHS, F3'H, DFR, ANS, LAR, UFGT, FLS, F3’5’H*. Although the authors identified the key genes responsible for light-induced color change, their regulatory regions have remained unexplored. Literature reports indicate that deletions/insertions in the promoter regions of anthocyanin biosynthesis genes often alter their regulation, thereby affecting trait expression (Nishihara et al. 2014; Moreau et al. 2012; Sato et al. 2011).

In this study, through a multi-year selection program, we developed white clover lines with stable, brightly colored inflorescences and conducted a comprehensive analysis of their key morphobiological traits and the underlying genetic mechanisms.

Materials and methods

Plant material and growth conditions

Plants were bred between 2021 and 2025 under greenhouse and field conditions. Seedlings were grown in 0.2 L containers. Elite plants were placed in 3 L containers. In field conditions, seedlings were transplanted in a grid pattern (checkrowed), with one plant per hole spaced at 0.5 m x 0.5 m.

The plot’s soil was sod-podzolic; its humus content was 1.6%; hydrolyzable nitrogen 7.5%; potassium 15 mg/100 g of soil; phosphorus 25 mg/100 g of soil, and its soil pHKCl was 4.5.

For initial PCR-analysis, we used five adult plants each of white-flowered (WF) and red-flowered (RF) clover, all grown in a greenhouse.

To validate the marker, we analyzed an expanded panel including: 1) seed progeny from 16 RF lines, and (2) 10 commercial varieties and 6 wild forms of WF clover (Table 1).

Table 1
List of white clover accessions used for marker validation and the corresponding red-flowered selection lines

No.	Type	Accession/Cultivar	Origin	No.	Red-flowered lines
1	Cultivar	Lugovik	FWRC FPA	21	№ 6/6
2	Wild	№ 762	Udmurtia	22	№ 8/8
3	Wild	№ 801	Buryatia	23	№ 29/1
4	Cultivar	VIK70	FWRC FPA	24	№ 1
5	Wild	№ 232	Altai, Ulagan pass, h = 2000 m	25	№ 2
6	Cultivar	Wilka	Netherlands	26	№ 217
7	Cultivar	Ritm	FWRC FPA	27	№ 3
8	Cultivar	Ladino	Netherlands	28	№ 17/8
9	Wild	№ 598	Archangelsk region, Nothern Dvina River	29	№ 6
10	Cultivar	Nanuk	Denmark	30	№ 15/3
11	Wild	Altai wild	Altai	31	№ 6/7
12	Wild	№ 517	Karelia, lake Sandal	32	№ 1/2
13	Cultivar	Merlin	Denmark	33	№ 16
14	Cultivar	Ovcak	Czechoslovak Republic	34	№ 7
15	Cultivar	Blanca	France	35	№ 12
16	Cultivar	Zapican	Uruguay	36	№ 5/5

DNA extraction

The experiments were carried out on 30 mg of leaf tissue from the adult plants grown in the greenhouse. To genotype the expanded sample set (varieties and wild forms), we used DNA from seedlings grown on Petri dishes, with 30 seedlings pooled per sample. Total genomic DNA was extracted using a modified SDS method (Klimenko et al. 2021).

Primer design

To amplify the promoter regions of eight genes, specific primers were developed using contigs from a previous transcriptome study (Zhang et al. 2018). The position of the ATG codon was determined by aligning the contigs against the *T. pratense* genome (version ARS_RC_1.1). Subsequently, the promoter

sequence, together with a portion of the first exon’s coding region, was aligned to the white clover genome (versions UTM_Trep_v1.0 and AgR_To_v5). The obtained data were loaded into the PrimerQuest tool (Integrated DNA Technologies, http://www.idtdna.com) to select primers according to the specified parameters.

PCR-amplification

The 20 µL PCR reaction mixture contained: 3 µL of 10× Taq Turbo buffer, 0.5 µL of 50× dNTP mix, 0.4 µL of Taq DNA polymerase (5 U/µL), 1 µL of DNA template (30 ng), and 1 µL each of forward and reverse primers. All reagent volumes are given per reaction. The samples were amplified on a Bio-Rad T 1000 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) under the following conditions: initial denaturation for 3 minutes at 94°C; 35 cycles of 30 seconds at 94°C, 30 seconds at 56/59/62°C (depending on the primer pair), 1 minute at 72°C; final elongation at 72°C for 5 minutes. Table 2 shows the characteristics of the developed primers and the lengths of expected PCR products.

Table 2
Primers designed to amplify the promoter region of flavonoid biosynthesis genes in white clover.

Gene	Primer	Sequence (5'→3')	Tm (°C)	Amplicon length (bp)
ANS	F	TGTTTCTATTGCCTTAGTTCCCT	62	747
	R	CCACTCAAAGATAAGCTTTCAACTC		
LAR	F	GCTCAGATAATAAAGAGTGTCTAAC	59	681
	R	TGTAACCAGTTCCACCAAATAC	61	
F3'H	F	ACATTGATTGCACGCACTTTAG	62	781
	R	GTTGAGAATGCAATGAGCAATAGA		
UFGT	F	CTCACCGCTACTTACCATTCC	62	800
	R	AACAGGGTGGCTTCCAAA		
DFR	F	CTCTACCAATTGAGCTATGTTTATGG	62	803
	R	GACCAACCATGAACCAATGAAG		
CHS	F	TGATTGCTTAGTTGATTAACCG	59	761
	R	GTGTGTTGCTATATTTGTCCTT		
FLS	F	GAAAGGAGATGTCACTCTTA	56	645
	R	TGTTGTGGCATATGTAGTTAG	58	
F3'5'H	F	CTCGCATCATGTACTTCATAGGT	62	776
	R	TGAGAAGCACAAGAGTACAACTTA		

PCR results were detected by electrophoresis in 1.6% agarose gel at 50 V for 90 minutes. A 100 bp DNA ladder molecular marker (Thermo fisher scientific, USA) was used to assess the size of amplification product fragments. The fragments were visualized using a Gel Doc™ XR+ imaging system (Bio-Rad, USA).

DNA sequencing

The PCR products obtained were purified using 'Clean-up Standard' columns (Evrogen, Russia), cloned into the pAL2-T vector following the manufacturer's protocol, and Sanger-sequenced from both ends to determine the primary nucleotide sequence.

Bioinformatic analysis

The obtained sequences were analyzed using the UGENE program (Okonechnikov et al. 2012). The PlantCare online application (Lescot et al. 2002) was utilized to study the cis-regulatory landscape. Sequence alignment and pairwise percent identity calculations were performed using the ClustalOmega software (https://www.ebi.ac.uk/jdispatcher/msa/clustalo). To determine the expected PCR product lengths, BLAST analysis was performed against the subgenomes of white clover (version UTM_Trep_v1.0) and the genome of *T.occidentale* (version drTriOcci1.hap1.1) when analyzing flavonoid bisynthesis gene regions.

Results

The breeding program commenced in 2021 with two plants selected from an experimental plot in a breeding nursery at Federal Williams Research Center of Forage Production and Agroecology. These plants were F3 hybrids of the cross “Atolai (Latvia) x Pechorsky (a wild form from Russia)” and were notable for their pale pink inflorescence color.

Multi-year selection for inflorescence color

In 2022, the first generation was evaluated in a soil greenhouse. Seedlings were transplanted into the ground at a spacing of 0.5 x 0.5 m. Among the 100 plants evaluated, 95 (95%) had white flowers, 3 (3%) pink, and 2 (2%) bright pink flowers. The colored genotypes were transplanted into 3-L containers for further hybridization.

In 2023, 613 F_2 generation plants were potted in 0.2 L containers. Among them, 200 plants (32.63%) had white flowers, 364 (59.38%) were pink and 49 (7.99%) were bright pink. Of these, 312 colored plants were transplanted to grow in the field; the distance between plants was 1 x 1 m. Based on the morphological characteristics, 25 elite plants were selected, potted in a greenhouse, and manually pollinated.

In 2024, the inflorescence colors of 536 F_3 plants grown in 0.2 L containers were evaluated. Twenty-one plants (3.92%) bloomed white, 471 plants (87.87%) pink, and 44 plants (8.21%) bright pink. Of these, 100 plants with colored inflorescences were planted in the field at a distance of 1 x 1 m. Based on the morphobiological characteristics, 17 elite plants were selected, their stolon parts were cut off for vegetative reproduction in a soil greenhouse.

In 2025, 960 F_4 genotypes were planted in the field on a highly acidic soil (pH = 4.5). The seedlings were planted at a distance of 0.5 m x 0.5 m. Among them, 4 plants (0.4%) were white, 906 (94.4%) were pink, and 50 (5.2%) had bright pink inflorescences. Seeds were obtained after negative selection. Stolons from elite plants of rich pink color were planted in a soil greenhouse.

Comparative analysis of morphological and productive traits

Comparative analysis of the morphological characteristics showed that the RF clover sample was less productive than the VIK 70 standard (see Table 3).

Table 3
Agronomically important traits of the F_3 white clover breeding line (year 2024).

Trait	VIK 70 standart	F_3 colored-flowered line	% of standart
Plant diameter, cm	77,00	47,69	61,94
Stolon diameter, cm	0,26	0,24	92,31
Leaf petiole height, cm	14,58	11,08	76,00
Leaf petiole diameter, cm	0,16	0,15	93,75
Leaf blade length, cm	2,99	2,15	71,91
Leaf blade width, cm	2,30	1,94	84,35
Peduncle height, cm	24,17	18,64	77,12
Peduncle diameter, cm	0,22	0,19	86,36
Inflorescence length, cm	2,28	1,83	80,26
Inflorescence diameter, cm	2,48	2,33	93,95
Flower length, cm	0,98	0,95	96,94
Flower diameter, cm	0,50	0,47	94,00
Number of flower heads per plant	98,00	59,00	60,20
Green mass yield, g/plant	180,00	95,40	53,00
Dry matter yield, g/plant	32,50	15,30	47,08
Crude protein content, %	20,00	18,00	90,00
Number of florets per head	88,40	80,00	90,50
Seed yield per plant, g	3,45	1,90	55,07
1000-seed weight, g	0,55	0,60	109,09
Winter hardiness, %	90,00	95,00	-

The plant belongs to the *hollandicum* variety. It has a semi-erect growth habit. The leaves have a distinct white mark and exhibit a color gradient from blue-green to burgundy. The flower heads are deep pink, and flowering continues until late autumn (Fig. 1).

Light and cold-induced color changes

Under low-temperature stress, the vegetative biomass of the plant accumulated anthocyanins, turning burgundy in color (Fig. 2)

Plants that did not change the color of their vegetative mass (green) subsequently bloomed with white inflorescences. Thus, these plants were discarded early in development.

We also found that the intensity of coloration of both green mass and inflorescences was regulated by exposure to light, resulting in higher brightness of pink coloration under field conditions. However, when the selected RF plants were placed in a shaded greenhouse, the inflorescence color changed to white (Fig. 3).

PCR screening for polymorphisms in candidate gene promoters

Prompted by our observations and prior reports of light-induced anthocyanin accumulation, we developed primers for amplification and further study of the promoter regions of eight candidate genes with differential expression. PCR analysis using eight primer pairs detected significant differences in amplicon length for the *F3'5'H* gene between the RF and WF samples (Fig. 4).

PCR analysis revealed a clear polymorphism (Fig. 4). DNA from five WF varieties yielded the expected ~ 750 bp product, whereas DNA from five RF plants produced a distinct ~ 850 bp amplicon.

Structural and phylogenetic analysis of the F3'5'H promoter

Sequence analysis of the polymorphic *F3'5'H* amplicon revealed that the length of the PCR product of the RF varieties was 865 bp, while that of WF was 775 bp. Pairwise alignment of the sequences identified several insertions at distinct positions within the *F3'5'H* promoter region in RF varieties (Fig. 5)

The observed high level of polymorphism in the *F3'5'H* promoter region between RF and WF plants is atypical for allelic variants within a species. Therefore, we identified and aligned the promoter region sequences of *F3'5'H* gene from: the white clover subgenomes (*T. occidentale* and *T. pallescens*), the genome of *T. occidentale* species, and our sequenced RF and WF samples. Figure 6 shows the identity percentages obtained after pairwise alignment of the sequences.

As shown in Fig. 6, pairwise identity analysis revealed the highest similarity (99.74%) between the WF and occi.sub sequences. In contrast, the RF sequence showed 99.08% identity to the *F3'5'H* gene from the whole genome of *T. occidentale* (occi.gen). Notably, the identity between this RF sequence and the *T. occidentale* subgenome within white clover was substantially lower (81.24%).

Cis-element landscape. Light and cold-responsive motifs

The resulting sequences were then analyzed for the presence of CREs using the PlantCARE program (Fig. 7).

Bioinformatic analysis of the *F3'5'H* promoter region revealed distinct cis-regulatory element (CRE) profiles between RF and WF plants (Fig. 7). In total, we identified 20 different groups of CREs across both sequences. The RF promoter contained 18 of these groups, while the WF promoter contained 15. Thirteen groups (65%) were common to both promoters. Each promoter also possessed unique CRE groups. The WF promoter harbored two specific elements: AuxRR-core (involved in auxin responsiveness) and STRE (function not yet defined in plants).

In contrast, the RF promoter possessed six unique CRE groups: AE-box, ERE, TATA, TC-rich repeats and TCT-motif. Of these, the functions of ERE and TATA remain uncharacterized in this context. TC-rich repeats are known cis-acting elements involved in defense and stress responsiveness. Critically, the remaining two motifs – AE-box and TCT-motif – are associated with light responsiveness. Their positions and characteristics within the RF promoter are detailed in Table 4.

Table 4
Location of cold- and light-inducible CREs in the promoter region of the *F3'5'H* gene

RF		**WF**	
Name of cis-element	Position and strand	Name of cis-element	Position and strand
Light-responsive cis-elements			
AE-box	404+	Box 4	404-
Box 4	286+,774-,479-		
TCT-motif	102-		
Cold-responsive cis-elements			
LTR	523-	LTR	448-

As summarized in Table 4, the RF *F3'5'H* promoter harbored three types of light-responsive CREs (AE-box, Box 4 and TCT-motif), whereas the WF promoter contained only a single Box 4 element. This striking disparity in the repertoire of photoresponsive elements (three distinct motifs in RF versus one in WF) provides a plausible cis-regulatory explanation for the light-induced anthocyanin accumulation specific to RF plants and its absence in WF plants.

As for changes in anthocyanin accumulation in response to cold exposure, we cannot draw any conclusions here, since both regions contain the low-temperature-responsive (LTR) element. This suggests that the expression of these genes is controlled by transcription factors of the cold-inducible regulon.

Discussion

Validation of SCAR-marker 865_F3’5’H

To validate the specificity and uniqueness of our newly developed DNA marker (provisionally called 865_*F3’5’H*), we expanded the analysis to include an extended panel comprising wild accessions and commercial varieties of white clover (Fig. 8).

As can be seen from the gel image, the 865-bp band was not detected in the DNA analysis of any of the three Russian and seven foreign varieties, as well as six wild forms of white clover. At the same time, DNA analysis of all 16 RF progeny pools showed the presence of this band. Notably, the 775-bp PCR product was present in all samples of varieties and wild forms of the white clover we analyzed, regardless of their country of origin. This band was also present in DNA samples from some progeny of the brightly colored forms, indicating segregation at this locus and providing evidence for their hybrid origin.

T.repens or T. occidentale?

In order to exclude the belonging of the brightly colored plants to the *T. occidentale* species, we also performed *in silico* analysis and aligned the sequences of all eight promoter regions of flavonoid synthesis genes to the *T. occidentale* genome and white clover subgenomes. The analysis showed that four genes can be used to hypothesize the species affiliation of brightly colored plants (Table 5).

Table 5
In silico and experimental PCR amplicon lengths for *CHS*, *ANS*, *UFGT*, and *F3'5'H*

Genes	**Genome**		**Genome**	**RF**	**WF**
	T.repens		***T. occidentale***		
	Subgenome	**Subgenome**			
	T. pallescens	***T. occidentale***			
Predicted amplicon length (bp)			**Experimental amplicon length (bp)**		
CHS	749	No product	No product	749	749
ANS	792	747	772	747, 772	747
UFGT	800	1278	559	800	800
F3’5’H	No product	776	866	865	775

In the case of *CHS*, only the reverse primer showed complete homology to the promoter region of the *T. occidentale* genome and subgenome; the forward primer exhibited mismatches. At the same time, both RF and WF samples produced a ~ 750 bp amplicon corresponding to a *CHS* gene copy originating from the *T. pallescens* subgenome. The predicted amplicon lengths for *ANS* and *UFGT* varied by 45–241 bp depending on the template (*T.occidentale* genome vs. subgenome), making these polymorphism easily detectable by gel electrophoresis. The most pronounced length polymorphism was observed for the

UFGT gene: 800 bp for the *T. pallescens* subgenome, 1278 bp for the *T. occidentale* subgenome, and 559 bp for its counterpart from the *T. occidentale* species. While the 800 bp *UFGT* amplicon present in RF and WF plants was identified as the *T. pallescens* subgenome copy (Table 5), the *ANS* promoter region exhibited unexpected polymorphism within the RF group. Hence, RF plants 1 and 4 possessed the *T. occidentale* genome copy of *ANS*, while plants 2 and 3 carried the subgenome variant. In contrast, amplification of the *F3'5'H* promoter from the *T. pallescens* subgenome was precluded by multiple primer-template mismatches.

The brightly colored plants are therefore not *T. occidentale* themselves, but contain introgressed *ANS and F3'5'H* alleles of *T. occidentale origin.* Consistent with this, crosses between RF and WF plants produced fertile F1 hybrids (Fig. 9), providing independent evidence for their close genomic relationship.

Direct crosses between diploid *T. occidentale* (2n = 16) and tetraploid *T. repens* (2n = 32) are typically unsuccessful due to chromosome number mismatch, resulting in seed failure. Pederson and McLaughlin (1995) likewise reported successful hybridization and backcrossing between RF and WF biotypes of the cultivar 'Tilman'. Several studies have since reported successful interspecific hybridization between *T. occidentale* and *T. repens* aimed at introgressing economically valuable traits into modern white clover cultivars (Gibson and Beinhart 1969; Hussain et al. 2016; Hussain & Williams 2016; Chen and Gibson 1970; Chen and Gibson 1972). The usual approach involved colchicines treatment to double the chromosome number of *T. occidentale* (from 2n = 2x = 16 to 2n = 4x = 32), rendering it compatible with tetraploid *T. repens* for subsequent hybridization. According to Hussain and Williams (2016), such hybrids were fertile. The presence of the genomes – one from colchicines treated *T. occidentale* and the two native subgenomes of *T. repens* (*T. occidentale* and *T. pallescens*) – allowed for multiple chromosome pairing configurations during meiosis. Thus, the brightly colored plants described here represent hybrids between *T. repens* and *T. occidentale*. The observed polymorphism in the *ANS* promoter amplification, together with the presence of the *T. occidentale* specific *F3'5'H* allele, points to a recent recombination event between homeologous chromosomes of the ancestral *T. occidentale* donor and its modern counterpart within the *T. repens* genome.

Conclusion

This study of white clover (*Trifolium repens*) led to the development of breeding lines with stable, atypical bright pink inflorescences and provided a genetic characterization of this trait. The identified molecular markers, particularly the SCAR marker 865 *F3'5'H*, are direct outcomes of this work. The work further demonstrates that comparative analysis of promoter sequences of flavonoid biosynthesis genes helps identify hybrid forms and detect haplotype-specific mutations.

Consequently, this research directly contributed to the breeding of the first Russian white clover cultivar with pink petals, 'Rubin'.

Statements & Declarations

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Competing Interests

The authors declare no conflict of interest.

Author Contributions

Study conception, SAO, IAA. Breeding and morphobiological analysis, IAA. DNA extraction, PCR and electrophoresis, DVA, SAO. Bioinfornatic analysis, primer design and visualization, SA. Original article preparation, SAO, IAA. Review and editing, SAO, IAA, DVA.

Data Availability

All data generated or analysed during this study are included in this published article.

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Figures



Figure 1

Representative phenotype of the selected F5 white clover breeding line



Figure 2

Effect of low temperature on pigmentation in white clover seedlings. Left: normal green coloration. Right: stress-induced burgundy coloration.



Figure 3

Loss of floral pigmentation under low-light conditions. Inflorescences of selected red-flowered (RF) plants revert to white when transferred to a shaded greenhouse, demonstrating the light-dependence of anthocyanin accumulation

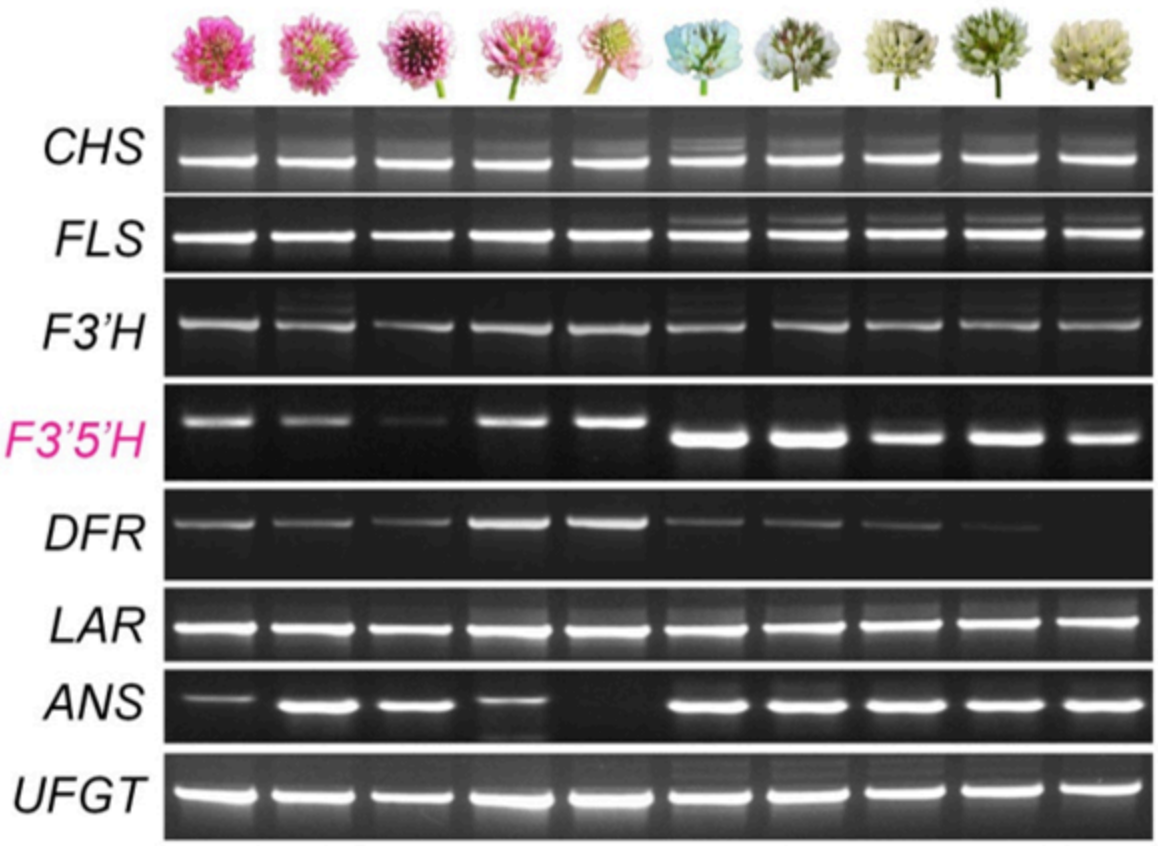


Figure 4

PCR amplification of promoter regions from anthocyanin/flavonoid biosynthesis genes in white clover. The electrophoregram shows amplicons from red-flowered and white-flowered varieties. A clear size polymorphism is evident for the *F3'5'H* gene (marked)



Figure 5

Structural polymorphism in the *F3'5'H* promoter.Alignment reveals length differences between RF and WF sequences caused by insertions specific to the RF plants.

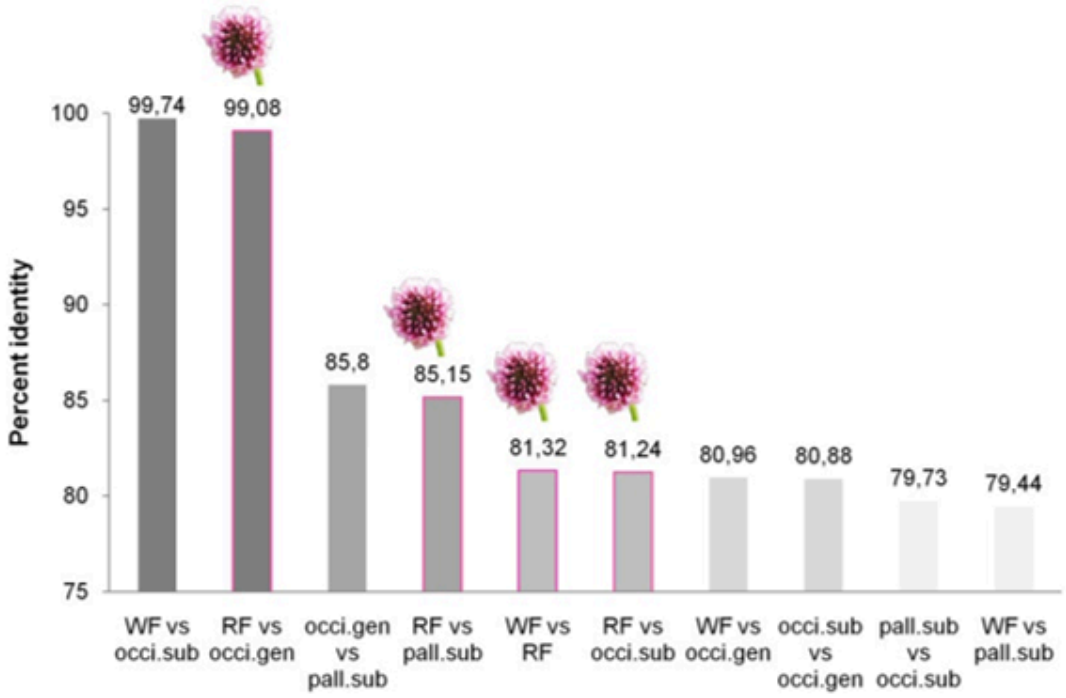


Figure 6

Pairwise percent identity matrix for the *F3'5'H* promoter region.Comparisons are shown between sequences from white-flowered (WF) and red-flowered (RF) plants, the *T.occidentale* subgenome (occi.sub), the whole *T.occidentale* genome (occi.gen), and the *T. pallescens* subgenome (pall.sub) of white clover.

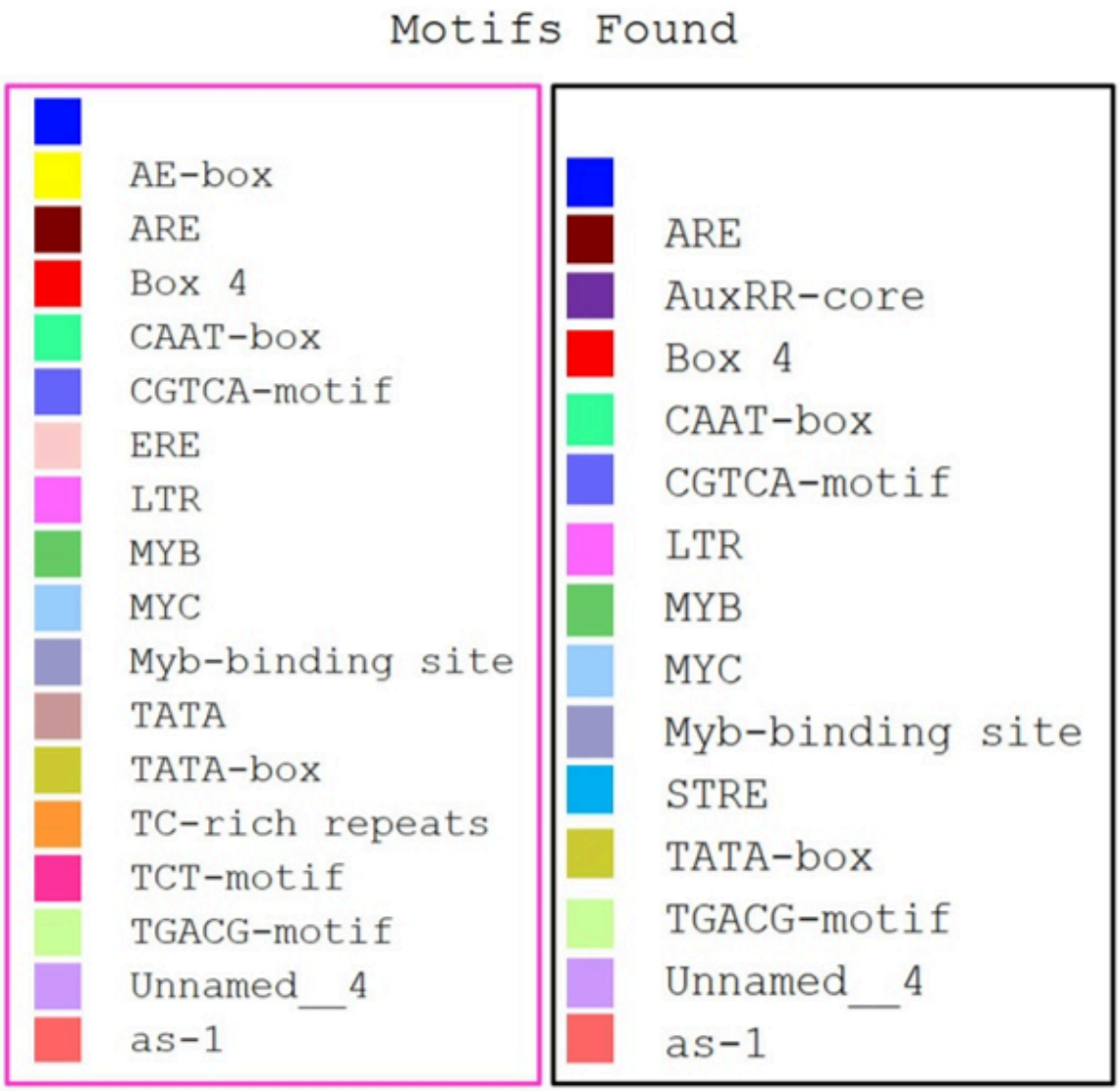


Figure 7

Comparative cis-regulatory element (CRE) profiles of the *F3'5'H* promoter in red-flowered (left) and white-flowered (right) plants.

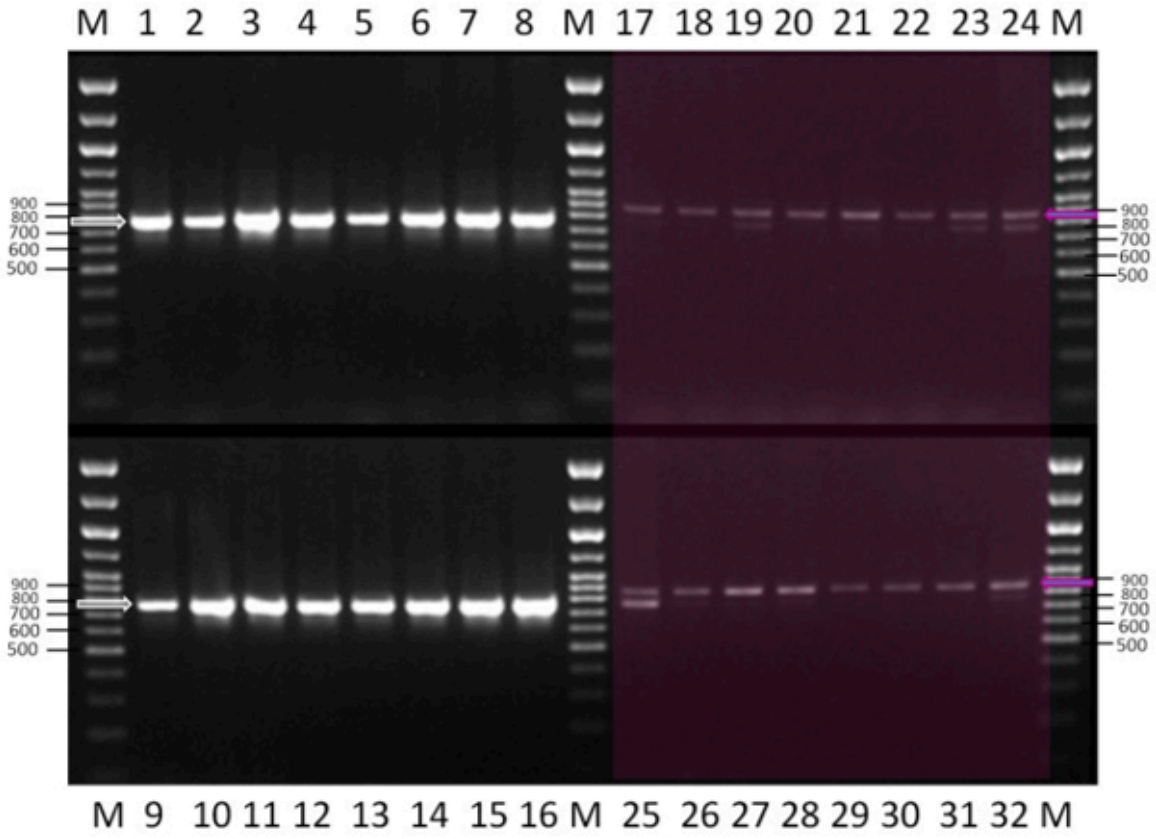


Figure 8

Validation of the diagnostic SCAR marker 865_*F3'5'H*on an extended white clover panel: WF plants (1–16); RF plants (17–32); M—molecular weight marker (Step 100 Long, Biolabmix). The 865-bp amplicon (pink arrow) is present exclusively in all RF lines (17-32) and is absent from all WF plants and varieties tested.



Figure 9

Fertile F1 hybrids obtained from reciprocal crosses between RF and WF *T. repens* plants.