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# VANILLA BREEDING - A REVIEW

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## ABSTRACT

**Breeding behaviour of vanilla needs a thorough relook taking into consideration the various reports of autogamy, self incompatibility and natural crossing. *Vanilla planifolia* flower is adapted to cross pollination, the anthers being physically separated from the stigma by a rostellum. In case of aided pollination, the rostellum is pushed back and the anthers are brought to contact with the stigma ensuring pollination. However, occasionally autogamy too is observed besides self incompatibility. Cultivar diversity, arising probably due to somatic crossing over, bud sports or sexual reproduction, too is encountered in the crop though vegetative propagation is the rule in vanilla .Vanilla germplasm maintained in the clonal repositories conserve both the primary and secondary gene pools. Given the scenario of viable sexual reproduction and the scope for fixing any new variation through vegetative propagation ,vanilla improvement is exciting. The present review is an attempt to look into the global scenario of conventional breeding of vanilla taking into consideration the conflicting reports on the breeding behaviour of the plant which may give new directions for the improvement of the crop by conventional breeding. New breeding strategies are also suggested.**

**Key words**: Conventional breeding, Diversity, Reproductive biology, Strategy, Vanilla, *Vanilla planifolia.*

Vanilla(*Vanilla planifolia* Andrews) with the sobriquet 'Prince of Spices' is the only orchid spice. It is a perennial succulent vine trailed on trees or other standards .Vanilla accounts for about 0.75 percent world import of spices in volume and in terms of value its share is six to seven percent of nearly 1500 million US$ of global spice trade (Vanilla Status Paper, Spices Board, Kochi,India, 2003). The major vanilla producing countries are India, Indonesia, Madagascar, Mexico, Reunion Islands and the Comoros besides the other countries such as China, Guadeloupe, French Polynesia, Fiji, Malawi, Tonga, Uganda, Zimbave, etc. which grow the crop to a lesser extent. Vanilla yields vanillin ($C_8 H_8 O_3$) from the processed beans (pods), which is used as a food flavourant besides in pharmaceuticals, perfumes and liquors. It is estimated that during 2001 the global trade of natural vanilla accounted for 103.18 million US $.However, currently cheap synthetic vanillin poses a tough competition for the natural vanillin. Synthetic vanillin is made from cheap sources like waste sulphate liquor of paper mills or coal tar extracts .During 2000-01 India imported 404.0 tonnes of synthetic vanillin worth Rs.1649.91lakhs(Vanilla Status Paper, Spices Board, Kochi, India, 2003).Vanilla is the only source of natural vanillin at present and high yielding varieties of the crop will be a boon in this regard.

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Vanilla, though sets seed, is conventionally propagated using cuttings. The viable sexual reproduction coupled with the vegetative method of propagation can be exploited in the improvement of vanilla. However, a clear understanding of the breeding behaviour of the plant is warranted to adopt an appropriate breeding strategy. This review tries to take a stock of the information available on the mode of sexual reproduction of vanilla, especially *Vanilla planifolia* and suggests breeding strategies.

**Cultivated species and germplasm**

History, taxonomy and ecology of vanilla is elaborately dealt by Bouriquet (1954).Although the number of species in the genus Vanilla reported varies from 90(Cameron and Chase,1999)to188(The International Plant Name Index, 2004) through 107 (Soto Arenas, 2003 and Porteres,1954), the cultivated species are only three at present viz., *Vanilla planifolia* Andrews syn. *V. fragrans* Ames (Salisb.) ( Mexican vanilla), *V. tahitensis* J.W. Moore ( Tahitian vanilla) and *V. pompona* Schiede (West Indian vanilla) (Sasikumar *et al.,* 1992).*Vanilla planifolia* is diploid (2n = 32) though variation in chromosome number is reported (Hoffman,1929; Heim,1954; Chardard,1963 and Martin, 1963). Even though the basic chromosome number of the genus is x = 8, different ploidy level is mentioned especially for *V. tahitensis* (Tonier,1951).

Vanilla germplasm is maintained in India at the Indian Institute of Spices Research (IISR), P. O. Marikunnu, Calicut, Kerala(about 300 accessions) and at the Indian Cardamom Research Institute (ICRI), Spices Board, P.O. Kailasanadu, Idukki, Kerala(about 21 accessions). Crop improvement work of vanilla is in progress at the Kerala Agricultural University, Vellanikkara, Trissur, Kerala, also. The accessions conserved in these clonal repositories mainly belong to the commercially cultivated species, *Vanilla planifolia*. The species which are endemic to India such as *Vanilla andamanica* Rolfe, *V. pilifera* Holtt, *V.walkeriae* Wright and *V.wightiana* Lindl.ex J.D. Hook are also being conserved. Weiss(2002)has mentioned of a germplasm collection, mainly Central American collections of vanilla, in CATIE (Centro Agronomico Tropical de Investigation y Ensenanza) of Turrialba (Costa Rica). Other germplasm collections of vanilla are maintained in French Polynesia and Fiji (Wong,2005), Reunion Islands and at the Institut de Recherches Agronomiques a Madagascar (IRAM) (Grisoni *et al.*,1997).

A minimum descriptor for vanilla is developed in India for characterizing the germplasm (Kuruvilla *et al.*,2000). And technical guidelines for safe movements of vanilla germplasm are also available (Pearson *et al.,*1991).

**Breeding behaviour**

The flowers are formed on axillary inflorescence which are generally simple but rarely branched. Vanilla flowers are large, pale greenish yellow, bisexual and zygomorphic. Petals and sepals together are called perianth. The lower petal is short, broad and modified in to a labellum which envelops a central structure called 'column' at its lower end. The tip of the column bears a single stamen with two pollinia covered by a hood like structure called 'rostellum' below which is the stigma. The rostellum acts as a physical barrier between the stigma and the anther (pollinia).With the flowers thus adapted to aided pollination, the information available on the breeding behaviour of vanilla is rather confusing. Major observations on the breeding behaviour of *V. planifolia* are reported from Mexico (Bory *et. al.*,2008). Indian studies are restricted to some observations on pollination time, stigma receptivity, fruit development, etc. Reproductive biology of *V. planifolia,* such as time of pollination, stigma receptivity, effect of pollen load on the size of the beans etc. were studied in India. (Bhat and Sudarsan,1998 and Bhat and Sudarsan,2000). These authors reported the ideal time for pollination from 6 am to 1 pm, and stigma receptivity up to 24 hrs. They also observed that complete transfer of pollen results in maximum fruit growth. The floral biology of cultivated vanilla though adapted to out crossing, differing natural self pollination rates up to

20% are reported in some *V. planifolia* 'cultivars' from Mexico, Puerto Rico and Central America (Childers and Cibes,1948;Soto Arenas, 1999; Weiss,2002;Lubinsky,2004).Self incompatibility too is reported in *V. planifolia* 'cultivar', 'Orega de Burro' (Castillo Martinez and Engleman,1990). It thus appears that there is even region wise variation in the occurrence of the self incompatible and self compatible forms of *V. planifolia*. This aspect needs to be studied in detail. Further, even though the floral biology basically favours allogamy, Soto Arenas (1999) could find very low cross pollination rate and very low observed heterozygosity in *V. planifolia*, making him to conclude that the dominant breeding behaviour is autogmay. However, based on the works reported, it appears that *Vanilla planifolia* can be better included under the group 'often cross pollinated species' of plants. It may also be worth noting here that natural hybridization between sympatric species of vanilla is also hypothesized (Nelsen and Seigismund,1999 and Lubinsky, 2004).

However ,in *V. wightiana* too natural fruit setting is reported (Rao *et al.*, 1994). Stray fruit set under natural condition is also seen in *V. aphylla* in India (personal observation).

Bees of the genus *Melipona,* humming birds, *Euglosa viridisima*, *Eulaema* spp. etc. are considered to be pollinators of vanilla (Soto Arenas,999 and Lubinsky *et al.,*2006).In the absence of the pollinators, hand pollination is resorted to ensure fruit set, especially in India and even in some parts of Mexico.

All these facts warrant a critical relook into the role of pollinators, out breeding rates, autogamy and self/cross compatibility of the plant including the role of apomixis.

## Seed dispersal and germination

Vanilla pods contain many minute black, globose seeds. Seeds of vanilla could be dispersed by air or water (Ariditti and Ghani, 2000) or even by bats (Soto Arenas,1999). It is even proposed that *V. planifolia* seeds could be dispersed by birds as the passing of the seeds through the intestinal gut helps quick germination (Bouriquet,1954;Arditti and Ghani, 2000). Seeds produced by vanilla generally do not germinate under natural condition though Delassus(1960)described seed germination of vanilla in the wild albeit the seedlings died prematurely. Recently ,Bory *et al.,* (2008) also indicated the possibility of natural seed germination of *V. planifolia* from the pods dropped on the plots. Childers *et al.,*(1959) noted that a small number of the seeds could germinate in ideal condition of humidity, temperature and nutrition. *In vitro* culture of the seeds under tissue culture conditions offers a viable safe option in this regard. *In vitro* seed culture of vanilla is standardized at the Indian Institute of Spices Research (IISR), Calicut, Kerala, India.

## Cultivar diversity

It is generally agreed that the present day vanilla cultivars originated from a narrow genetic base (Soto Arenas,1999;Lubinsky,2003; Sasikumar, 2004). History gives ample evidence that the colonial rulers took special interest in introducing the crop to their colonies (Potty, 2003). In most cases the first introduction came from just a handful of cuttings. Being perpetually propagated vegetatively from this original germplasm, a wide genetic base in the primary gene pool of vanilla is very unlikely especially in the secondary areas of domestication of the crop which may lead to genetic vulnerability. However, over the years of domestication and selection by farmers some new variants ('sub cultivars') have been recognized in Mexico and Reunion Islands. Somatic crossing over (Nair and Ravindran, 1994),spontaneous mutation (bud sports) or even sexual recombination and natural seedlings (see section- **Seed dispersal and germination**), epigenetic variation as well as fresh unofficial introductions (Bory *et al.,*2008) are all attributed to the origin of variation. In Reunion Islands the planters distinguish two types of variants of *V. planifloia* viz., 'Classique' with light green flat leaves and tapering pods and 'Mexique' or 'Bleue' with dark bluish leaves with a central gutter and curved sides producing cylindrical pods. 'Aiguille', 'Grosse

Vanille', 'Sterile', 'Variegata' are the other minor variants recognized in the species (Bory *et al.*,2008). Four major types of *V. planifolia* variants are known in Mexico (Soto Arenas, 2003). 'Mansa' or 'Dura', with two sub types based on stem and leaf colour namely, 'Amarilla' and 'Verde' is the most common one(Diaz,1989).'Rayada' or 'Variegata', 'Albomargina' and 'Oreja de Burro' are the other types. These four types can be distinguished based on the stem and leaf colour, leaf margin nature etc. 'Oreja de Burro' is self incompatible too (Castillo Martinez and Engleman,1993). However, Lubinsky (2003) is of the view that these variants may not represent clones of the same species or even can be inter-specific hybrids. Intraclonal variation in clonally propagated crops is of course a fact, as reported in black pepper (Pradeep Kumar *et al.*, 2003; Shahanas *et al.,* 2003).



A preliminary Indian study on the genetic variability of six accessions of *V. planifolia* using PAGE indicated variability in peroxidase, esterase and amylase profile (Vanilla Status Paper,Spices Board, Kochi,India,2003).

**Inter-specific hybridization**

The variation that exists among the cultivated species of vanilla or even in some related species can be combined to produce new types. The secondary gene pool of vanilla may contain useful genes for self pollination, root rot and virus resistance, larger fruits, reduced photo sensitivity, better aroma profile and pod indehiscence for incorporating to the cultivated vanilla (Rao *et al.,*1994;Leclerc-Le Quillee *et al.,*2001). Interspecific compatibility between some vanilla species though doubtful (Soto Arenas,1999), attempts may be made to hybridize the different species and raise the progenies through *in vitro* seed culture. The xenia/ metaxenia aspects too can be looked into *inter alia* as it is observed that pollen of some vanilla species has a positive effect on the pod size of *V. planifolia* (personal observation). In Madagascar successful inter-specific hybrids between *V. planifolia* x *V. tahitensis* and *V. planifolia* x *V. pompona* have been produced (Delassus,1960; Dequaire,1976; FOFIFA,1990). Lubinsky (2004) also hypothesized the spontaneous occurrence of hybrids between the sympatric species such as *V. planifolia, V. pompona* etc. Nelsen and Seigismund(1999)too suggested the possibility of natural hybridization of *Vanilla* spp. of the Caribbean Islands. Interspecific hybrid between *V. planifolia* x *V. aphylla* is reported from India too(Minoo *et al.,* 2006).

Above fifty percent pod set is reported in an interspecific hybrid of vanilla by Kerala Agricultural University (Anon.2001). Reciprocal variation in fruit set of interspecific hybrids between *V.planifolia* x *V. aphylla* too is observed (Nissar *et al.*,2006)Hybrids numbering 250, probably interspecific ones, are reported from Madagascar too (Grisoni *et al.,*1997).

**Breeding strategies**

Keeping in view the breeding objectives such as better quality profile of the produce, self fertilization, plant type, high yield, disease resistance etc. one or the other of the following known strategies may be adopted to improve the crop.

**Inter 'cultivar' hybridization**: The variability that is observed in the so called 'cultivars' can be exploited to produce new varieties of vanilla having better quality profile through hybridization followed by *in vitro* seed culture. Recombinants with new traits are a possibility.

**True Vanilla seedling selection**: Vanilla being a heterozygous vine propagated vegetatively, selfing can lead to release of the residual variability. Selfed seeds can be cultured *in vitro* and new variants can be picked up from the seedling progenies.

**Mutation**: *In vitro* and *in planta* mutation induction of the vegetative tissue/seeds can also be a gainful breeding strategy for vanilla especially in view of the limited variability in the germplasm.

**Germplasm selection**: Collection and evaluation of the germplasm may also be useful in vanilla improvement. Polyploidy breeding and inter-specific hybridization are worth attempting in improving the crop.

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