Detection of a True Breeding Homeotic Gene Mutant Pps-1 with Partially Petaloid Sepals in Opium Poppy (Papaver somniferum L.) and Its Genetic Behavior

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A spontaneous true breeding homeotic gene mutant Pps-1 with distinct partial petaloid sepal was detected in the population of downy mildew (DM)-resistant elite accession I-14 during our studies for the identification of disease resistance sources in opium poppy. The trait was found to be stable and inherited truly in the subsequent generations. Genetic studies were carried out through systematic reciprocal crosses with the parental wild-type genotype I-14, and segregation pattern of phenotypic characteristics in F1 and F2 populations clearly indicated single recessive nuclear gene control of the mutant character. The studies have demonstrated that the mutant phenotype is due to mutations at the Pps-1 locus that possibly corresponds to B-class function (according to ABC model) with negative control function. The mutant Pps-1 being single-whorl homeotic mutant might greatly help in providing insight into mechanisms of flower development in opium poppy.

The information on floral developmental genetics has exploded in recent years (Albert et al. 2005) and extensive documentation of the developmental morphology of Arabidopsis flowers has facilitated the linkage of genes and morphology. These studies served as the benchmark by which mutant defects have been identified and gene functions defined (Coen and Meyerowitz 1991; Weid et al. 2004). Morphine is a preferred analgesic for cancer patients and codeine is cough depressant (Kramer and Jaramillo 2000). A recent study of the expression of floral identity genes in poppies, buttercups, and their relatives have shed more light on the evolutionary origin of petals (Baum and Whitlock 1999; Kramer et al. 2003).

Opium poppy (Papaver somniferum L.) is one of the oldest medicinal plants cultivated for life-saving alkaloids such as morphine and codeine (Weid et al. 2004). Morphine is a preferred analgesic for cancer patients and codeine is cough depressant (Katip 1995). Besides these 2, opium poppy produces approximately 80 more alkaloids belonging to various tetrahydroisoquinoline-derived classes (Weid et al. 2004). Seeds obtained from the capsules are edible and used in confectionary (Nergiz and Otles 1994), and fatty oils in seeds are used for culinary and pharmaceutical purposes (Krist et al. 2005). It is an annual winter herb and the plants are highly variable in morphological characteristics. In addition to these, many spontaneous mutants with altered floral structures like complete conversion of petals into stamens and stamens into petals has also been identified (Belyaeva and Nevkrytaya 1979). All these spontaneous mutants suggest that there is a considerable mutability of genes controlling floral behavior. Papaver somniferum and several of its relatives are currently studied in terms of developmental genetics, and have become model organisms (see Discussion).

We recovered a distinct partial petaloid sepal spontaneous mutant in which nearly half the area of sepals on the margins is modified into petal-like structures. The studies have demonstrated that the mutant phenotype is due to mutations at the Pps-1 locus. Here, we report the phenotype of Pps-1 and its genetic behavior through systematic reciprocal crosses with the wild type indicating its probable location in B function with negative control function.
Materials and Methods

The plant materials consist of a true breeding flower mutant Pps-1 with partial petaloid sepals (Figure 1) spontaneously originated in the downy mildew (DM)–resistant accession I-14 of opium poppy. The mutant line Pps-1 has been inbred for 6 selfing cycles and is completely distinct, uniform, and stable. The parental genotype I-14 has also been inbred for 9 selfing cycles and is being used in crossing programs as the main source of DM resistance in opium poppy.

The experiments were carried out at the research fields of Central Institute of Medicinal and Aromatic Plants, Lucknow, India, during Rabi season (November–April). The parental accession I-14 has narrow leaves with very deep leaf incisions and white petals. The mutant genotype Pps-1 differs from its parent I-14 in only one character where margins of both the sepals are modified into petal-like structures.

We crossed Pps-1 mutant line with the parental line I-14 taking it both as male and female parents during 2002–2003 crop seasons. The F1 seeds were then sown in the field along with their parents to collect seeds of the F2 generations and raising reciprocal F1s simultaneously with F2 during 2003–2004 crop season. Different generations (reciprocal F1s and F2s) and parents (Pps-1 and I-14) were sown in the field in randomized block design with 3 replications during 2004–2005 crop season and observations were collected on single-plant basis. Row-to-row distance of 50 cm and plant-to-plant distance of 10 cm was maintained. Normal agronomic practices were carried out to have good crop population. Chi-square analysis was applied to test the goodness-of-fit for frequency distributions in the F2 generations.

Results and Discussion

Homeotic mutations in flowers have been related to the deviated development of floral organs in abnormal locations. In this study, the typical Pps-1 expression has been observed.
to be a true breeding homeotic gene mutation of opium poppy in which sepals are partially converted into petaloid structures on the margins. An opposite effect has been reported from PISTILLATA floral mutant of Arabidopsis thaliana where petals have been replaced by sepal-like organs (Hill and Lord 1989). The mutant Pps-1 is interesting because as a naturally occurring mutation it affects only the first whorl of floral organs producing partially petaloid sepals. The character is stable and inherits truly as a typical recessive trait in the subsequent generations. A true breeding inbred line named Pps-1 with this mutant character has been stabilized through subsequent selfing and selection for 6 generations. In the present study, the phenotypic characteristics of the Pps-1 in comparison to wild-type population of I-14 have been analyzed genetically and correlated to the possible mechanism parallel to ABC model reported in other systems.

Phenotypic Characteristics of Pps-1

In a selfed population of the genotype I-14 of P. somniferum while being screened for disease resistance characters, a true breeding spontaneous flower mutant Pps-1 with partial petaloid sepals was observed. This mutant spontaneously originated in the DM-resistant accession I-14 of opium poppy (Figure 1). It was found that Pps-1 was fully fertile and there was no effect in other 3 floral whorls. The mutant line Pps-1 like the parental line I-14 had narrow leaves with very deep leaf incisions. It also had white petals and with same level of resistance to DM caused by Peronospora arborescence as the parents I-14. Pps-1 mutant thus differed from its parental genotype I-14 only in this understandably homeotic gene mutation at Pps-1 locus in which nearly half the area of both the sepals turns into petal-like structures along the margins of sepals, whereas the central portion remains wild type (Figure 1).

Genetic Analysis

Genetic analysis can provide the basis to understand gene functions and regulation and has been successful in identifying many floral genes (Baum et al. 2002). With the distinct morphotypic mutant Pps-1 differing only for the sepal development, the genetic analysis through F1 and F2 analysis was carried out in this study.

The phenotypic characteristics of all the F1 plants in reciprocal crosses involving Pps-1 and I-14 during both the years (2003–2004 and 2004–2005 crop seasons) showed normal sepals indicating thereby that the typical mutant character (Pps-1) was recessive in nature and there was no cytoplasmic control for the mutant trait. Segregation pattern of the F2 populations of both the reciprocal crosses also gave good fit of the monogenic Mendelian ratio (P ≥ 0.50–0.30) of normal wild type (I-14) and the mutant characters (Pps-1) indicating that mutant character is perhaps controlled by a single recessive nuclear gene “Pps-1” (Table 1). Phenotypic characteristics of both the parents during both the years also showed that the mutant character is true breeding and may be used in breeding and molecular studies to understand the mechanisms of homeotic gene expression in flowering plants.

The classical ABC model of flower development put forward by Coen and Meyerowitz (1991) outlines the existence of 3 classes of gene functions termed A, B, and C that work through in overlapping domains to determine specific organ identity. As sepal identity is reported to be encoded by A function genes alone, mutants in this class exhibit complete homeotic transformations of organ identity. The genes corresponding to these classes have been well characterized and in Arabidopsis are represented by APETALA 1 (AP1) and APETALA 2 (AP2) in the A class, APETALA 3 (AP3) and PISTILLATA (PI) in B class, and AGAMOUS (AG) in the C class (Bowman et al. 1989, 1991). As the A function is poorly defined and seems to largely depend on the suppression of C function, studies of petal development and evolution have generally been focused on the B-group genes. Kramer and Irish (1999) carried out a detailed study of the ontogenetic changes in B-group gene expression in 4 species namely, icelandic poppy (Papaver nudicaule), bleeding heart (Dicentra eximia) and buttercups (Ranunculus bulbosus and Ranunculus ficaria). The functions of AP1 gene, generally contributing to sepal development have not been found to be highly conserved (Theissen et al. 2000). This suggests that the regulation of A function and their interaction with B and other transactors need to be re-evaluated through more genetic and molecular studies involving various mutants of sepals and petals showing homeotic transformations. This can be

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**Table 1.** Segregation pattern of homeotic gene mutant in different generations of the reciprocal crosses involving Pps-1 mutant and parental accession I-14 having wild-type phenotype

<table>
<thead>
<tr>
<th>Genotype/cross</th>
<th>Generation</th>
<th>Number of observed plants</th>
<th>Segregation ratio</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wild type</td>
<td>Mutant type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pps-1</td>
<td>P$_1$</td>
<td>0</td>
<td>140</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>I-14</td>
<td>P$_2$</td>
<td>180</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pps-1 × I-14</td>
<td>F$_1$ (P$_2$ × P$_2$)</td>
<td>165</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>I-14 × Pps-1</td>
<td>rF$_1$ (P$_2$ × P$_1$)</td>
<td>172</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pps-1 × I-14</td>
<td>F$_2$ (P$_1$ × P$_2$)</td>
<td>185</td>
<td>55</td>
<td>3:1</td>
<td>0.554</td>
</tr>
<tr>
<td>I-14 × Pps-1</td>
<td>rF$_2$ (P$_2$ × P$_1$)</td>
<td>170</td>
<td>50</td>
<td>3:1</td>
<td>0.605</td>
</tr>
</tbody>
</table>
achieved better if novel mutants differentially and morphologically become available like Pps-1.

On the other hand, such distinct mutants may be used as potential morphological markers in breeding programs for crop improvement. The I-14 genotype has been found previously to be highly resistant to DM disease caused by *P. arborescens* (Sattar et al. 1995; Dhawan et al. 1998) and has been involved in breeding experiments for the transfer of DM resistance to develop high-yielding DM-resistant varieties of opium poppy. Genetically, the DM resistance has not been found to be linked to the Pps-1 mutation as all the progenies of the parental line (I-14) and the mutant line (Pps-1) observed were invariably showing disease resistance phenotype. However, the linkage of this marker character can be studied with other important traits and subsequently can be used as an effective morphological marker in the breeding programs for crop improvement.

Mutant Pps-1 obtained and isolated to stability level is significant not only because it represents a deviation in A-whorl about which very little is known at the moment but also because it is not a laboratory-induced mutation as is the case in most of the commonly studied floral mutants. Probably this is the first report of selection of a true breeding partial homeotic gene mutant of A-function genes of flower development spontaneously arising in natural populations.

It has been demonstrated during earlier reports that A-function genes (Coen and Meyerowitz 1991; Soltis et al. 2002; Buzgo et al. 2004; Kramer and Jaramillo 2005) and all 4 SEPALLATA genes (SEP1, SEP 2, SEP3, and SEP4) (Ditta et al. 2004; de Folter et al. 2005) are responsible for sepal formation. However, it needs to be further ascertained looking at the morphology and breeding behavior of Pps-1 whether only A-class genes or interference of B-class genes is also there that are known to be involved in the development of petals and stamens only. One of the reasonable hypotheses might be that this phenotype is due to simple outward shift in the B-domain boundary resulting in partial petaloid sepal mutant phenotypes.

However, the role of a specific “B” function in suppressing the expression of “A” and thus limiting it to only sepal formation by transactors lying in a separate domain not interacting with other part of A for petal formation should not be ruled out. In this study, however, there is no indication for an antagonism or a down regulation of A function by B function.

Single-whorl homeotic mutant condition is not a common occurrence, and existence of these types of mutations suggests that further pursuits of the *Pps-1* locus in opium poppy could thus prove very useful to other studies. Continued investigation of the partial petaloid sepal mutant (Pps-1) of opium poppy and other similar mutants will help us to compare and contrast the relationships between the different floral whorls and may help to provide insight into the evolution of the flower itself. By integrating data on gene expression with functional studies of various plant mutants, it is expected to get clearer picture of flowers’ development in plants.

Papaveraceae and Ranunculaceae are both members of the order Ranunculales (Angiosperm Phylogeny Group II 2003). Ranunculales occupy an important position in angiosperm phylogeny: they are the basalmost eudicots. Therefore, *P. somniferum* and several of its relatives are currently studied in terms of developmental genetics and have become model organisms. These taxa include *P. somniferum* (Irish 2006), *Eschscholzia californica* (California poppy, Papaveraceae; Becker et al. 2005; Groot et al. 2005; MacLeod et al. 2006), and *Aquilegia* (Ranunculaceae; Kramer et al. 2003).

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References


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