



Short Communication

Flower pattern formation of orchids: Case studies with respect to ABCDE model

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Abstract

Orchids are known for their beautiful flowers with specialized organ identity and pattern formation. Genetic models for pattern formation of flowers are well established in actinomorphic flowers like *Arabidopsis*. However, the orchid flowers show zygomorphic symmetry and contain a highly differentiated labellum or lip. Besides, there is no marked difference in terms of structures of sepals and petals in many cases. The stamens and carpels are fused and form the column or gynostemium with anther cap containing pollinia at top. Few models like 'Orchid code theory', 'Homeotic Orchid Tepal (HOT) model', 'P-code model' etc. have been proposed recently to explain genetic control of flower patterning in orchids. We have studied the suitability of those proposed models with some spontaneous bud variants as well as somaclonal variants showing alteration of floral organ patterns of all the flowers in the spike. The basic model of flower development with some alteration was found suitable in case of *Cymbidium*. Occurrence of structural male sterility due to mutation in B class homeotic gene in *Cymbidium* is also reported for the first time. The "P-code" model of perianth formation in orchids was found to be applicable for the recorded peloric mutants of *Phalaenopsis*. Few other variants of *Eria tomentosa*, *Epidendrum* sp. and *Arundina graminifolia* are also reported here. Whether all these changes are due to alteration of gene sequences or epigenetic mutations, needs to be verified in details.

Key words: Homeotic mutation, MADS-Box Genes, peloric mutations, perianth

Except Apostasioideae subfamily, the flowers of orchids show zygomorphic symmetry and contain a highly differentiated labellum or lip. Besides, mostly there is no marked difference in terms of structures of sepals and petals. The stamens and carpels are fused

and forms the column or gynostemium with anther cap containing pollinia at top (Cameron 2004). Such highly specialized floral structures make the orchids an excellent model for examining the interaction and network of genes involved in floral morphogenesis.

Genetic control of floral organ specification have been a major area of interest by plant scientists starting from the year 1994 with focus on specification of floral organ, their development and evolutionary role (Weigel and Meyerowitz 1994, Purugganan et al. 1995, Munster et al. 1997). Initially the model was established in *Arabidopsis thaliana* and then further extended for many other species and in all cases MADS-box-containing transcriptional regulators were found to play key role. The 'ABCDE model' has been proposed, where the identity of the floral organs in each of the four flower whorls (sepal, petal, androecium and gynaecium) is determined by unique combinatorial gene regulation activities of floral identity genes popularly named as A, B, C, D and E. Sepals are proposed to be specified by activity of A & E; petals by A, B & E; stamens by B, C & E; carpels by C & E and ovules by C, D & E classes of homeotic genes. Diversification of these genes played a key role in diversity of floral architecture in plant kingdom (Weigel and Meyerowitz 1994; Theissen and Saedler 2001; Zahn et al. 2005; Wang et al. 2011). The standard ABCDE model is able to explain flower development in most of the actinomorphic flowers.

Orchid code theory (Li et al. 2013) suggests that B class of *AP3/DEF* genes which is duplicated twice

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or more in orchids (Chang et al. 2010) play a crucial role in lateral petal and lip identity and the rest genes have unchanged function. Homeotic Orchid Tepal (HOT) model (Pan et al. 2011) for orchidaceae have been proposed based on expression patterns of the B, C, D-class and other MADS-box genes. As per the model, all the B-class MADS-box gene members are expressed during early inflorescence stage and involved in orchid perianth identity. However at late inflorescence stage, *PI* and *AP3B* determine formation of sepals. Combination of *PI*, *AP3A2* and *AP3B* controls the formation of the lateral petals whereas *PI* and *AP3A2* control lip formation possibly in combination with other MADS-box genes (*AGL6-like*, *SQUA-like* or unknown genes). The most comprehensive model ('P-code' or Perianth-code) for perianth formation in orchids was proposed in April, 2015 (Hsu et al. 2015). As per this model, a higher order heterotetrameric complex known as SP (sepal/petal) complex formed by 'OAP3-1/OAGL6-1/OAGL6-1/OPI' leads to sepal/petal formation, whereas the L (lip) complex of 'OAP3-2/OAGL6-2/OAGL6-2/OPI' is exclusively required for the formation of lips. Hence, depending upon whether *OAP3-1* and *OAGL6-1* or *OAP3-2* and *OAGL6-2* are being expressed in a whorl; the organ identity of petals or lips is defined. Most of the research work in orchid flower development is concerned with B class MADS-box genes. The information on role of C class genes is limited. However, Wang et al. (2011) isolated two C-class genes, *CeMADS1* and *CeMADS2*, from *Cymbidium ensifolium* and characterized their role in gynostemium development in normal flowers as well as in the multipetal mutant whose gynostemium was replaced by a newly emerged flower.

In present study we recorded some variants of flower development of orchids from various genera and studied them in light of existing models. A spontaneous bud mutant appeared in one unidentified *Cymbidium* hybrid (Figs. 1a and b). In the mutant flower the two lateral petals, lip and pollinia were absent and it was found to have only two sepals and the gynostemium without anther cap. The flower thus also displayed structural male sterility in *Cymbidium* (Fig. 1c). As per 'orchid code theory' of Li et al. (2013) and 'P-code theory' of Hsu et al. (2015), 'B-class' genes are essential for formation of petals, lip & stamen and absence of their expression may lead to absence of the concerned floral organs. Two sepals were present, although it was one less than normal three sepals. This may result either due to fusion of two lateral sepals

into one or some other kind of regulation. The development of lateral sepals is not completely independent of B-class genes in orchids unlike the *Arabidopsis* model (Hsu et al. 2015). Hence complete absence of B class gene expression may also have some effect on development of sepals. Overall, the phenomenon may be best explained in terms of complete absence of expression of any B-class genes during the development of this flower leading to absence of petals, lip and stamen which may also happen due to epigenetic changes in the gene.

Another bud mutant was recorded (Fig. 2) in *Cymbidium* hybrid 'Soul Hunt' which contains three normal sepals. However the number of lateral petals was four in place of two besides three lips, columns and pollinia in place of one each. Wang et al. (2011) reported one multipetal mutant in *Cymbidium ensifolium* where gynostemium was replaced by a newly emerged ectopic flower which resulted from absence of expression of *CeMADS2* (a C-class gene) gene in the mutant flower. In the variant we are reporting, the first whorl has shown normal development with one dorsal and two lateral sepals. However, ectopic flower development happened in rest three whorls, albeit absence of two lateral petals in one of them. Overall this mutant type possibly has arisen due to unregulated ectopic expression of A, B and C class of genes in different whorls. With the existing organ identity models in orchids, a complete explanation for this observation will not be possible without gene expression study in all the organs. Though comprehensive models for sepal-petal-lip (perianth) formation are available, any detailed model considering the role of C-class gene and its interaction with B-class genes is still lacking.

In the population of 28 imported tissue cultured plants of *Phalaenopsis* hybrid 'Detroit', six plants were found to display different degree of alteration in the petals of all flowers in the spike (two plants each for three types). In all the cases, the two lateral petals were found to transform into lip like forms with gradual degree of changes. There was also concomitant reduction in flower size. The three variants along with the wild type have been represented in Fig. 3. In orchids, a floral mutant with the morphological features of lateral petals with a keeled callus similar to that of a lip is referred as a peloric mutant (Rudall and Bateman 2002) which are observed in both natural populations and micro propagated plants (Pan et al. 2011). Cubas et al. (2004) suggested that these changes mainly arise due to epigenetic mutation arising from DNA

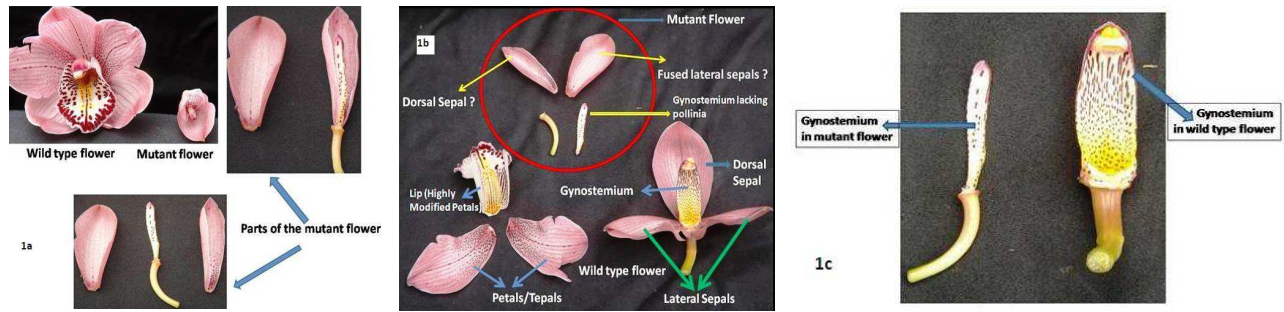


Fig. 1. Pattern formation in wild type vs. mutation flower of *Cymbidium* (1a & 1b). Absence of pollinia in mutant flower leading to structural male sterility (1c)



Fig. 2. Variant flower of *Cymbidium* hybrid 'Soul Hunt' showing ectopic flower organ development. 2a: normal wild type flower and 2b: Variant flower

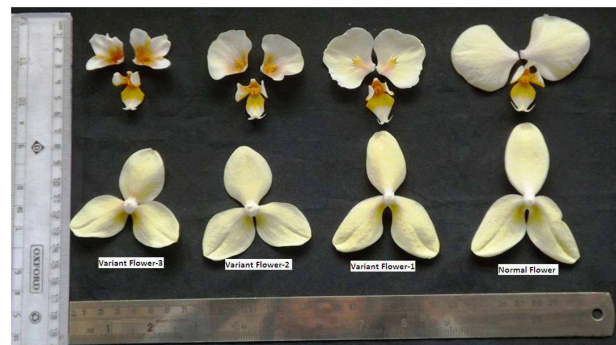


Fig. 3. Variants of petal to lip conversion in micro propagated plants of *Phalaenopsis* hybrid 'Detroit'. The gradual conversion of lateral petals into lip like structure may be clearly seen

methylation which is also heritable and recorded during plant tissue culture (Wang et al. 2013). With the Perianth or P-model proposed by Hsu et al. (2015), this gradual change from petal to lip may be very well explained in terms of levels of expression of L-complex and SP-complex genes in the lateral petals of the respective variants. The differentiation of petals from lips arises mainly depending upon whether *OAP3-1* and *OAGL6-1* or *OAP3-2* and *OAGL6-2* are expressing in that particular part. Simultaneous expression of both *SP* and *L*-complex genes or absence of both of them disturbs the P-code balance and results into intermediate structures. In present study, the lateral petals had keeled callus without any bifurcation at tips in 'Variant-1'. In 'Variant-2', bifurcation was initiated with increased prominence of keeled callus. In 'Variant-3', the lateral petals were divided in three lobes (*lips are also tri-lobed*) and colour pattern of keeled callus was almost similar to lips. Gradual increase in ratio of L-complex vs. Sp-complex genes may possibly lead to this. When SP-complex will be completely suppressed and only L-complex will express, petals will completely get converted into lips. In order to

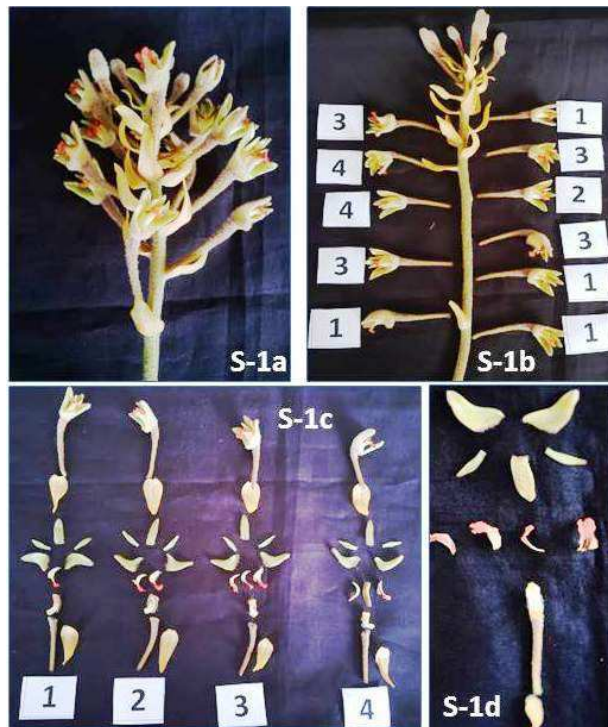
exactly quantify the effects, gene expression study in the petals of respective variants for L and SP complex genes by real time PCR will be very useful. Interestingly concomitant flower size reduction with petal to lip conversion turned the standard flowers into miniature types possibly due to pleiotropic gene action. We also recorded few other variations in flower pattern formation in some other genera of orchids, which have been presented and described in Supplementary Figs. 1-3 (available online at <http://www.isgpb.co.in>).

The available models for flower development are effective and valid, although not complete. More number of mutant types may be recorded and included for case study based on the proposed models. Orchid breeders may use such changes for creation of novelty through fixation of mutants. Random mutation breeding through chemical and physical mutagens may be attempted besides directed changes through RNAi approach, especially for L and SP complex genes.

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Supplementary Figures



Supplementary Fig. 1. Occurrence of variants of flower pattern formation in *Eria tomentosa*. S-1a: spike of *E. tomentosa* containing four different patterns. S-1b: Representation of different flowers identified in same spike as per their position. S-1c: Dissected flowers shown as per representation in spike. All the four types contain same numbers of sepals (03 nos.), petals (02 nos.) and gynostemium (01 no.). The flower no. 1 is the taxonomic standard of normal flower as described by Rajkumar *et al.* (2014). The flower no. 2 shows one extra lip. The flower nos. 3 and 4 has two extra lips in addition to the normal one lip. However, the last one has one intermediate lip like structure. S-1d: One flower recorded in another spike shows four lips. With the three available models of orchid pattern formation, the increase in number of only lips can't be explained



Supplementary Fig. 2. S-2a shows a normal flower (left) and one variant flower (right) of *Epidendrum sp.* and S-2b: Dissected flowers of these two. The wild type flower contains five tepals, one lip and one gynostemium. The variant flower has only two tepals and two lips which were fused and without any gynostemium. This may happen due to lack of expression of C-class gene in the third and fourth whorl of flower leading to absence of column. Expression of B-class gene in absence of C-class gene in third whorl may have converted the stamen into lip. Absence of expression of A-class gene in first whorl may possibly lead to absence of all the three sepals



Supplementary Fig. 3. S-3a shows normal wild type flower of *Arundina graminifolia* (common name-Bamboo orchid). It contains three sepals, two petals, one lip and one gynostemium inside the lip. However, in one variant flower (s-3b) we have recorded four sepals, three petals, two lips and one column (exposed outside the lips). Hence the variant have one additional sepal, petal and lip. Although it seems be resultant of ectopic expression of A & B-class genes, how these has arisen can't be exactly explained by the three available models. However, the alteration has created a novel variant with greater ornamental value