Fine Mapping and Candidate Gene Analysis of the *Up* Locus Determining Fruit Orientation in Pepper (*Capsicum* spp.)

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Research Article

Keywords: Pepper, Fruit orientation, Fine-mapping, Candidate gene analysis

Posted Date: February 24th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-235544/v1

Abstract

Fruit orientation is an important horticultural and domesticated trait, which is controlled by a single semi-dominant gene (*up*) in pepper. However, the gene underlying *up* locus has not yet been identified. In this study, the previously detected major QTL *UP12.1* was firstly verified using an intraspecific backcross population (n=225) stem from the cross of BB3 (*C. annuum*) and its wild relative Chiltepin (*C. annuum* var. *glabriusculum*) using BB3 as the recurrent parent. Then, a large BC_1F_2 population (n=1827) was used for recombinant screening to delimit the *up* locus into an interval with ~169.51 kb in length. Sequence comparison and expression analysis suggested that *Capana12g000958,* encoding a developmentally regulated G-protein 2, was the most likely candidate gene for *up*. The findings of this study will form a basis for gene isolation and reveal of genetic mechanism underlying the fruit orientation domestication in pepper.

Key Message

The *up* locus determining fruit orientation in pepper was fine mapped into a region with a physical length of ~169.51 kb on chromosome P12. *Capana12g000958,* encoding a developmentally regulated G-protein 2, was proposed as the strongest candidate via sequence comparison and expression analysis.

Introduction

Plant organ orientation is governed by antagonistic interactions of the ubiquitous gravitropic mechanism and auxin-dependent antigravitropic offset mechanism (Roychoudhry et al. 2013). It is a crucial important constituent trait determining the morphogenesis of higher plants. The dynamic control model of growth direction has been well established in roots and shoots. In this model, the TIR1/AFB-Aux/IAA-ARF-dependent auxin signaling pathway plays a dominant role in gravity sensing by regulating the magnitude of the antigravitropic offset component (Roychoudhry et al. 2013). However, there is comparatively little attention has been paid to the genetic regulation of fruit/pedicel orientation, which is fundamentally determined by the lateral organ pedicel's adaxial-abaxial polar growth in flowering plants (Venglat et al. 2002).

In *Arabidopsis*, several molecular regulators that govern alterations in pedicel orientation have been identified. They include the *BREVIPEDICELLUS* (*KNAT1/BP*) (Douglas et al. 2002; Venglat et al. 2002), *ASYMMETRIC LEAVES1/2* (*AS1/2*) (Xu et al. 2003), *CORYMBOSA1* (*CRM1/BIG*) (Yamaguchi et al. 2007), *KNOTTED-like2/6* (*KNAT2/6*) (Ragni et al. 2008), *ARABIDOPSIS THALIANA HOMEOBOX GENE1* (*ATH1*) (Li et al. 2012) and *LEAFY* (*LFY*) (Yamaguchi et al. 2012). The Arabidopsis *brevipedicellus* mutant that disrupted in the *KNAT1/BP* gene displays reduced pedicel lengths and downward-oriented flowers (Douglas et al. 2002; Venglat et al. 2002). *KNAT1/BP* negatively regulates *KNAT2*, *KNAT6*, and *ATH1* to ensure that pedicels have a normal upward-pointing orientation (Ragni et al. 2008; Li et al. 2012). *AS1* and *AS2*, two critical regulators of leaf polarity, can also induce pedicels to grow downward by downregulating the expression of *KNAT1/BP*, *KNAT2*, *KNAT6*, and *ATH1* (Li et al. 2012). The *CRM1/BIG*, an auxin transport-related gene, and *LFY*, a hypomorph allele of the meristem identity regulator, are involved in the regulation of *KNAT1/BP* and *AS2* expression (Yamaguchi et al. 2007, 2012). In other non-model plants, *NtSVP* in tobacco, *SlAGO7* in tomato, and *CsUp* in cucumber were found to participate in the developmental regulation of pedicel/fruit orientation (Wang et al. 2015; Lin et al. 2016; Sun et al. 2019).

Pepper (*Capsicum* spp.) belongs to the Solanaceae family and is one of the earliest domesticated crops in Central and South America with versatile applications (Cheng et al. 2016b). It is widely used in the fields of vegetable consumption, medicine (Hernández-Ortega et al. 2012), biological control (Castillo-Sánchez et al. 2012), and military

(Reilly et al. 2001) nowadays. Fruit orientation is one of the domesticated traits in pepper (Paran and Van Der Knaap 2007). Currently, most cultivated species of pepper have transformed from the wild erect type of fruit orientation to the pendant one. This change may be associated with an increase in fruit size, better protection from sun exposure, and predation by birds (Paran and Van Der Knaap 2007). Fruit orientation is also an important horticultural trait for pepper because it influences pollination, yield, and harvesting approaches (Lee et al. 2008; Wang et al. 2014a). However, the underlying genetic mechanism of this trait remains poorly understood.

Previous genetic analyses showed that the pepper fruit's erect trait is controlled by a single recessive gene which was named *up* (Lippert et al. 1965; Cheng et al. 2016a). Two linked markers, namely A_2C7_{469} and *up*CAPS, were developed with a genetic distance of 1.7 cM and 4.3 cM from the *up* locus (Lee et al. 2008), respectively. Furthermore, two major and four minor quantitative trait loci (QTLs) were detected under four different environments based on an ultra-high-density bin map. Of which, a stable and major QTL, namely *FP-12.2*, was identified at 199.6 Mb on the pepper chromosome P12 in the CM334 genome, explaining over 40% of the phenotypic variation (Han et al. 2016). In addition, a major QTL, named *Up12.1*, was identified at 36.54–41.06 Mb on the chromosome P12 in the Zunla-1 genome based on a high-density single nucleotide polymorphism (SNP) map. There were 65 protein-coding genes were predicted within this QTL region based on the current annotation of the Zunla-1 genome (Cheng et al. 2016a).

In this study, the previous detected major QTL *UP12.1* was firstly verified using an intraspecific backcross population stem from the cross of BB3 (*C. annuum*) and its wild relative Chiltepin (*C. annuum* var. *glabriusculum*) using BB3 as the recurrent parent. Furthermore, a large BC_1F_2 population was constructed and used for recombinant screening and fine mapping of the *up*. The candidate genes embedded in the fine mapping region were then analyzed by sequence and expression comparisons. The expression profile of a strong candidate, *Capana12g000958*, was elaborately examined in various tissues at different developmental stages. The findings of this study will form a basis for the gene isolation and revealing of the underlying genetic mechanism of fruit orientation domestication in pepper.

Materials And Methods

Plant materials

The pepper inbred line BB3 (*C. annuum*) and its wild relative Chiltepin (*C. annuum* var. *glabriusculum*) were used as the female and male parents, respectively, to construct a backcross population (BC_1F_1, n = 225) with BB3 as the recurrent parent to verify the *UP12.1* interval previously detected based on the BA3 (*C. annuum*) × YNXML (*C. frutescens*) F_2 population (Cheng et al. 2016a). The BB3, Chiltepin, and their F_1 and BC_1F_1 population were grown at SCAU Main Campus Teaching & Research Base, Guangzhou, China (23°N, 113°E).

A total of 1,827 BC_1F_2 individuals were generated by artificial self-pollination with the heterozygous individuals from the BC_1F_1 population to narrow down the *up* candidate interval. Fruit orientation phenotypes were evaluated with at least five flowers/fruits were recorded from stage S3 to S7 (Fig. 1) for each plant based on the ELV (E: erect, LP: lateral pendant, VP: vertical pendant) classification method as described previously (Cheng et al. 2016a).

Marker genotyping

Insertion and deletion (InDel) loci were identified based on sequence comparisons between the reference genome sequences of Zunla-1 and Chiltepin (Qin et al. 2014). Primer pairs flanking the InDel loci were designed by using

Primer3web (version 4.1.0; http://primer3.ut.ee/). DNA was extracted from young leaves by using the modified CTAB (Murray and Thompson 1980) and then preserved at − 20°C before genotyping. The PCR was performed using a 20 μL reaction mixture, which contains 2.0 μL DNA template (50 ng/μL), 2.0 μL PCR buffer (10×), 2.0 μL Mg^{2+} (25 mM), 1.5 μL forward and reverse primer (1 μM), 0.2 μL dNTPs (10 mM), and 1U *Taq* DNA polymerase. PCR reactions were performed as follow: 94°C for 3 min, 32 cycles of 94°C for 30 s, 55°C for 30 s, and 1 min at 72°C; and a final extension at 72°C for 10 min. PCR products were genotyped using 6% polyacrylamide gel electrophoresis.

Gene cloning

The primer sequences used for candidate gene cloning were listed in Supplementary Table S1. The PCR amplicons of candidate genes were ligated to the pMD-19T cloning vector (Takara, Tokyo, Japan). At least three randomly selected positive colonies for each amplicon were sequenced and assembled. Nucleotides and amino acid sequences were aligned using DNAMAN (version 9).

Expression analysis

For comparative expression analysis, the mixed samples comprised of the flower buds (white arrow 1), main stem (white arrow 2) close to the first branching point and the pedicel (white arrow 3) were excised as a whole at four developmental stages including S1 to S4 (Fig. 1), respectively, for RNA isolation. To characterize the expression profile of *Capana12g000958*, the flower bud/fruit and pedicel samples at seven developmental stages (S1 to S7, as described in Fig. 5) were excised separately from the BB3, Chiltepin, and BB3×Chiltepin (F_1), respectively, for RNA isolation. Every sample was collected in three biological replicates, wrapped in tin foil, frozen directly in liquid nitrogen, and then stored at − 80°C for subsequent experiments.

Total RNA was isolated by using the Eastep® Super Total RNA Extraction Kit (LS1040; Promega, Madison, America) following the manufacturer's instructions. First-strand cDNA was synthesized by using a cDNA synthesis kit (Takara, Tokyo, Japan). qRT-PCR was performed in triplicate by using SYBR Green PCR master mix (Takara, Tokyo, Japan) on a BioRad CFX96 system (Bio-Rad, CA, USA). The primer sequences of the candidate genes and *Ca-Actin* were listed in Supplementary Table S2. PCR was performed with an initial denaturation step set at 94°C for 3 min, followed by 39 cycles of denaturation and annealing at 94°C for 10 s and 55°C for 30 s, respectively. The relative expression level was analyzed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). All reactions were performed in triplicate with three biological replicates for each sample.

Results

Dynamic change and inheritance of fruit orientation

Through continuous observation, we found that the orientation of flower buds displayed no difference at the stage S1 when corolla was tightly wrapped by the calyx between BB3 and Chiltepin (Fig. 1). From stage S3 to S7, BB3 and Chiltepin exhibited an obvious difference in pedicel or fruit orientation with the significant change that occurred from the stage S2 to S3 (Fig. 1). After stage S3, all pedicels and fruits from the BB3 plants displayed vertical pendant phenotype, while that of the Chiltepin plants exhibited erect orientation, and all of the BB3 × Chiltepin (F_1) plants showed a lateral pendant (LP) pedicel/fruit orientation phenotype (Fig. 1). Among the BC_1F_1 population (n = 225), 111 plants showed a vertical pendant (VP) fruit orientation similar to that of BB3, and 114 presented the lateral

pendant (LP) fruit orientation as with that of the BB3×Chiltepin (F_1). Chi-square tests revealed that the observed segregation in the BC_1F_1 population fitted the expected ratio 1:1 ($\chi 2 = 0.04$, $P > 0.05$). These findings indicated that variations in fruit orientation between BB3 and Chiltepin were controlled by a single semi-dominant gene.

Verification of the major QTL *Up12.1*

Fourteen polymorphic InDel markers (Supplementary Table S3) between BB3 and Chiltepin were developed within the *Up12.1* interval and then were used to genotype the 225 BC_1F_1 individuals. Linkage mapping demonstrated that all markers had a tight linkage less than 2.34 cM with fruit orientation (Fig. 2a). Out of the 14 polymorphic markers, seven markers including the FO-551, FO-818, FO-771, FO-704, FO-78, FO-616, and FO-508 were found to be co-segregated with the *up* locus among the 225 BC_1F_1 individuals (Fig. 2a). This finding suggested that *Up12.1* was also responsible for variation in fruit orientation between BB3 and Chiltepin. The *up* locus was therefore delimited within the interval between marker FO-572 and FO-277 with a physical length of ~ 2.43 Mb (Fig. 2a).

Fine mapping of the *up* locus

A large BC_1F_2 population consisting of 1,827 individuals was developed to screen recombinant events by using the markers FO-572, FO-277, as well as FO-616 (Fig. 2b). A total of 11 recombinant plants were identified between FO-572 and FO-277, and eight out of them underwent recombination between the FO-572 and FO-616 (Fig. 2c). Additional nine polymorphic InDel markers (Supplementary Table S4) were developed within the interval between FO-572 and FO-277 and then were used for genotyping of the 11 recombinants. Results showed that three markers, namely FO-771, FO-676 and FO-946, were found to be co-segregated with the fruit orientation (Fig. 3). As a result, the *up* locus could be eventually localized between the markers FO-818 and FO-951 with a physical distance of ~ 169.51 kb (Fig. 2b and Fig. 3).

Sequence comparisons of the candidate genes

A total of seven protein-coding genes were annotated within the fine-mapping interval of the *up* locus (Table 1). Of which, *Capana12g000958* was highly orthologous (Bit score = 614 and E-value = 0) to the *Arabidopsis thaliana* developmentally regulated G-protein 2 (*DRG2*) gene (Supplementary Figure S1). *Capana12g000960* and *Capana12g000966* showed a high degree of sequence similarity (Identity = 99.70%) to each other and both of them encode ELF4-LIKE3 protein (Supplementary Figure S2). The rest of the four candidate genes including *Capana12g000959*, *Capana12g000962*, *Capana12g000964* and *Capana12g000965* had no significant (E-value > 1e-5) homologous genes in the public database to date (Table 1).

Table 1
List of seven candidate genes for the pepper *up* locus.

Gene	Start	End	Gene symbol	Homologous species	Homologous protein	E-value	Bit score
Capana12g000958	37,644,791	37,650,731	*DRG2*	*Arabidopsis thaliana*	Developmentally regulated G-protein 2	0	614
Capana12g000959	37,683,574	37,688,329	\	\	\		
Capana12g000960	37,689,586	37,689,918	*EFL3*	*Arabidopsis thaliana*	Protein ELF4-LIKE 3	1.6E-38	128
Capana12g000962	37,733,635	37,734,657	\	\			
Capana12g000964	37,739,818	37,740,560	\	\	\		
Capana12g000965	37,783,070	37,785,471	\				
Capana12g000966	37786745	37787077	*EFL3*	*Arabidopsis thaliana*	Protein ELF4-LIKE 3	1.6E-38	127

We tried to clone and compare the full cDNA sequences of all the above-mentioned candidate genes between BB3 and Chiltepin. However, we could not obtain any amplification product for *Capana12g000962*, *Capana12g000964* and *Capana12g000965*, using all the tested cDNA samples from roots, stems, leaves, flowers and fruits as template. We checked their expression in another 46 pepper transcriptome data and found they almost were not expressed (Supplementary Figure S3). As for the other four genes, there was no variation for coding sequences of *Capana12g000958* (Supplementary Figure S4), *Capana12g000960* and *Capana12g000966* (Supplementary Figure S2), but two non-synonymous SNPs were found in that of *Capana12g000959* (Supplementary Figure S5) between BB3 and Chiltepin.

Comparative expression analysis of the candidate genes

To further determine which candidate may be responsible for the differentiation of fruit orientation, we compared the expression levels of *Capana12g000958*, *Capana12g000959* and *Capana12g000960/66* in the mixed samples of flower buds, main stem close to the first branching point and pedicel from stage S1 to S4 among the BB3, Chiltepin and BB3×Chiltepin (F_1). The results showed that *Capana12g000958*'s expression was gradually increased from stage S1 to S4 in BB3, Chiltepin and BB3×Chiltepin (F_1). Notably, *Capana12g000958* displayed significantly difference between BB3 and Chiltepin at all four stages (Fig. 4a). The expression levels of *Capana12g000959* (Fig. 4b) and *Capana12g000960/ Capana12g000966* (Fig. 4c) did not change much from stage S1 to S4 in BB3, Chiltepin and BB3×Chiltepin (F1). In addition, there was no obvious expression difference for *Capana12g000959, Capana12g000960/ Capana12g000966* at all stages between BB3 and Chiltepin with the exception of that at stage S4 for *Capana12g000959* (Fig. 4b and 4c).

Expression profile of *Capana12g000958* in pepper

Sequence and expression comparisons collectively indicated that *Capana12g000958* was the most likely candidate gene for *up*. As a result, we conducted an elaborate sample collection to characterize the expression profile of

Capana12g000958 in pepper. Results showed that the expression level of *Capana12g000958* represented an upward tendency from stage S1 to S4 in the flowers (Fig. 5a), which was similar to that in the mixed samples (Fig. 4a), and then it maintained a constant low expression level from stage S5 to S7 of fruits in BB3, Chiltepin and BB3×Chiltepin (F_1) (Fig. 5a). Furthermore, *Capana12g000958* was expressed significantly higher in BB3 than that in both Chiltepin and BB3×Chiltepin (F_1) at stage S4 in the flower (Fig. 5a). With respect to the *Capana12g000958*'s expression in the pedicle, a trend of rising from stage S1 to S4 and falling from stage S5 to S7 was observed in BB3, Chiltepin and BB3×Chiltepin (F_1). Intriguingly, the expression of *Capana12g000958* in BB3 was significantly higher than that in both Chiltepin and BB3×Chiltepin (F_1) after stage S2 (Fig. 5b). In addition, tissue-specific expression analysis showed *Capana12g000958*' expression was higher in the flower-related tissues including receptacles, anthers, and petals than that in the root, stem and leave (Supplementary Figure S6).

Discussion

Fruit orientation is an important horticultural trait for pepper because it has significant impact on pollination efficiency, yield, and harvesting approaches (Lee et al. 2008; Wang et al. 2014a). Herein, in order to avoid the deficiency of sterility and incompatibility of the interspecific cross between BA3 (*C. annuum*) and YNXML (*C. frutescens*) (Cheng et al. 2016a), we turned to use the intraspecific population derived from the cross of BB3 (*C. annuum*) and its wild progenitor Chiltepin (*C. annuum* var. *glabriusculum*) to fine map the *up* locus. Linkage mapping demonstrated that the major QTL *Up12.1* was also responsible for fruit orientation variation in the BB3 × (BB3 × Chiltepin) population (Fig. 2a). The result of this study adds new evidence to our previous deduction that the *Up12.1* could be the same QTL as *FP-12.2*, which was also identified in an intraspecific RIL population of *C. annuum* (Han et al. 2016), indicating that the origin of the *up* gene should occur before the species differentiation between *C. annuum* and *C. frutescens*.

The completion of pepper genome sequencing has markedly accelerated the mining of target genes through forward genetics approach although it's still a draft version (Kim et al. 2014; Qin et al. 2014; Hulse-Kemp et al. 2018). In this study, there is an inconsistence between the genetic and physical order within the preliminary mapping interval from marker FO-508 to FO-620 (Fig. 2a, 2b and Supplementary Table S3). This entire interval was found to be located on the scaffold445 (Fig. 2) whose orientation has not yet been defined because it was anchored only by one SNP (Cheng et al. 2020). Thus, in addition to the FO-572 and FO-277, we also used the FO-616 to screen recombinants from the large population. The recombinant events of FO-616, FO-277 and FO-508 further confirmed the existence of scaffold inversion (Fig. 2b). Nevertheless, because the co-segregation region was located on another scaffold (scaffold1796), this inversion should make no difference to the final fine mapping (Fig. 2). Based on the analysis of recombinants identified from the large population, the candidate interval for *up* locus was narrowed into a region between FO-818 and FO-951 with a physical length of 169.51 kb (Fig. 2). To our knowledge, this is the first time such a precise interval has been reported for the *up* locus to date.

Seven protein-coding genes were annotated in this fine mapping interval (Table 1), however, none of them showed homology to the known regulators governing alterations of pedicel orientation (Cheng et al. 2016a), indicating that the pepper *up* gene is possibly a new member that participated in the pedicel orientation regulation pathway. Further expression analysis showed that three out of the seven candidates, *Capana12g000962*, *Capana12g000964* and *Capana12g000965*, were very likely to be pseudogenes because they were not expressed in all the tested samples from this study as well as in another 46 pepper samples from different sources (Supplementary Figure S3). For *Capana12g000960/66*, there was no difference either in the expression level or sequence comparison between BB3 and Chiltepin (Fig. 4c and Supplementary Figure S2). Sequence comparison between BB3 and Chiltepin revealed that

there were two non-synonymous SNPs in the coding sequences of *Capana12g000959*, but they do not exist in the Zunla-1 and YNXML (Supplementary Figure S5), both of which exhibit erect fruit orientation. With regard to *Capana12g000958*, we found its spatial and temporal differential expression between parental lines in the pedicle was highly corresponding to the fruit orientation change (Fig. 1 and Fig. 5b). Furthermore, a SNP located in the promoter region was in line with the fruit orientation phenotype of eight pepper breeding lines (958pro_SNP4_37651506, Supplementary Figure S7) despite there was no difference in the coding region. Taken together, we suggested that *Capana12g000958* was the strongest candidate gene for *up*.

Capana12g000958 shared ~ 88% of amino acid sequence similarity with the *Arabidopsis thaliana* DRG2 (Supplementary Figure S1), which belongs to the highly conserved GTP-binding protein (also known as G protein) family that found in archaea, plants, fungi and animals, indicating their pivotal roles in fundamental pathways (Ma 1994; O'Connell et al. 2009). Plant G-proteins have been implicated in regulation of almost every aspect of growth, development, hormonal perception, response to biotic and abiotic stresses (Assmann 2002; Pandey and Vijayakumar 2018). Intriguingly, several G proteins were identified as vital regulators of plant organ growth direction. For example, in *Arabidopsis*, the *AGB1* encoding the β-subunit of the G protein negatively regulates auxin-induced cell division and affects the hook angle (Ullah et al. 2003; Chakravorty et al. 2012). Rop2 GTPase, a kind of small G protein, can regulate the early phase of organogenesis's directional cell expansion (Fu et al. 2002; Jones et al. 2002). A complex, composing of ROP GTPases, its activators and effectors, and AGC1.5 subfamily kinases can regulate the polar dynamic distribution of apical growth (Li et al. 2020). In rice, the *Dwarf 1* and *DEP1* encoding the α-subunit and γ-subunit of heterotrimeric G protein, respectively, can regulate the panicle architecture (Ashikari et al. 1999; Huang et al. 2009). At the post-transcriptional level, small Ras GTP-binding protein was found to be related to fruit bending in cucumber (Wang et al. 2014b). All above evidences prompt speculation that *Capana12g000958* might be a signaling molecule involved in the dynamic control of fruit orientation in pepper. Further investigation would be necessary to analyze the function of *Capana12g000958* in future.

Declarations

Acknowledgments

This research was supported by the National Natural Science Foundation of China (31701921, 31672162), Guangdong Basic and Applied Basic Research Foundation (2019A1515011784), and Guangzhou Science and Technology Plan Projects (201704020019).

Author contribution statement

FH, JWC, and KLH conceived and designed all experiments; FH, JCD, JZ performed the experiments; FH and JWC analyzed the data; FH, JWC and KLH wrote the manuscript; All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors declare that they have no conflict of interest.

Ethical statement

Authors declare that this study complies with the current laws of China.

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Figures

Figure 1

Dynamic development of fruit orientation in BB3, F1 and Chiltepin (a) BB3 with vertical pendant (VP) flower and fruit. (b) F1 with lateral pendant (LP) flower and fruit. (c) Chiltepin with erect (E) flower and fruit. S1, S2, S3, S4 and S7 represent different stages of flower buds (S1: the corolla is wrapped by calyx. S2: the calyx split slightly and the flower stalk bends. S3: the flower will bloom the next day. S4: fully opened flower) and mature fruit (S7: 55 days after flowing), respectively. 1, 2 and 3 represent the sampling site of the sessile flowers, pedicel and main stem close to the first branching point, respectively

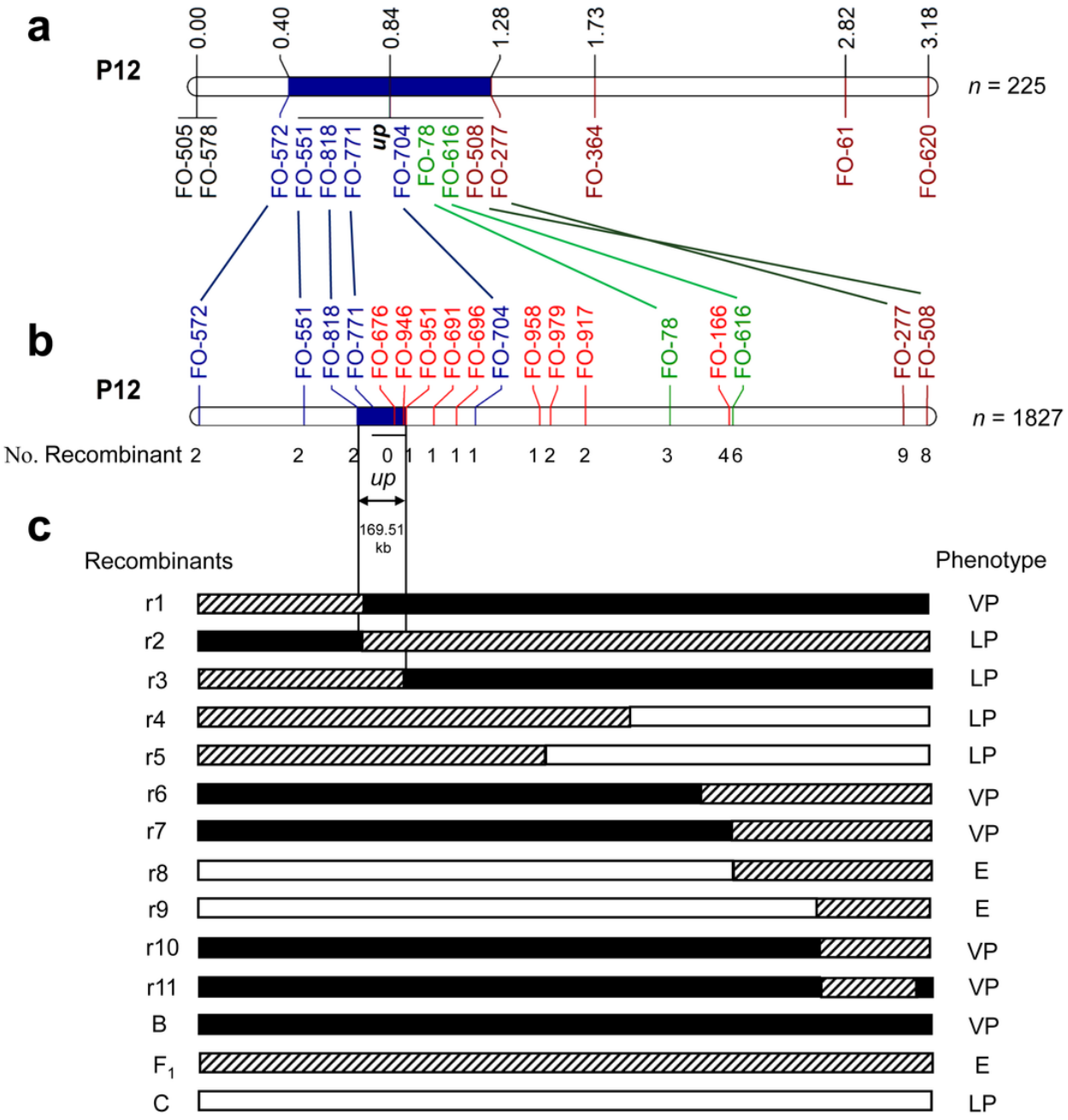

Figure 2

Fine-mapping of the up gene (a) genetic map of the preliminary mapping interval from the BC1F1 (BB3 × Chiltepin) population. The dark blue segment indicates the candidate region of the up gene. The solid line represents the collinearity analysis of physical and genetic maps. The black, blue, green and brown labeled markers were anchored

to the Scaffold5893, Scaffold1796, Scaffold772 and Scaffold445, respectively. (b) The up falls within the interval between FO-818 and FO-951 in the fine map generated from analysis of 1827 BC1F2 segregants. The red markers are newly developed for fine mapping. The numbers below the line are the recombinants. (c) Graphical genotypes of BC1F2 recombinants and their corresponding fruit orientation phenotypes. The genetic composition of each recombinant category is shown in different colors. Black, white, and grid rectangles denote homozygous BB3 genotype (VP, vertical pendant), homozygous Chiltepin genotype (E, erect), and heterozygous F1 genotype (LP, lateral pendant), respectively. r1-r11 represents recombinants

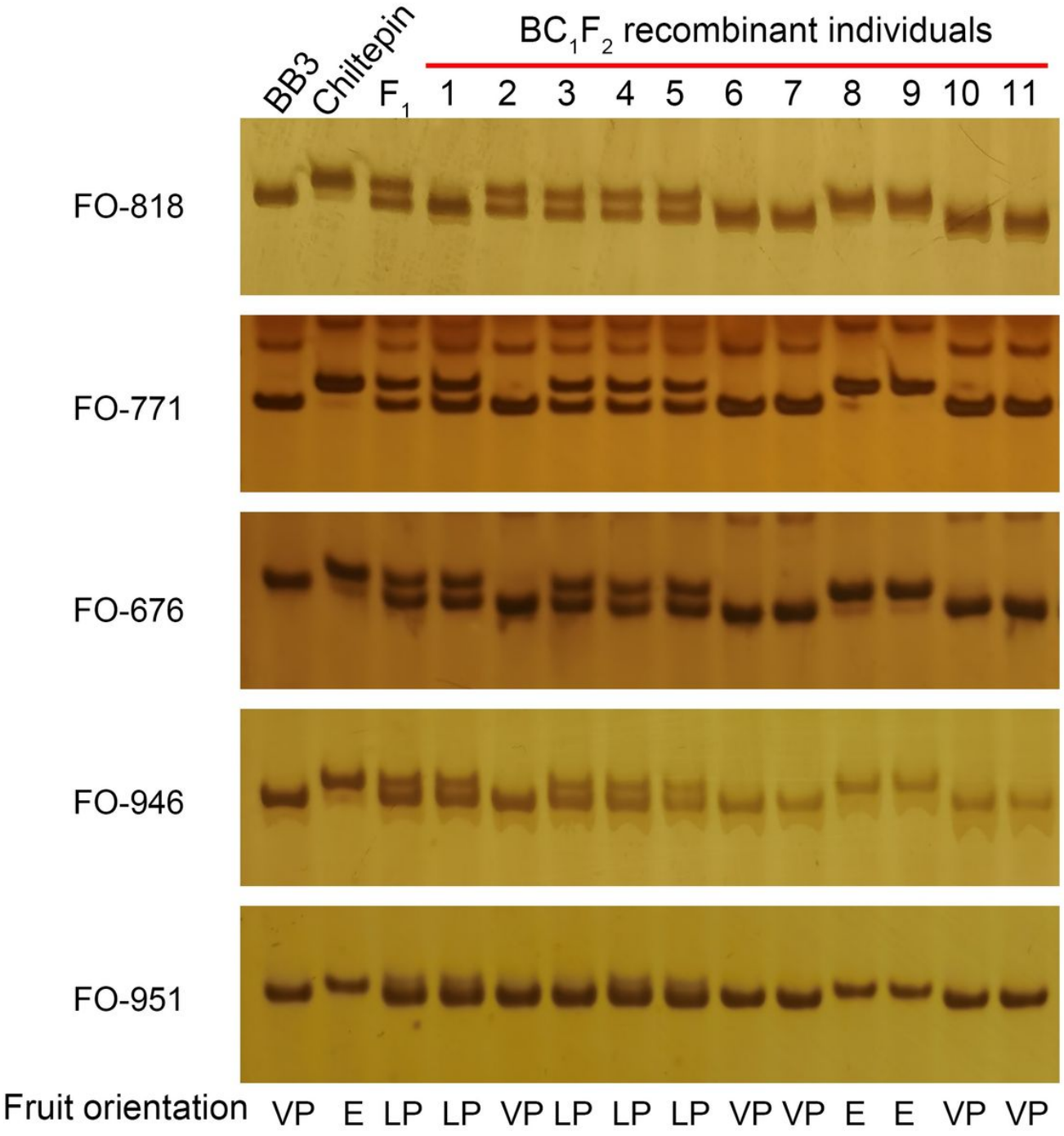

Figure 3

Genotyping results of several markers located in the fine mapping interval from the 11 BC1F2 recombinants E, VP anf LP represent the erect, vertical pendant and lateral pendant fruit orientation phenotypes, respectively. S1 ~ S4

represent different stages of flower buds. S5 ~ S7 represent different development stages of fruit (33, 44 and 55 days after flowing), respectively

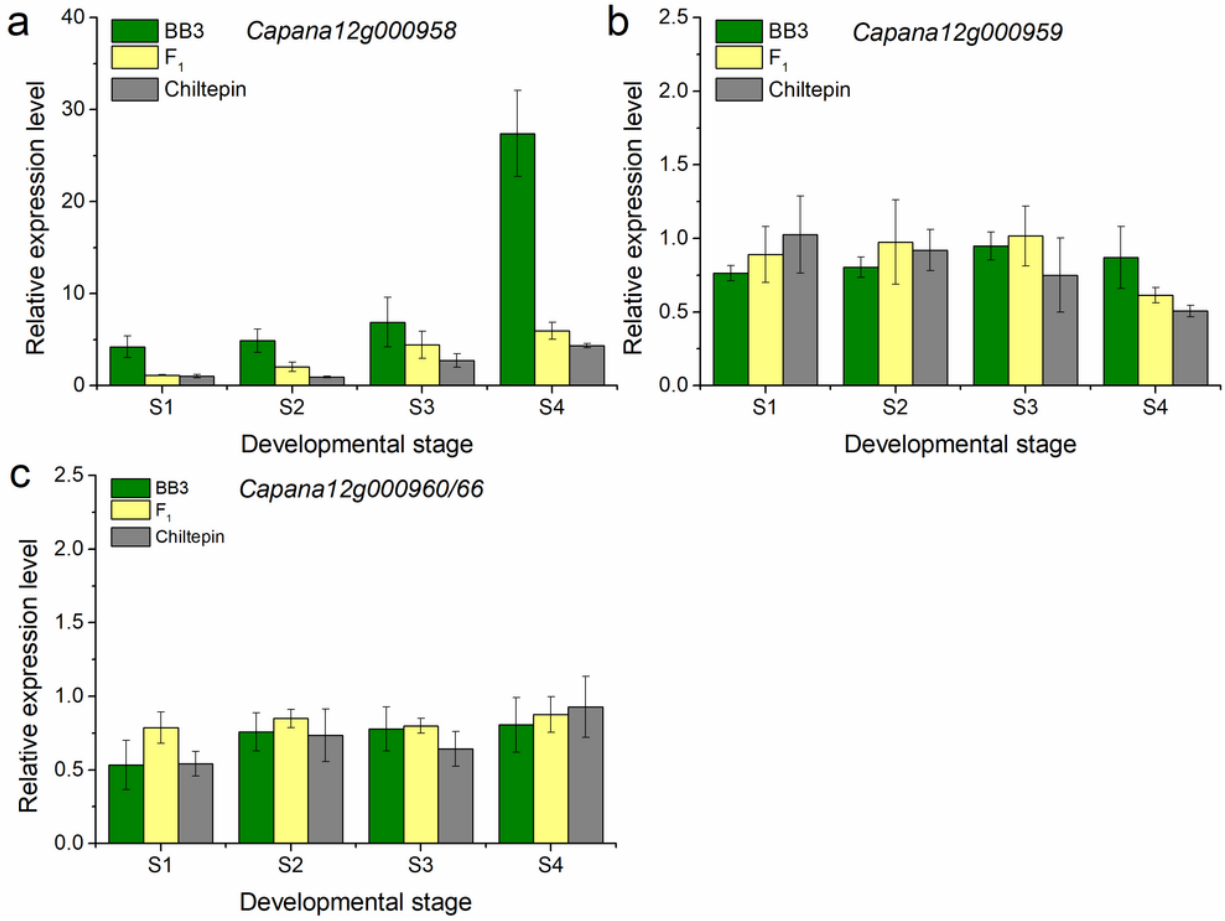

Figure 4

Relative expression of the candidate genes in the mixed samples (stems, pedicels and flower buds) of BB3, F1 and Chiltepin

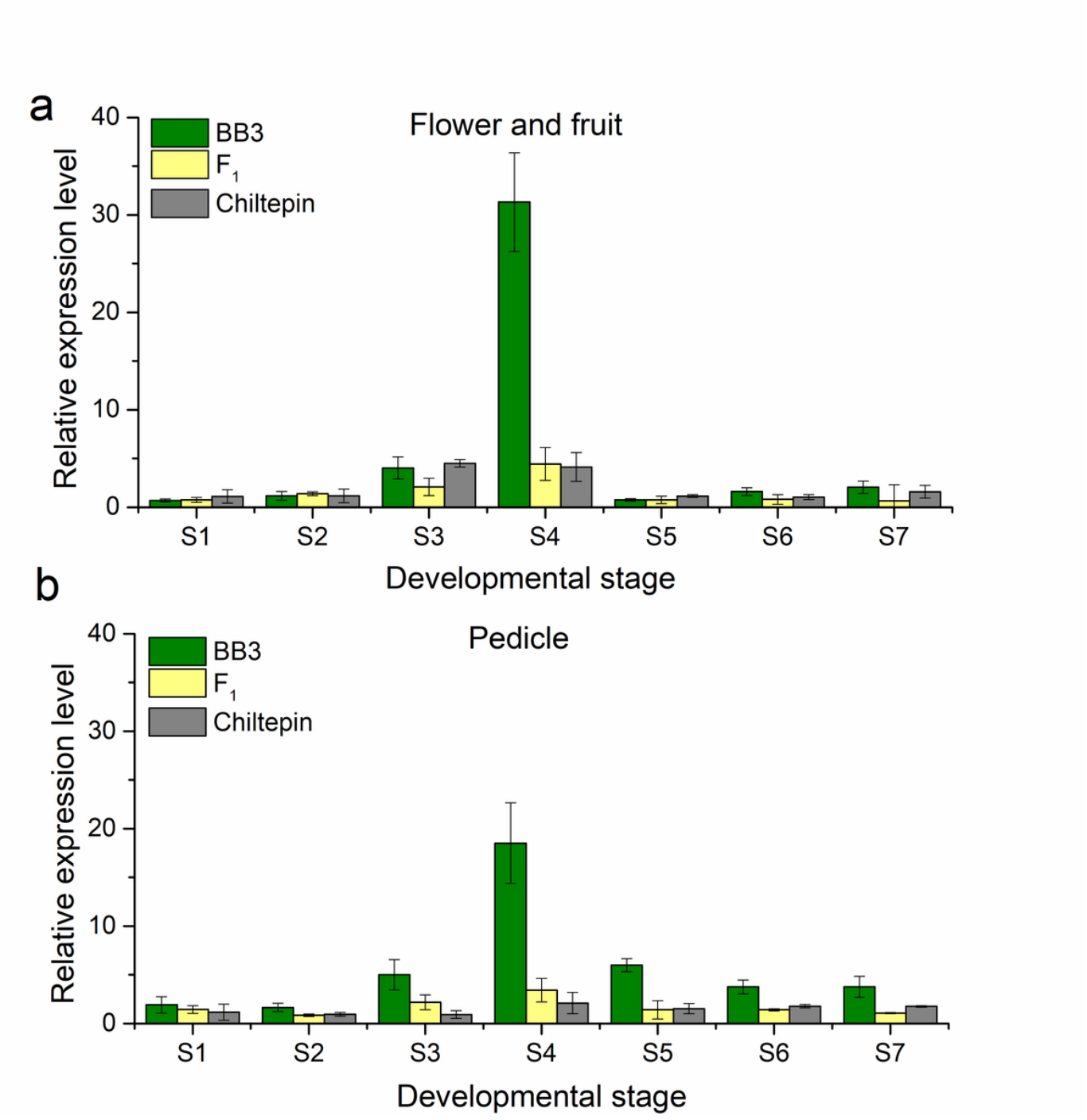

Figure 5

Expression profile of Capana12g000958 based on qPCR analysis in BB3,Chiltepin and BB3×Chiltepin (F1). (a) The expression level of Capana12g000958 in the flowers from stage S1 to S4, and in fruits from S5 to S7. (b) The expression level of Capana12g000958 in the in pedicles from stage S1 to S7

Supplementary Files

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