Fine mapping and candidate gene analysis of the *up* locus determining fruit orientation in pepper (*Capsicum* spp.)

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Abstract

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

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 a 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 BC1F2 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 the *up* locus. There is no difference within the coding sequences of *Capana12g000958* between BB3 and Chiltepin, while a SNP in the upstream of *Capana12g000958* showed a complete correlation with the fruit orientation among a panel of 40 diverse pepper inbred lines. These findings will form a basis for gene isolation and reveal of genetic mechanism underlying the fruit orientation domestication in pepper.

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 HOME-OBOX 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). *ASI* 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 A32 expression (Yamaguchi et al. 2007, 2012). In other non-model plants, NiSVP in tobacco, SIAGO7 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 A2C7 up and upCAPS, were developed with a genetic distance of 1.7 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 pepper 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 the fine mapping region of the major QTL, named UP12.1, which contains 2.0 Mb on the pepper chromosome P12 in the Zunla-1 genome based on a high-density single nucleotide polymorphism (SNP) map.

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 BC1F2 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, Capana12p000958, 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 (BC1F1, 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) F2 population (Cheng et al. 2016a). The BB3, Chiltepin, and their F1 and BC1F1 population were grown at SCAU Main Campus Teaching & Research Base, Guangzhou, China (23°N, 113°E).

A total of 1827 BC1F2 individuals were generated by artificial self-pollination with the heterozygous individuals from the BC1F1 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

Based on sequence comparisons between the reference genome sequences of Zunla-1 and Chiltepin (Qin et al. 2014), a total of 723 pairs insertion and deletion (InDel) markers were designed by using Primer3web (version 4.1.0, http://primer3.ut.ee/). Of these, 461 pairs primers were designed in the region of the major QTL Up12.1. 262 pairs primers were designed at different stages of the fine mapping process. All markers were genotyped for selecting polymorphism markers between BB3 and Chiltepin using 6% polyacrylamide gel electrophoresis. 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 MgCl2 (25 mM), 1.5 μL forward and reverse primers (1 μM), 0.2 μL dNTPs (10 mM), and 1U Taq DNA polymerase. PCRs were performed as follows: 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 and the upstream and downstream of *Capana12g000958* cloning are listed in Supplementary Table S1. The PCR amplicons of candidate genes were ligated to the pMD-19 T cloning vector (Takara, Tokyo, Japan). At least three randomly selected positive colonies for each amplicon were sequenced and assembled. The primer sequences (F: TCG CTCATGTGATACGGAGAA; R: CCTGCTTTCTATCACATGAGAA) were used for identifying the SNP loci (958upstream_SNP4_37651506) in the upstream region of *Capana12g000958* among 40 inbred lines with diverse genetic backgrounds by sequencing. 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, main stem close to the first branching point and the pedicel 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 (F1), respectively, for RNA isolation. In addition, the fully opened flower and pedicle were collected from 18 inbred lines with diverse genetic background and fruit orientation for verifying the expression differences of *Capana12g000958*. 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 Bio-Rad CFX96 system (Bio-Rad, CA, USA). The primer sequences of the candidate genes and *Ca-Actin* are listed in Supplementary Table S2. PCR was performed with an initial denaturation step set at 94 °C for

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**Fig. 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 flowering), respectively. White arrows 1, 2, and 3 represent the sampling site of the sessile flowers, pedicel and main stem close to the first branching point, respectively.
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 (F1) plants showed a lateral pendant (LP) pedicel/fruit orientation phenotype (Fig. 1). Among the BC1F1 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 (F1). Chi-square tests revealed that the observed segregation in the BC1F1 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**

Among the 461 InDel primers, a total of 14 polymorphic markers (Supplementary Table S3) were selected and used to the genotype of 225 BC1F1 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 BC1F2 segregants. The red markers were newly developed for fine mapping. The numbers below the line are the recombinants. e Graphical genotypes of BC1F2 recombinants and their corresponding fruit orientation phenotypes. The genetic composition of each recombinant category was 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 represent recombinants.
225 BC₁F₁ individuals (Fig. 2a). This finding suggested that \textit{Up}12.1 was also responsible for variation in fruit orientation between BB3 and Chiltepin. The \textit{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 \textit{up} locus**

A large BC₁F₂ 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 \textit{up} locus could be eventually localized between the markers FO-818 and FO-951 with a physical distance of ~ 169.51 kb (Figs. 2b and 3).

A total of seven protein-coding genes were annotated within the fine-mapping interval of the \textit{up} locus (Table 1), of which, \textit{Capana12g000958} was highly orthologous (Bit score = 614 and E-value = 0) to the \textit{Arabidopsis thaliana} developmentally regulated G protein 2 (\textit{DRG2}) gene (Supplementary Figure S1). \textit{Capana12g000960} and \textit{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 \textit{Capana12g000959}, \textit{Capana12g000962}, \textit{Capana12g000964}, and \textit{Capana12g000965} had no significant (E-value > 1e-5) homologous genes in the public database to date (Table 1).

**cDNA sequence comparisons of the candidate genes**

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 \textit{Capana12g000962}, \textit{Capana12g000964}, and \textit{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 \textit{Capana12g000958} (Supplementary Figure S4), \textit{Capana12g000960} and \textit{Capana12g000966} (Supplementary Figure S2), but two nonsynonymous SNPs were found in that of \textit{Capana12g000959} (Supplementary Figure S5) between BB3 and Chiltepin.

![Fig. 3 Genotyping results of several markers located in the fine mapping interval from the 11 BC₁F₂ recombinants. E, VP, and LP represent the erect, vertical pendant and lateral pendant fruit orientation phenotypes, respectively.](image)

**Table 1** List of seven candidate genes for the pepper \textit{up} locus

| Gene            | Start   | End     | Gene symbol | Homologous species   | Homologous protein            | E-value   | Bit score |
|-----------------|---------|---------|-------------|----------------------|------------------------------|-----------|-----------|
| \textit{Capana12g000958} | 37,644,791 | 37,650,731 | DRG2        | \textit{Arabidopsis thaliana} | Developmentally regulated G protein 2 | 0         | 614       |
| \textit{Capana12g000959} | 37,683,574 | 37,688,329 | –           | –                    | –                           | –         | –         |
| \textit{Capana12g000960} | 37,689,586 | 37,689,918 | \textit{EFL3} | \textit{Arabidopsis thaliana} | Protein ELF4-LIKE 3 | 1.60E-38 | 128       |
| \textit{Capana12g000962} | 37,733,635 | 37,734,657 | –           | –                    | –                           | –         | –         |
| \textit{Capana12g000964} | 37,739,818 | 37,740,560 | –           | –                    | –                           | –         | –         |
| \textit{Capana12g000965} | 37,783,070 | 37,785,471 | –           | –                    | –                           | –         | –         |
| \textit{Capana12g000966} | 37,786,745 | 37,787,077 | \textit{EFL3} | \textit{Arabidopsis thaliana} | Protein ELF4-LIKE 3 | 1.60E-38 | 127       |
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 (F1). The results showed that Capana12g000958’s expression was gradually increased from stage S1 to S4 in BB3, Chiltepin and BB3 × Chiltepin (F1). 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 Capana12g000958 (Fig. 4b and c).

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 (F1) (Fig. 5a). Furthermore, Capana12g000958 was expressed significantly higher in BB3 than that in both Chiltepin and BB3 × Chiltepin (F1) at stage S4 in the flower (Fig. 5a). With respect to the Capana12g000958’s expression in the pedicel, a trend of rising from stage S1 to S4 and falling from stage S5 to S7 was observed in BB3, Chiltepin, and BB3 × Chiltepin (F1). Intriguingly, the expression of Capana12g000958 in BB3 was significantly higher than that in both Chiltepin and BB3 × Chiltepin (F1) after stage S2 (Fig. 5b).

In addition, tissue-specific expression analysis showed Capana12g000958’s expression was higher in the flower-related tissues including receptacles, anthers, and petals than that in the root, stem, and leave in BB3 (Supplementary Figure S6). Then, we investigated the expression differences of Capana12g000958 in the flower and pedicle from another 18 inbred lines including 8 lines showing vertical pendant fruit orientation and 10 lines showing erect fruit orientation (Fig. 5c). Results showed the expression of Capana12g000958 was higher in the vertical pendant lines than that in the erect ones. Taken together, we suggested that Capana12g000958 was the strongest candidate gene for the up locus.

Fig. 4 Relative expression of the candidate genes in BB3, F1, and Chiltepin S1 ~ S4 represent of the different stages of mixed samples (stems, pedicels, and flower buds) divided based on the flower buds. * represents significance at the 0.05 level (Student’s t test)
A SNP located in the upstream of \textit{Capana12g000958} is highly associated with fruit orientation

\textit{Capana12g000958} was regarded as the strongest candidate gene for the \textit{up} locus based on systematic expression comparisons between BB3, Chiltepin, and another 18 inbred lines with diverse genetic background and fruit orientation. However, there was no difference within the coding sequences of \textit{Capana12g000958} between the BB3 and Chiltepin. Hence, we compared the sequences in the upstream (3328 bp) and downstream (2211 bp) of \textit{Capana12g000958} between BB3, Chiltepin and Zunla-1 (Supplementary Figures S7 and S8). Except for the SNP (958upstream\_SNP4\_37651506), we did not find any other sequence variation that is consistent with the fruit orientation of BB3, Chiltepin, and Zunla-1. Furthermore, we observed a perfect correlation between the 958upstream\_SNP4\_37651506 and fruit orientation among a panel of 40 inbred lines with diverse genetic background (Fig. 6).

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 cross between BA3 (\textit{C. annuum}) and YNXML (\textit{C. frutescens}) (Cheng et al. 2016a), we turned to use the BC$_1$F$_1$ population derived from the cross of BB3 (\textit{C. annuum}) and its wild progenitor Chiltepin (\textit{C. annuum} var. \textit{glabriusculum}) to fine map the \textit{up} locus. Linkage mapping demonstrated that the major QTL \textit{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 \textit{Up12.1} could be the same QTL as \textit{FP}-12.2, which was also identified in an intraspecific RIL population of \textit{C. annuum} (Han et al. 2016), indicating that
the origin of the \textit{up} gene should occur before the species differentiation between \textit{C. annuum} and \textit{C. frutescens}.

The completion of pepper genome sequencing has markedly accelerated the mining of target genes through forward genetics approach although it is 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, b 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 \textit{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 \textit{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 \textit{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, \textit{Capana12g000962}, \textit{Capana12g000964}, and \textit{Capana12g000965}, were very likely to be pseudogenes.
because they were not expressed in all the tested samples from different sources (Supplementary Figure S3). For \textit{Capanal12g000960/66}, 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 \textit{Capanal12g000959}, but they do not exist in the Zunla-1 and YNXML (Supplementary Figure S5), both of which exhibit erect fruit orientation. With regard to \textit{Capanal12g000958}, we found its spatial and temporal differential expression between parental lines in the pedicle was highly corresponding to the fruit orientation change (Figs. 1 and 5b). Further analysis showed that the expression differences of \textit{Capanal12g000958} in the fully flower and pedicle from another 18 inbred lines with diverse genetic background and fruit orientation were in accordance with that between BB3 and Chiltepin (Fig. 5c). Taken together, we suggested that \textit{Capanal12g000958} was the strongest candidate gene for up.

\textit{Capanal12g000958} shared ~88% of amino acid sequence similarity with the \textit{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 \textit{Arabidopsis}, the \textit{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 \textit{Dwarf 1} and \textit{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 posttranscriptional 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 \textit{Capanal12g000958} might be a signaling molecule involved in the dynamic control of fruit orientation in pepper.

Fruit orientation is an important domestication trait in pepper, which has long been chosen by human (Paran and Van Der Knaap 2007). Studies have shown that crop domestication might have proceeded during relatively short periods through the occurrence of nucleotide polymorphisms. During domestication, the SNP was strongly selected by ancient humans. For example, a SNP in the upstream region of the \textit{qSH1} gene can cause loss of seed shattering during rice domestication (Konishi et al. 2006). Similarly, several maize domestication genes contain critical polymorphisms that have resulted from prehistoric artificial selection in the upstream region (Wang et al. 1999; Clark et al. 2004). In this study, a SNP located in the upstream region of \textit{Capanal12g000958} (958upstream_SNP4_37651506, Supplementary Figure S7) was in line with the fruit orientation phenotype of 40 pepper inbred lines (Fig. 6) despite that there was no difference in the coding region. Therefore, we speculated that this SNP might affect the transcriptional expression of \textit{Capanal12g000958}. Further investigation would be necessary to analyze the function and mechanism of \textit{Capanal12g000958} in future.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00122-021-03867-2.

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**Author contributions** FH, JWC, and KLH conceived and designed all experiments; FH, JCD, and 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.

**Declarations**

**Conflict of interest** Authors declare that they have no conflict of interest.

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

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