An International Journal of Floriculture Science and Landscaping
The Journal of the Greens and Gardens, Volume 01, No. 02 : 1-6. 2019

Review Paper

Studies on *Bougainvillea* breeding: issues and challenges

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Received: March 08, 2019
Accepted: March 15, 2019

Abstract

Bougainvillea (*Bougainvillea* Commers.) is a popular ornamental plant grown for its colourful and attractive bracts. Out of 16 species, 4 have ornamental importance. There are around 1000 cultivars under different species available all over the world. Due to its high demand in nursery trade and landscape use, the demand for new and novel cultivars is growing steadily. Breeders all over the world, especially from tropical countries, are putting hard efforts to develop new cultivars with special traits, mainly dwarf and thorn less varieties, to match the changing taste and lifestyle.

This paper highlights some basic issues and challenges faced by the amateur breeders besides the ways to solve the breeding problems.

Key words: Somatic cell, germ cell, polyploidy, sterility, incompatibility, dominant, recessive, inheritance.

Introduction:

Bougainvillea (*Bougainvillea* Commers.), a member of the family Nyctageniaceae, is a popular ornamental plant grown in tropical and sub-tropical gardens of the world. Their colourful bracts and wide adaptability into different agro-climatic conditions are the main reasons for their popularity. Easy to grow, floriferous habit, and their capability to transform landscapes have made them a most popular garden plant. Bougainvilleas are widely available in Asian countries (China, India, Korea, Malaysia, Philippines, Singapore, Thailand etc.) beside in Australia, Tropical America (Roy *et al*, 2015).

Being one of the most popular garden plants, Bougainvilleas draw attention of the plant lovers and breeders from all over the world. At present, around 1000 varieties are available around the globe especially in tropical countries of the world. Though there are 16 species of *Bougainvillea*, following 04 of them have horticultural importance with colourful bracts - *B. spectabilis* Wild., *B. glabra* Choisy, *B. peruviana* Humb. And Bonp. and *B. x buttiana* Holtum & Standl. Present day varieties belong to these four species. Several breeding methods (hybridization, mutation breeding, bud sport, colchicines treatment) have been used for the development of these varieties (Roy, 2019).

With the changing life style, housing pattern and new landscape usages, the demand for new and novel varieties is growing day by day. As a result, large scale breeding programmes have been initiated for the development of the new varieties. There are some hurdles also in the breeding programme viz. self sterility, seed formation, compatibility amongst the species etc. These need to be addressed for successful breeding programme.

Breeding Perspectives:

Breeding in bougainvillea are highly subject to following issues or limitations.

- Somatic cells turn into germ cells.
- Synopsis and pairing in meiosis.
- Self-Incompatibility.
- Dominance / recession on alleles of traits.

Somatic Cell to Germ Cells in Inheritance:

We may see many morphological alteration of traits during daily maintenance work of bougainvillea germpalsm collection. Most of them were temporary changes caused by environmental and other factors. A few of them are permanent due to mutation resulted by certain changes of chromosome or gene. Mutations may happen in somatic cells of buds or germ cells in stamens and pistil of flowers. In the latter case, the genetic variation could pass the change to its offspring by sexual reproduction while in the former the trait of mutation may be inherited by asexual reproduction, such as cuttings and layering.

In contrast to humans and animals, plants do not set aside a specialized and permanent organ for future sexual

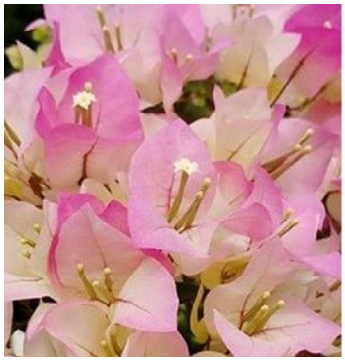
reproduction. When it needs and blooms, germ cells can arise from somatic cells into the flower. In Bougainvillea, majority of cultivars have been developed from mutation followed by hybridization and finally through seedlings.

Issues:

- Can mutation of desirable traits pass to the germ cells so that seedlings may inherit the trait you are looking for?
- After years waiting, experienced bougainvillea lovers may wonder why bract color of seedlings developed by themselves were always mauve in 'Eva', red in species *B. x battiana*, despite the fact that both female and male parents involved for breeding have no mauve / red colour involved.

Case Study:

A compact seedling from hybridization of *B. x buttiana* 'Imperial Thai Delight' and 'Sunvillea Cream' was got. The two cultivars are sterile both on pollen and seed bearing. The former cultivar regained fertility by spontaneous polypolidy mutation and fertility of the latter was restored by colchicine treatment. Bract colors of the two are pinkish-white and yellow, but color of the hybrid seedling is red, why?



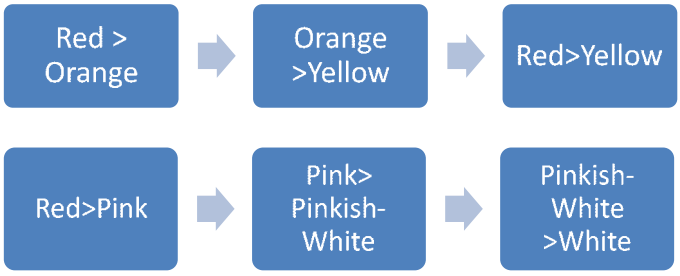
'Imperial Thai Delight'

'Sunvillea Cream'

Seed bearing on the seedling of
'Tetra Imperial Delight' x 'Sunvillea Cream'

Explanations:

Before looking for answers, it is better to know how the cultivars in breeding derived from. According my personal observations, rule of color change through bud sport in species *B. x buttiana* mainly as follows:



Pinkish-white and yellow are directly or indirectly bud sport from red. Red was the ancestor color of the two parent cultivars in the breeding programme. In the first case lots of evidences are available such as chimera or reversion red in bract. In my opinion the ancestor of 'Imperial Delight' is *B. x buttiana* 'Barbara Karst', a red cultivar and a seedling of 'Mrs. Butt' and 'Sanderiana'. As to 'Sunvillea Cream', a bud sport mutant of 'Sunvillea Pink', and 'Sunvillea Pink' was bud sport from 'Konatu' (a Japanese variety). However, 'Konatu' was highly resemble to 'Pixie', a dark pink or purple-red bract.

Plant usually needs a mechanism of cell restoration and filtering to control and select suitable germ cells for its next generation and in order to ensure their progenies will be strong enough to withstand environmental challenges for their survival. Factually, most of mutations on traits which are interesting came from defective changes, on plant itself. Therefore, in most of cases, the trait combined in bud sport at somatic cell, when somatic cell was turned into germ cells restore the parental trait and, sometimes, throwback to its ancestral color of trait.

- That might be one of the reasons why color of my compact seedling was red even though there are no red on both female and male parent.
- There is exception of every rule. Some exceptions one might get when there is handling of large quantity or got lucky in breeding. It is understandable, that mutation also might happen in meiosis, grow of zygote and even during seed germination. It can't be regarded as an error on theory.

Synopsis and Pairing in Meiosis:

Why the cultivars of *B. x battiana* and *B. x specto-peruviana* are mostly both pollen and seed sterile? Similarly, only limited cultivars under *B. glabra* and *B. spectabilis* are fertile?

Moreover, why certain members in interspecific hybrid, such as cultivars in specto-glabra are pollen fertile? The reason for such phenomena is still not clear. It seems, contrary to common theory, the cultivars should be sterile in interspecific hybrids.

All these questions can be resolved from knowledge of synopsis and pairing in process of meiosis. Issues related to content and structure of chromosomes of the three common species *viz. B. glabra, B. spectabilis and B. peruviana*, are matter of in depth study. But it is confirmed that almost all cultivars in both *B. x buttiana* and *B. x specto-peruviana* are sterile, incapable of having fertile pollens and seeds bearing due to pairing troubles in meiosis, despite exceptions with seed collection on these sterile group are often seen (Ohri, and Zadoo, 1986).

There are lots of cultivars in the species of *B. x buttiana* such as 'Mrs. Butt', 'Barbara Karst', 'Poultoni', 'Scarlet Queen' and 'Miss Manila', in which each of these red cultivars also have their respective sorts color of mutants. Cultivars in *B. specto-peruviana* group staring from 'Mrs. H.C.Buck', the parent of many bi-colored mutants, 'Mary Palmer', and all cultivars directly and indirectly are bud sport of 'Mary Palmer' including 'Thimma', 'Lipstick', 'Shubhra', 'Ice Cream' ('Odisee' in India, 'Splash' in Philippines) and 'Magic Ice Cream'.

These sterile cultivars are now major part of present Bougainvilleas and undoubtedly are the main obstacles in breeding. How to overcome the problem on pairing in meiosis is really a critical issue to deal with. Fortunately, sterile diploid interspecific cultivars can regain fertility by colchicine treatment, one of ways of doubling sets of chromosomes, which could deal with problems of pairing. The author has got success on restoring fertility of some sterile cultivars and developed hundreds of seedlings with polyploidy, most of them are still waiting for bloom (Khoshoo and Zadoo, 1969).

Cultivars with partial success – By the treatment of cholchicine of the following cultivars, got seedlings from them but couldn't isolate an independent tetraploid mother cultivar. Eg. 'Sunvillea Cream', 'Mona Lisa Yellow', 'Poultoni', 'Partha', 'Miss Manila'.

Cultivars with complete success - After colchicine treatment got seedlings and could isolate an independent tetraploid mother of *Bougainvillea* cultivars 'Mrs. H.C. Buck', 'Ice Cream', 'Magic Ice Cream' and the latest one 'Lipstick' so far. The isolation process is still continuing and the chance of getting success is there.

Colchiploid *B.x spectoperuviana* 'Tetra Lipstick'

Colchiploid *B.x spectoperuviana* 'Magic Ice Cream'

Pollen grains of 'Tetra Lipstick'

Pollen grains of 'Tetra Magic Ice Cream'

Seeds of 'Chitra' x Offspring of ('Imperial Delight' and 'Sunvillea Cream')

Self-incompatibility:

Self-incompatibility (SI) is one of the most important systems used by many flowering plants to prevent self-fertilization and thereby generate and maintain genetic diversity within a species (Silva and Goring, 2001). The SI response is comprised of a self-and nonself-recognition process between pollen and pistil that is followed by selective inhibition of the self-pollen (tube) development. Classic genetic studies have established that the self-/ nonself-recognition in most species is controlled by a single multi-allelic locus, the S-locus, and that pollen inhibition occurs when the same "S-allele" specificity is expressed by both pollen and pistil. Sexual reproduction in many flowering plants involves self incompatibility (SI), which is one of the most important systems to prevent inbreeding (Takayama

and Isogai, 2005; Kao and McCubbin, 1996; Wang *et al.* 2003).

Self incompatibility does exist in fertile bougainvillea, but so far discussions mainly focus on a fertile cultivar, same or different pots. Does SI also exist between cultivars with bud sport relationship? For example, 'Mrs. McClean' was bud sport from 'Mrs. Butt', 'Mary Palmer' was bud sport from 'Mrs. H C Buck'. If these four sterile cultivars have regained their fertility by colchicine treatment, can we get seeds from hybridization of 'Mrs. Butt' x' Mrs. McClean', and 'Mrs H.C. Buck' x 'Mary Palmer' ?

The author has successfully got 4 colchiploid cultivars with highly genetic relationship in *B. specto-peruviana* group viz. 'Mrs. H.C.Buck', 'Lipstick', 'Ice Cream' and 'Magic Ice Cream'. In the process 'Lipstick' was isolated from 'Mary Palmer', 'Ice Cream' was bud sport from 'Mary Palmer', 'Magic Ice Cream' was indirectly bud sport from 'Ice Cream'.

Meanwhile, a small scale SI test was done using following two cultivars – 'Mrs H.C. Buck' and 'Magic Ice Cream' a few months ago. In each cultivar rate of seed setting by hand pollination was around 30% when hybridized with other cultivars without SI limit.

Reciprocal crosses were done by hand pollination on 13 floral tubes of samples under study but all bracts fallen after 2 weeks and got no seed setting was there. Though the number of sample was small, it did show a preliminary result that SI might exist among related mutants. If it is true that will be a big barrier and will highly influence breeding of Bougainvillea. Because most cultivars in bougainvillea derived from mutation do have a closed relationship genetically. Bougainvillea has its own mechanism to prevent inbreeding, leaving no ways of seed setting among them (Hiscock *et al*. 2003; Kao and Tsukamoto , 2004; Ohri, and Zadoo, 1975).

Self Incompatibilty Studies - Some tetraploid cultivars, besides hundreds of tetraploid seedlings were raised, as follows:

1. Chitra
2. Seedlings of tetra 'Imperial Delight' x tetra 'Sunvillea Cream' (Seedlings)
3. Tetra 'Mrs. H.C. Buck' (HCB)
4. Tetra 'Lipstick'
5. Tetra 'Ice Cream'
6. Tetra 'Magic Ice Cream' (MIC)

Following cases of reciprocal cross, by hand pollination, the focus was mainly on the seedlings with compact trait No. 2 and the objective was to get more compact seedlings and additionally bi-colored compact seedlings.

Achievements - Finally, 36 seedlings were produced and half of them had compact trait. It is expected to get 18 tetraploid compact seedlings the current year.

Seedlings with compact internodes

Table 1: Number of seedlings produced from crossing

Sl. No.	Crosses	No. of seedlings produced
01.	**Seedling x 'Chitra'**	**00**
02.	Seedling x MIC	02
03.	Seedling x Lipstick	02
04.	HCB x Seedling	06
05.	Ice Cream x Seedling	03
06.	**Chitra x Seedling**	**14**
07.	MIC x Seedling	05
08.	Lipstick x Seedling	02
09.	Seedling x Ice Cream	01
10.	Others	01
	Total:	**36**

Explanations – Examination of the data revealed that the cross under sl. No. 6 (Chitra x compact seedling), 14 seedlings were produced but got 0 in case sl. No. 01 (Seedling x 'Chitra') when both of parent cultivars were fertile. The reason for such result is not very clear, as combination of A x B is Ok but B x A is not OK. Similar results were also obtained in case 'Chitra' and 'Mrs. Eva' when crosses 'Chitra' x 'Eva' was Ok with high seeds setting but reverse combination 'Eva' x 'Chitra' was not fruitful with very low rate of seed setting. The reason might be related to SI, together with factor of dominance / recession of multi S alleles (Entani *et al.* 1999; Jordan *et al.* 1999; Ohri, and Zadoo, 1975; Mukhopadhaya and Lakshmanan, 1957; Ohri, and Zadoo, 1979).

Dominance/Recession Effects of Traits:

The phenotypic appearance of a plant is the expression of hundreds of traits and their combinations. This is mainly determined by genes of female and male parents and majority of traits are not a concept of 'mix' of inputs but a relationship of dominant /strong and recessive / weak from related alleles genes of parents. Therefore, color/ apex/ base/ size/ shape and texture of bract and leaf, compact or long internode, shape and length of thorn, even color/shape/size of seed etc. are different kinds of traits (Rastogi *et al*., 2019).

Pollen / gametes and ovules are the result of meiosis happened at germ cells which was transformed from somatic cells when bougainvillea bloom, as mentioned before. In process of meiosis, contents of chromosomes and its DNA and genes on pollen and ovules have also been determined (Shiba *et al*. 2002; Cooper, 1931). For instances, there are 4 sets of chromosomes in 'Chitra', *i.e., glabra / peruviana / spectabilis/peruviana* and trait of bi-colored might only exist one of them, owing to there is no significant variance on contents of chromosomes between species *glabra* and *spectabilis*, Chitra will meet less pairing trouble in meiosis and be capable of generating fertile gametes. Finally, 1/2 opportunities of gametes (2n) might carry the bicolor genes, when people or insects put the 2n pollen grains from 'Chitra' onto stigma of another deploid fertile cultivar with 1n gametes, if it's got success and produced an zygote, and finally got a seed. The seed will naturally own 3 sets of chromosomes mean a triploid, in which, a certain allele of genes, say bract color, normally own genes two from male and one from female parent, the final appearance you looks are determined by dominance and recession of alleles, *i.e.,* despite pollens have got 1/2 chance of bi-colored trait, still need to compete with the genes from female parent (Pancho *et al.,* 1960). The opportunities of being a bi-colored seedling of the hybridization case 'Chitra' as male parent will depend on the female cultivar, if female cultivar is a bi-colored also such as 'Tetra Lipstick' or 'Tetra Ice Cream', opportunity is higher than single colored cultivars.

Therefore, taking dominant /recessive issues into account, as follows:

- If male parent owns a big size of leaf, and you want leaf size of seedlings be smaller, you should select a small leaf cultivar as the female parent.
- If someone highly expect a compact seeding, should select cultivars having shot inter-node of trait.
- If there is lots of red cultivars in collection and do not like to get more, should avoid to select red cultivars or which is bud sport from red as one of parents, as because red trait is too strong.

Conclusion:

Therefore, if you are interested in breeding of Bougainvillea, it is better to know something about the cultivars, as described hereunder.

- Is it a fertile cultivar? Pollen fertile or seed fertile or have both.
- Is it derived from a seedlings or a mutant? It highly relates to traits selected and is a genetic trait or just a mutation of somatic cell. The latter will probably be restored or filtered out at stage of somatic cell turn into germ cell, hard to pass to subsequent generations.
- What is the relationship of the selected parent, highly related or not? This may face problem of SI (self-incompatibility).
- Finally, the breeding work may be started when one has sufficient knowledge about dominance and recession of the traits and ultimately selecting the right cultivars for breeding programme.

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