# Cloning and Expression Analysis of ABP Gene Associated with Spur Development in *Impatiens uliginosa* Postprint

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**Date:** 2022-10-27T00:00:00+00:00

## Abstract

To investigate the structure and expression characteristics of the ABP gene in *Impatiens uliginosa*, this study utilized *Impatiens uliginosa* as experimental material. The ABP gene was cloned via RT-PCR, and homology analysis and phylogenetic analysis of its encoded protein sequence were conducted using DNAMAN and MEGA. Furthermore, the spatiotemporal expression pattern of the ABP gene was analyzed by qRT-PCR. The results demonstrated: (1) The full-length cDNA of the ABP gene from *Impatiens uliginosa* was 627 bp, encoding 208 amino acids, and was designated as *IuABP*. Its protein possessed the typical structure of Cupin superfamily proteins. (2) Homology analysis indicated that the amino acid sequence of the ABP gene from *Impatiens uliginosa* exhibited 71% homology with species such as *Impatiens glandulifera*, *Rosa chinensis*, and *Manihot esculenta*. Phylogenetic analysis revealed that IuABP clustered with *Impatiens glandulifera*, reflecting the closest genetic relationship. (3) qRT-PCR analysis showed that the *IuABP* gene was expressed in three developmental stages and two tissues of the *Impatiens uliginosa* spur. During spur development, the expression level of *IuABP* in the spur limb displayed a trend of initial decrease followed by increase, peaking at the full flowering stage, while its expression in the spur tube gradually decreased. The study also found that *IuABP* expression was highest in the spur limb at the full flowering stage. These results provide a theoretical foundation for further investigation into the function of the ABP gene in spur development and its expression regulatory mechanisms in *Impatiens uliginosa*.

## Full Text

# Cloning and Expression Analysis of the ABP Gene Related to Spur Development in *Impatiens uliginosa*

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**Abstract**

This study aimed to investigate the structural and expression characteristics of the ABP gene from *Impatiens uliginosa*. Using *I. uliginosa* as experimental material, the ABP gene was cloned via RT-PCR. DNAMAN and MEGA software were employed for homology analysis and phylogenetic analysis of the encoded protein sequence, while qRT-PCR was utilized to analyze the spatiotemporal expression patterns of the ABP gene. The results revealed: (1) The full-length cDNA of the ABP gene from *I. uliginosa* was 627 bp, encoding 208 amino acids, and was designated as *IuABP*. The protein possessed the typical structure of Cupin superfamily proteins. (2) Homology analysis demonstrated that the amino acid sequence of the ABP gene from *I. uliginosa* shared 71% homology with those of *Impatiens glandulifera*, *Rosa chinensis*, *Manihot esculenta*, and other species. Phylogenetic analysis indicated that IuABP clustered with *Impatiens glandulifera*, suggesting the closest genetic relationship. (3) qRT-PCR analysis showed that *IuABP* was expressed across all three developmental stages and in both examined parts of the spur. During spur development, *IuABP* expression in the spur blade exhibited a trend of initial decline followed by increase, reaching its peak at the blooming stage. In contrast, expression in the spur cup gradually decreased. Notably, *IuABP* expression was highest in the spur blade during the blooming stage. These findings provide a theoretical foundation for further investigation into the function and regulatory mechanisms of *IuABP* in spur development.

**Keywords:** *Impatiens uliginosa*, spur development, ABP gene, gene cloning, expression analysis

## Introduction

Floral spurs represent an evolutionary innovation that not only enhances pollination efficiency and reproductive success but also drives rapid diversification in certain plant lineages, making them a key trait regulating biological invasion.

Research on plant spurs illuminates speciation mechanisms and deepens understanding of plant-pollinator interactions. At the cellular level, spur elongation in *Aquilegia* primarily depends on anisotropic cell expansion, a pattern also observed in *Centranthus ruber*. However, contrasting mechanisms have been reported: in two *Pelargonium* species, spur formation results from accelerated hypanthium growth and extended developmental timing, while in *Linaria*, cell division rather than cell expansion drives spur length variation. Several genes regulating cell division and expansion, including *TCP*, *ARF6/8*, and *BEH*, are highly expressed in the *Aquilegia* spur cup. Virus-induced silencing of these genes disrupts the balance between cell division and expansion, causing spurs to become shorter and curve inward. Collectively, spur elongation relies on both cell division and anisotropic expansion, with genes such as *ABP*, *TCP*, *ARF*, and *BEH* playing crucial regulatory roles.

ABP (auxin binding protein), comprising ABP , ABP , ABP , and NPA-binding proteins, is widely distributed in plants and participates in auxin responses at the plasma membrane. ABP regulates rapid processes including cell expansion, enlargement, and cell cycle progression. Studies have shown that *ABP1* is highly expressed in young stems and buds of ramie, in maize seedlings (though less in roots), and at extremely high levels during active growth phases of tobacco cells, with expression declining as differentiation concludes. ABP distribution varies significantly across cellular locations. Beyond its characteristic high expression in rapidly growing tissues, ABP mediates auxin-induced cell expansion to regulate plant size. Overexpression of *Arabidopsis ABP1* in tobacco promotes leaf epidermal cell expansion, while constitutive overexpression in maize or tobacco suspension cells positively regulates cell size. Conversely, downregulation of *ABP1* in *Arabidopsis* leaves reduces cell volume, and its absence in embryos impairs normal cell expansion, resulting in uniform cell diameters. Thus, ABP1 is essential for cell division, volume increase, and meristem elongation.

*Impatiens uliginosa*, a member of Balsaminaceae, is an annual or perennial herb with broad distribution, rapid growth, high biomass, year-round flowering, and strong stress resistance. The entire plant has medicinal value and can be used for nail dyeing, representing an important ornamental, ecological, medicinal, and economic resource. As an ornamental species, *I. uliginosa* exhibits diverse flower colors, unique floral morphology, and variable spur lengths, numbers, and colors, making it valuable for research. To date, no studies on the *ABP* gene in *I. uliginosa* have been reported. Therefore, cloning and analyzing the molecular mechanisms and expression characteristics of the *ABP* gene in this species is significant for understanding its regulatory role in spur development. Building upon our previous transcriptome sequencing of *I. uliginosa*, this study cloned the spur development-related *ABP* gene and employed bioinformatics approaches and qRT-PCR to analyze its evolutionary relationships, structural features, and tissue-specific expression. Specifically, we addressed: (1) the phylogenetic relationships of *I. uliginosa* ABP protein; (2) its basic physicochemical properties and structural characteristics; and (3) its expression patterns across different developmental stages and parts of the spur.

## Materials and Methods

### 1.1 Plant Materials

Plant materials were collected from *I. uliginosa* cultivated in the experimental greenhouse at Southwest Forestry University. Spur tissue from the spur cup and spur blade was sampled at three developmental stages: bud stage (S1), beginning flowering stage (S2), and blooming stage (S3) for qRT-PCR analysis [Figure 1: see original paper].

**FIGURE:1** Three key stages of flower development and two different parts of the floral lip in *Impatiens uliginosa*. S1, Bud stage; S2, Beginning flowering stage; S3, Blooming stage.

### 1.2 Total RNA Extraction and ABP Gene Cloning

Total RNA was extracted from *I. uliginosa* floral organs using the OMEGA RNA extraction kit. RNA was reverse-transcribed into cDNA using the TransScript kit and stored at -20°C. Based on the *IuABP* gene identified in the *I. uliginosa* transcriptome, specific primers were designed and synthesized by Sangon Biotech: *IuABPF* (5 -ATGTTGCGCCTCGTTTTC-3 ) and *IuABPR* (5 -TTAATTGGTTCCTCCAAGAACACC-3 ). Using spur cDNA as template, the ABP gene was amplified via RT-PCR in a 20 L reaction under the following conditions: 95°C for 5 min; 35 cycles of 95°C for 5 s, 56°C for 30 s, 72°C for 48 s; final extension at 72°C for 10 min; and storage at 4°C. PCR products were purified, ligated into the pMD19-T vector, transformed into *E. coli* DH5$\alpha$ competent cells, and positive clones were sequenced by Sangon Biotech.

### 1.3 Sequence Analysis of *I. uliginosa* ABP Gene

The basic physicochemical properties of the *I. uliginosa IuABP* gene were analyzed using the ExPASy online tool (https://web.expasy.org/protparam/). The SMART online tool (http://smart.embl-heidelberg.de/) was used to predict protein domains. Subcellular localization was predicted using TargetP (https://services.healthtech.dtu.dk/service.php?TargetP-2.0). The three-dimensional structure was modeled using SWISS-MODEL (https://swissmodel.expasy.org/interactive). Multiple sequence alignment was performed using DNAMAN v9.0, and phylogenetic analysis was conducted using MEGA-X software with the neighbor-joining method (bootstrap = 1,000).

### 1.4 Spatiotemporal Expression Analysis of *I. uliginosa* ABP Gene

Total RNA was extracted from spur tissues across three developmental stages (bud, beginning flowering, and blooming) and two parts (blade and cup), and reverse-transcribed into cDNA. qRT-PCR primers for *IuABP* were: qABPF (5 -CGGGCTTTGTGGCTCAATAC3 ) and qABPR (5 -TTCGCAAACAGCGCGAAATC ). The reference gene was *IuActin* with

primers ActinF (5 -TGAATGTCCCTGCTGTTTG-3 ) and ActinR (5 -ACCTTCCGCATAACTTTACC-3 ). Using cDNA from the three stages and two parts as templates, relative gene expression was quantified on a LightCycler 480 II (Roche) real-time PCR system in a 20 L reaction with the following program: pre-denaturation at 95°C for 5 min; 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 20 s, extension at 72°C for 20 s. Each sample was analyzed in triplicate, and expression levels were calculated using the 2^(-ΔΔCT) method. Expression patterns were analyzed with the spur cup at the blooming stage defined as the calibrator (value = 1).

## Results

### 2.1 Cloning and Sequence Analysis of *I. uliginosa* ABP Gene

Using gene-specific primers designed from the *I. uliginosa* spur transcriptome and spur cDNA as template, the ABP gene fragment was successfully cloned via RT-PCR [Figure 2: see original paper]A. ExPASy-ProtParam analysis revealed that *IuABP* has a full length of 627 bp, encoding 208 amino acids with a molecular weight of 22,108.6 Da and a theoretical isoelectric point of 6.95. The protein comprises 3,162 atoms, has an instability index of 27.46 (classifying it as stable), and a grand average of hydropathicity of 0.405, indicating hydrophobic properties.

SMART analysis identified a typical Cupin-1 domain spanning 147 amino acids (E-value: 7.85e-36) and a 20-amino-acid signal peptide, confirming that *IuABP* belongs to the Cupin superfamily [Figure 2: see original paper]B. The protein lacks transmembrane domains and is predicted to localize to the cell wall. SWISS-MODEL prediction of the three-dimensional structure revealed the characteristic small barrel fold typical of Cupin superfamily proteins [Figure 2: see original paper]C.

**FIGURE:2** PCR amplification and sequence analysis of the ABP gene from *Impatiens uliginosa*. A: M, Marker 2000; 1, ABP. B: Conserved domains of ABP protein. C: Predicted spatial structure of ABP protein.

### 2.2 Phylogenetic Analysis of *I. uliginosa* ABP Gene

BLASTP analysis against the NCBI database showed that IuABP shares high similarity with ABP proteins from *Impatiens glandulifera* (XP_{047342663}.1), *Capsicum annuum* (XP_{016567466}.1), *Prunus persica* (XP_{007202573}.1), *Nicotiana tomentosiformis* (XP_{009600028}.1), *Fragaria vesca* (XP_{004287628}.1), *Erythranthe guttata* (XP_{012830892}.1), *Carya illinoinensis* (XP_{042972396}.1), *Rosa chinensis* (XP_{024182988}.1), *Juglans regia* (XP_{018844294}.1), *Morella rubra* (KAB1209489.1), *Pistacia vera* (XP_{031267949}.1), *Solanum tuberosum* (XP_{006342915}.1), *Manihot esculenta* (XP_{021596589}.1), and *Hevea brasiliensis* (XP_{021647439}.1). Multiple sequence alignment using DNAMAN v9.0 demonstrated that IuABP shares 71% similarity with ABP

proteins from these species [Figure 3: see original paper]. Phylogenetic tree construction using MEGA-X (neighbor-joining, bootstrap = 1,000) revealed that *I. uliginosa* ABP clusters with *Impatiens glandulifera*, indicating the closest genetic relationship [Figure 4: see original paper].

**FIGURE:3** Homologous amino acid sequence alignment of the ABP gene from *Impatiens uliginosa*.

**FIGURE:4** Phylogenetic tree based on the amino acid sequence of the ABP gene from *Impatiens uliginosa*.

### 2.3 Spatiotemporal Expression Analysis of *I. uliginosa* ABP Gene

*IuABP* was expressed across all three developmental stages (bud, beginning flowering, and blooming) and in both parts (blade and cup) of the *I. uliginosa* spur [Figure 5: see original paper]. In the spur blade, *IuABP* expression initially decreased then increased, peaking at the blooming stage. Conversely, in the spur cup, expression was highest at the bud stage and gradually declined thereafter. The highest overall expression was detected in the spur blade at the blooming stage, followed by the blade at the bud stage, suggesting that *IuABP* plays an important role in cell growth and expansion in the spur blade. These results imply that *IuABP* functions significantly in spur cell development.

**FIGURE:5** Spatiotemporal expression pattern analysis of ABP genes across three key flower development stages and two different parts of the floral lip in *Impatiens uliginosa*. Different lowercase letters indicate significant differences ($P < 0.05$).

## Discussion and Conclusion

ABP significantly promotes plant cell division and elongation, establishing it as a potential auxin receptor protein. Increasing numbers of ABP genes have been isolated and functionally characterized across various plants. This study successfully cloned the spur development-related gene *IuABP* from *I. uliginosa*, which has a full-length cDNA of 627 bp encoding 208 amino acids and is classified as a hydrophobic, stable protein. The $\beta$-barrel structure of the Cupin domain confers thermostability and functions in amino acid storage. *IuABP* contains a typical Cupin-1 domain and exhibits the characteristic small barrel fold, consistent with previous reports on the *Rosa chinensis RcABP19* gene structure, suggesting that *IuABP* belongs to the Cupin superfamily and may participate in amino acid storage during spur development.

Homology analysis revealed that *I. uliginosa* ABP shares approximately 71% identity with ABP genes from peach, *Impatiens glandulifera*, and rose. Studies on peach *ABP1* have demonstrated its involvement in auxin signal transduction during fruit development. As ABP is known to receive and transport auxin signals, inducing rapid cell expansion and elongation, whether *I. uliginosa* ABP protein shares similar functions with peach ABP1 requires further investigation.

Phylogenetic analysis confirmed the closest relationship between *I. uliginosa* ABP and *Impatiens glandulifera*.

As a novel auxin receptor, ABP1 regulates non-transcriptional cytoplasmic responses and induces transcription of early auxin-responsive genes such as *PLT*, *Aux/IAA*, and *ARF*, thereby participating in cell expansion, proliferation, and auxin feedback regulation. Studies have shown that auxin-induced protoplast swelling is mediated by extracellular ABP1, and overexpression of maize *ABP1* in tobacco leaves enhances cellular auxin sensitivity. Our qRT-PCR analysis revealed that *IuABP* expression in the spur blade increased gradually during development, peaking at the blooming stage, while expression in the spur cup was highest at the bud stage and decreased progressively. This pattern aligns with reports of high *ABP1* expression during active cell growth and reduced expression as differentiation concludes. The differential expression between blade and cup suggests developmental stage- and tissue-specific regulation, indicating that *IuABP* may function through distinct mechanisms in these tissues. The expression pattern in the spur cup may promote cell division and elongation at the beginning flowering stage, with this effect diminishing sharply by the blooming stage. We hypothesize that *IuABP* plays a crucial role in *I. uliginosa* spur development, potentially regulating cell division and elongation via auxin modulation, though the specific regulatory mechanisms require further investigation.

In conclusion, *IuABP* represents a new member of the *I. uliginosa* ABP subfamily, possessing the typical Cupin superfamily domain. The qRT-PCR results indicate that *IuABP* promotes spur cell growth in *I. uliginosa*, but its precise mechanism requires further study. Future experiments could employ virus-induced gene silencing (VIGS) to validate *IuABP* function in spur cell development. This research not only establishes a foundation for investigating the molecular mechanisms underlying spur development in *I. uliginosa* but also provides essential data and theoretical support for spur development studies, floral shape improvement, and new cultivar breeding in *Impatiens*.

## References

BRAUN N, WYRZYKOWSKA J, MULLER P, et al., 2008. Conditional repression of auxin binding protein1 reveals postembryonic shoot development coordination between cell division and cell expansion in *Arabidopsis* and tobacco[J]. *Plant Cell*, 20(10): 2743-2752.

CHEN JG, SHIMOMURA S, SITBON F, et al., 2001. The role of auxin-binding protein 1 in the expansion of tobacco leaf cells[J]. *Plant J*, 28(6): 607-617.

CHEN JG, ULLAH H, YOUNG JC, et al., 2001. ABPl is required for organized cell elongation and division in *Arabidopsis* embryogenesis[J]. *Genes Dev*, 15(7): 902-911.

CULLEN E, FERNÁNDEZ-MAZUECOS M, GLOVER BJ, 2018. Evolution of

nectar spur length in a clade of *Linaria* reflects changes in cell division rather than in cell expansion[J]. *Ann Bot*, 122(5): 801-809.

FERNÁNDEZ-MAZUECOS M, GLOVER BJ, 2017. The evo-devo of plant speciation[J]. *Nat Ecol Evol*, 1(4): 0110.

FU L, WANG HH, HUANG SL, et al., 2021. Alternative splicing of rice *OsEnS51* and structural analysis of encoding protein[J/OL]. *Mol Plant Breed*. http://kns.cnki.net/kcms/detail/46.1068.S.20210901.1702.006.html.

GAO QX, LI YZ, LIU SL, 2001. Distribution and expression of auxin binding protein1 (ABP1) in the thin cell layers of tobacco[J]. *Acta Bot Sin*, 43(10): 1018-1023.

HODGES SA, ARNOLD ML, 1995. Spurring plant diversification: are floral nectar spurs a key innovation?[J]. *Proc Royal Soc Lond Series B Biol Sci*, 262(1365): 343-348.

HODGES SA, FULTON M, YANG JY, et al., 2003. Verne grant and evolutionary studies of *Aquilegia*[J]. *New Phytol*, 161(3): 113-120.

HUANG Y, LIU F, GUO QQ, et al., 2008. Cloning and expression of auxin-binding proteins 1 gene in ramie [*Boehmeria nivea* (Linn.) Gaud.][J]. *Acta Agron Sin*, 34(8): 1358-1365.

JONES AM, IM KH, SAVKA MA, et al., 1998. Auxin-dependent cell expansion mediated by overexpressed auxin-binding protein 1[J]. *Science*, 282(5391): 1114-1117.

KIM HJ, TRIPLETT BA, 2004. Characterization of GhRac1 GTPase expressed in developing cotton (*Gossypium hirsutum* L.) fibers[J]. *Biochim Biophys Acta*, 1679(3): 214-221.

LUO C, HUANG WL, ZHU JP, et al., 2019. The complete chloroplast genome of *Impatiens uliginosa* Franch., an endemic species in Southwest China[J]. *Mitochondrial DNA B*, 4(2): 366-367.

MACK JL, DAVIS AR, 2015. The relationship between cell division and elongation during development of the nectar-yielding petal spur in *Centranthus ruber* (Valerianaceae)[J]. *Ann Bot*, 115(4): 641-649.

PUZEY JR, GERBODE SJ, HODGES SA, et al., 2011. Evolution of spur-length diversity in *Aquilegia* petals is achieved solely through cell-shape anisotropy[J]. *Proc Biol Sci*, 279(1733): 1200-1205.

QIAO LY, ZHANG WP, ZHANG L, et al., 2012. Research advances in auxin binding protein ABP1[J]. *Guangdong Agric Sci*, 39(7): 230-232.

REN HR, FU XD, ZHI QJ, et al., 2019. Cloning and expression profile analysis of auxin binding protein gene *RcABP19* in *Rosa chinensis*[J]. *Plant Physiol J*, 55(7): 983-992.

STEFFENS B, FECKLER, PALME K, et al., 2001. The auxin signal for protoplast swelling is perceived by extracellular ABP1[J]. *Plant J*, 27(6): 591-599.

STEPHANIE JC, CRISTINA WC, KATE SB, 2021. Brassinosteroids regulate petal spur length in *Aquilegia* by controlling cell elongation[J]. *Ann Bot*, 128(7): 931-942.

TONG ZK, LI Y, CAI B, et al., 2022. Cloning and expression analysis of *SAUR* gene of *Impatiens uliginosa* Franch.[J] *Acta Bot Boreal-Occident Sin*, 42(1): 21-28.

TSAI T, DIGGLE PK, FRYE HA, et al., 2018. Contrasting lengths of *Pelargonium* floral nectar tubes result from late differences in rate and duration of growth[J]. *Ann Bot*, 121(3): 549-560.

VERVOORT A, CAWOYV, JACQUEMARTAL, 2011. Comparative reproductive biology in co-occurring invasive and native *Impatiens* species[J]. *Int J Plant Sci*, 172(3): 366-377.

YANT L, COLLANI S, PUZEY J, et al., 2015. Molecular basis for three-dimensional elaboration of the *Aquilegia* petal spur[J]. *Proc Biol Sci*, 282(1803): 20142778.

ZHANG C, ZHAO KL, HU JJ, et al., 2018. Analysis of ABP1 family in *Arabidopsis thaliana* and Gramineae[J]. *Mol Plant Breed*, 16(11): 3526-3532.

ZHANG R, MIN Y, LYNN DH, et al., 2020. A role for the auxin response factors ARF6 and ARF8 homologs in petal spur elongation and nectary maturation in *Aquilegia*[J]. *New Phytol*, 227(5): 1403-1415.

ZHANG W, SHI MY, YU J, et al., 2013. Cloning and expression analysis of auxin-binding proteins 1 from *Prunus persica* L. fruit[J]. *J Beijing Agric Coll*, 28(2): 1-4.

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