Breeding of ornamentals: tuberose (*Polianthes tuberosa* L.)

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In the floriculture industry there is always demand and necessity for new and novel varieties. The colour, form and scent of the flower are the primary novelty markers in the global flower industry. Genetic diversity plays an important role in breeding. *P. tuberosa* is grown all over the world for cut flower production, for floriculture trade and as a source of oil. Breeding has successfully developed high yielding varieties in India, but there is no new colour. Literature survey indicates that development of coloured tuberose is possible through creation of genetic variability through conscious/selective breeding. Collection of coloured germplasm is the most important step in developing new flower colour tuberose through hybridization and induced *in vitro* mutagenesis.

Keywords: Coloured tuberose, germplasm, genetic diversity, hybridization, pigments, tuberose.

FOR a modern science-based and industrialized floriculture there is always demand and necessity for new varieties. Global flower industry thrives on novelty traits such as flower colour, form and scent which are primary novelty markers and key determinants in consumer choice. Ornamental species fall into two main categories. The first group of plants are capable of sexual reproduction, but are propagated vegetatively for commercial purpose. The second group are apomicts. In obligate apomicts, hybridization fails to generate any variability. The present-day colourful ornamentals have evolved through complex inter-specific crosses among elemental species, open pollination, indiscriminate intervarietal hybridization, spontaneous and induced mutation, selection and management of chimera. Creation of genetic variability is pre-requisite for development of new variety. Genetic diversity plays an important role in breeding because hybrids between genetically diverse parents manifest greater heterosis than those between closely related parents[1–4]. It is important to understand the science behind flower colour inheritance, polyploidy, and potential sterility of one or more parents before hybridization. It is not always easy to attain hybridizer's goal, but by understanding some of the genetics involved, one can make good decisions as to which crosses might lead to success. Normally for developing new variety through hybridization in any ornamental crop, we start crossing among varieties/species available at hand. The cross may be a success or not. If it is a success, the seedlings that are selected from segregations, are established and claimed as a new variety. But this variety may not have any/much market value. Hence scientific manpower and time are wasted. Therefore, we must acquire relevant knowledge before starting hybridization.

For crop improvement and more specifically development of new varieties, a number of plant breeding methods like cross-breeding, induced mutagenesis and molecular breeding are available. Plant breeders have produced a large variety of flowers by classical genetic techniques. However, some colours are still lacking for many ornamental plant species because of limitations in the gene pool of any single species. There are limitations on the genes responsible for the flower colour spectrum in a single species. The final visible colour depends on a number of factors like type of anthocyanin accumulation, modifications to the anthocyanidin molecule, co-pigmentation and vacuolar pH. Each of these factors is regulated by a number of genes. For undertaking a meaningful improvement programme, it is most imperative that basic genetic information is obtained through study of breeding systems, and experimental hybridization involving both cultivated and elemental species from the wild[5]. Information generated by such studies has helped in the circumscription of 'gene pools' and their utilization in the creation of new and novel cultivars of commercial importance keeping in view the direction of market trend. An understanding of breeding and genetic system of a plant is important because these control its heredity and variation. Breeders should acquire the knowledge regarding the fundamental techniques used in plant breeding, from classical plant breeding (e.g. recurrent selection, inbred line extraction, backcrossing, hybrid varieties and mutagenesis) to modern molecular tools (e.g. marker-assisted selection and genetic modified crops) to develop new ornamental plants.

The genus *Polianthes*, with the popular species *Polianthes tuberosa* L. is placed in the family Agavaceae (tribe Polianthеae)[6–11]. The genus *Polianthes* L. is endemic to Mexico and includes about 15 species, 3 varieties and a few cultivars. These species range in colour from white,

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orange red, red to striped. All the species are wild with the exception of *P. tuberose* which has never been found anywhere except under cultivation. Tuberose has been classified into three types on the basis of flowers. 'Single' cultivars are flowers with one row of corolla segments. These are extensively used for extraction of essential oil. 'Semi-double' bearing flowers are those with two to three rows of corolla segments. The spikes are straight and flowers are usually white. It is generally cultivated for cut flower purpose. 'Double' cultivars have more than three rows of corolla segments. Flowers are white in colour but tinged with pinkish red. The single flower type is often called 'Mexican Tuberose' while the double flower type is known as 'The Pearl'. *P. tuberosa* is a day-neutral plant (flowering is not strictly controlled by photoperiod). *P. tuberosa* is grown all over the world for cut flower production, for floriculture trade and as a source of oil in Egypt, China, France and Morocco.

Considering the important position of tuberose in Indian floriculture, voluminous research has been conducted on this crop on standardization of agro-techniques, utilization of agro-chemicals, development of new and improved varieties, post harvest, breeding system, disease management, etc. at different research institutions and universities. A large number of publications are available as a result of successful experiments. All these aspects have been reviewed by Datta[12]. The time has come to assess the impact of research on improvement of tuberose and its contribution to the floriculture industry.

Although *P. tuberosa* is an ornamental species of economic importance worldwide, the development of new cultivars has not been successful. Only two major varieties (single and double) and few improved varieties have been cultivated, all of which have white flowers. Development of new varieties is one of the major objectives of floriculture activities. Breeding for development of new varieties in India is restricted to single and double varieties. Breeding has successfully developed high yielding varieties. But there is no new colour. There are selections of improved types of the cultivar in different tuberose growing regions[13–18]. At present, total germplasm including new varieties available in India are:

Single types: Local Single, Pune Local Single (Pune), Calcutta Single (Calcutta), Hyderabad Single (Hyderabad), Kahikuchi Single (Assam), Mexican Single (Mexico), Navsari Local (Gujrat), Nilakottai Local, Sikkim selection, Rajat Rekha (Gamma ray-induced mutant, NBRI), Prajwal (Shringar × Mexican single, IIHR), Phule Rajani (MPKV, Rahuri, Maharastra), Shringar (single × double, IIHR), Arka Nirantara, GKTC-4, STR-501, Variegated Single Local.

Double types: Calcutta Double, Pune Local Double, Hyderabad Double, Pearl Double, Suvasini (Single × Double, IIHR), Vaibhav (Mexican Single × IIHR-2, IIHR), Swarna Rekha (Gamma ray-induced mutant, NBRI), STR-505, Arka Sugandhi.

Despite best efforts, no colour tuberose variety has been developed in India.

This review also highlights the breeding work done elsewhere on tuberose for development of new colour. Different genus and species included in the breeding system, their pigment composition, role of pigments in developing colour shades, etc. are also mentioned. For breeding work, the breeder should first develop all background knowledge on materials and the breeding system. An attempt has been made to review the breeding work on tuberose from the beginning. For developing a new variety, creation of genetic variability is a pre-requisite. Breeder should have an up-to-date knowledge about all available techniques and their merits and demerits for development and improvement of new varieties. The breeder should be aware of the potential and limitations of various approaches and should deliberately choose the most appropriate and economical strategy for reaching the aim under prevailing circumstances of variety improvement. Hybridization involves crossing two plants to produce a genetically and phenotypically superior offspring when compared to either of the parents. During this process, selection is an important step. There are limitations of the sexual system which limits the progress of conventional breeding, because it is usually not possible to incorporate genes from non-related species or to incorporate small changes without disturbing the particular combination of genes that make a particular type unique. An understanding of the breeding system is important for developing a methodology for genetic improvement. Breeding is the art and science of changing the genetics of plants to produce the desired characteristics. The genetic system of a taxon controls its heredity and variation and one of its important components is the breeding system[19–21]. Among other features, the latter encompasses a study of the mating system dealing with the nature and extent of self- or cross-incompatibility, self-compatibility and self-incompatibility, and amphi- or apomixes (including vegetative reproduction). These data together with a knowledge of other characteristics (e.g. time interval between pollen emission and stigma receptivity, style size vis-à-vis stamen size, pollen grain number per anther and per flower, seed number per plant, seed dormancy, etc.) exercise considerable influence on the genetical architecture and in turn affect evolutionary patterns, pathways and potentialities, and population size and structure including the extent and nature of its variability[22]. Such a study also helps to chalk out a meaningful breeding methodology for genetic improvement and build a sound taxonomic system. For optimum utilization of any commercial crop, proper understanding of evolutionary dynamics, elemental ecogeographical distribution, species and cultivar range, knowledge of its breeding system, agro-technology, techno-economics, genetic variability, etc. is essential.

The genetic variability available in tuberose is limited and this is in fact the major constraint in conventional breeding of tuberose. The main breeding objectives of tuberose are: to develop varieties with: enhanced vase life; resistance to various diseases; resistance to various insect pests; demand in domestic and international market for varied colour and fragrance; production of tuberose oil; improved yield and quality; and new and novel colours. In India, major breeding objectives were to develop high yielding varieties utilizing available variability for various important morphological characters[23]. *Polianthes* hybridzing efforts are mainly concentrated with 'tuberose' (*Polianthes tuberosa* L.) due to its ready availability, large flowers and outstanding fragrance. White is the sole 'real' colour for tuberose flowers. While these flowers are naturally white, plant breeders have produced hybridized cultivars in different colours – pinkish-lavender, pale pink, deep yellow and pale yellow. Breeders try to integrate colours from other plants that are near kin to tuberoses. Original tuberoses, however, always possess the classic white flowers. There are some important species of *Polianthes* worth mentioning, viz. *P. tuberosa* (white), *P. palustris* (white), *P. durangensis* (purplish), *P. montana* (white), *P. longiflora* (whitish purple), *P. plaitphylla* (white tinged with red), *P. granini-folia* (deep red), *P. geminiflora* (light orange red), *P. gracilis* (white), *P. blissi*, *P. pringlie* (white), *P. sesiliflora* (white), *P. nelsonill* (white), *P. graminiflora* Rose[13,14,24].

Few reports on early hybridization work are available. Bundrant[25] during his hybridization work mentioned that he collected tuberose from a local nurseryman in San Antonio, Texas around 1972. During that period only one 'Mexican Single' was in commerce[26]. Three distinct cultivars then known were assumed to have originated through mutation. Hybridization work started to develop further genetic variability in commercial cultivars. The genus *Polianthes* includes not only those species originally included in *Polianthes*, but all those formerly placed in the genera Bravoa, Pseudobravoa, Manfreda, Prochnyanthes Runyonia and the herbaceous species of Agave[27]. Traub[28] believed hybrids between *Polianthes*, Prochnyanthes, Pseudobravoa to be possible. The first hybrid in this group was produced using *Polianthes* (Bravoa) *genminiflora* and *P.* (Prochnyanthe) *bulliana* in 1899 (ref. 29), but the first cross involving the tuberose was reported in 1911 as *Polianthes* × *blissii*, a cross between *P. geminiflora* and *P. tuberosa*. Sixty nine years elapsed before the next hybrid was recorded. Verhoek-Williams[8] reported having crossed *P.* (Manfreda) *virginica* with *P. tuberosa*. Bundrant[25] was successful in developing three hybrids: *P.* × *blissii*, *P.* × *bundrantii* (*P. tuberose* × *P. howardii*) and *P. tuberose* × *P.* (Manfreda) *maculosa*. *P. tuberosa* has the characters of dichogamy and self-incompatibility. Cross between single and double cultivars produced fruits and seeds when the female parents were fertilized 2–3 days after anthesis. Reciprocal crosses produced many single and few double plants in the progenies, and 12 seedlings with improved characteristics were selected[30]. Howard[31] was interested in hybridizing various new *Polianthes* species with the tuberose to develop coloured flowers having the tuberose fragrance. Some coloured forms of the popular scented tuberose would be a great asset. He was motivated to initiate hybridization work from the *Polianthus* × Blissi Worsley Hybrid. This was developed by Worsley[32]. The hybrid was intermediate between its parents, having the delicious fragrance of male parent *P. tuberosa* combined with the rich rose pink colour of female parent *P. geminiflora*. He was successful in developing a myriad of new *Polianthes* hybrids. He reported that *Polianthus tuberosa* not only crosses freely with other *Polianthes*, but also with Manfreda and Prochnyanthes[33]. Howard[34] carried out experiments to combine with colour and fragrance in hybrids, the characteristics of the tuberose. He reported a new hybrid *P.* × *Bundrantii* (*P. tuberose* × *P. howardii*) which is similar to tuberose. The hybrid flowers had maroon interiors and rose pink exteriors that were tipped green and with fragrance. Two major cultivars, namely, white-coloured cvs. 'single' and 'double', were cultivated for commercial production. Crosses and back crosses among *P. tuberosa* 'single', *P. tuberosa* 'double', *P.* × *howardii* and *P.* × *blissii* were made and several hybrids showing pink, reddish-purple, purple, orange and yellow flower colours were selected. However, the long spikes of flowers in these coloured hybrids were only suitable for cut flowers. Four hybrids showing a dwarf plant type were selected for use as pot and/or bedding plants[35]. Gurav *et al.*[36] studied genetic variability, heritability and genetic advancement of different yield contributing characters in nine tuberose varieties. They reported that characters like weight of spike, 100 floret weight, rachis length and length of flower stalk offer great scope for effective selection and crop improvement as these attributes exhibit greater to moderate genetic advancement coupled with high heritability and higher variability estimates. Huang *et al.*[37] extensively studied breeding for coloured tuberose and pigment composition of hybrids. They bred and selected more than 50 hybrids having pink, reddish-purple, purple, orange and yellow flower colours[38] by crossing with *P. howardii*, a species native to Mexico[39]. Colour fluctuation was observed during experimental cultivation of these hybrids. Temperature and light intensity are two environmental factors affecting petal pigmentation[40–42]. Huang *et al.*[37] analysed pigment composition of petals of some selected hybrids and their parents. Effects of temperature and shading on flower characteristics and pigmentation in one anthocyanin-containing hybrid were also investigated. Among the selected nine bred lines, one hybrid contained only carotenoids, four hybrids had only anthocyanins and the other four had both carotenoids and anthocyanins in their petals. The main anthocyanidin in the anthocyanin containing petals was cyanidin with some hybrids also containing delphinidin. Variation of pigment contents contributed the diversity of flower colours in hybrid tuberoses. One of the lines, '77A05', of which the main pigment was anthocyanin showed reddish-purple flowers when cultivated at 20°C under natural light, whereas the colour was white at 30°C. It was almost white at 25°C with 45% shading of natural light, but pale with 25% shading. Cultivation of '77A05' in open fields was carried out at four different altitudes, 25, 75, 500 and 1200 m in Taiwan. Days to flowering from planting at 25 and 1200 m were 80.3 and 89.5 respectively. The higher the altitude, the longer the flower stalk, but there was no significant difference in inflorescence length. The anthocyanin content at 500 and 1200 m was approximately two and three times higher than that at 25 m respectively. The site selection for cultivation of coloured tuberose when the primary pigment is anthocyanin must be carefully considered. Effects of light intensity and other factors have been reported in other ornamentals[43–46]. Verhock-Williams[8] made extensive intergeneric crosses between *Manfreda* and *Polianthes*. The University of Arkansas started breeding work between *Polianthes* and *Manfreda* in 2003 in order to obtain cultivars more tolerant to hot and dry conditions, and reported that the flower colour of *Polianthes* is dominant over that of *Manfreda virginica* (L) Salisb. and *M. maculosa* (Hooker) Rose and these hybrids showed hybrid vigour characterized by larger plant size and extended blooming time. Some hybrids were successfully over-wintered suggesting that *M. virginica* may confer additional cold-hardiness[33]. Double tuberose has been reported sterile[9,47] and cannot be used as pollen parents. The double cultivar has been subjected to artificial selection for a long time and currently is not known to exist in the wild; the pistil and stamens have become petaloid segments or staminoid. Vegetative propagation has favoured this transformation and most individuals in cultivation are sterile[48]. Shen *et al.*[47] found that double cultivar is fertile in early flowering stage, when the female parent in 2–3 days after anthesis can be used as both pollen and seed parents. It is also reported that there was no seed production in both selfed single varieties and selfed double varieties due to self-incompatibility[47]. However, Huang *et al.*[49] found that selfed progenies could be produced. On the other hand Verhock-Williams[8] demonstrated that *P. geminiflora* is self-compatible. Petals of two common tuberose cultivars, 'single' and 'double' were white, whereas those of *P. howardii* were reddish purple. The nine hybrids had flower colours of orange, pink, purple and yellow. Some showed different colours in their inside and outside petals. From pigment analysis, 'single' and 'double' had neither carotenoids nor anthocyanins, '84A07' had only carotenoids, four hybrids, '82R16', '84D03', '84D04' and '84E14' had only anthocyanins and other four hybrids '82O04', '84G02', '84J08' and '85A05' and *P. howardii* had both carotenoids and anthocyanins. The variation in petal colours in the hybrids is due to carotenoids, anthocyanins and their combinations: the ratio of anthocyanins and carotenoids being the important factor. Additional colour variations can be obtained by changing this ratio. Certain double forms of *P. tuberosa* are sterile[50] and, thus, cannot be used as pollen parents[31]. Howard[51] collected three species *Polianthes* species #73–75 from the city of Oaxaca. Flowers were bright-orange-red externally, and yellowish on the inner surface. He also collected another rare *Polianthes* species similar to *P. geminiflora*; flowers were scarlet with green segments and tubes more inflated. He found another species of *Polianthes* in the northern Oaxaca with red and yellow flowers. He collected another species in 1974 which was very handy and easy to grow; flowers were orange-red with yellowish interiors and were among the more successful species to hybridize with *P. tuberose*. He further collected another species from a mountain range in the city of Guanajuato, central Mexico which was quite different from those of earlier collections. Colours of the flowers varied from a good coral-red, through shades of pink and rose, cream to nearly white. Howard[52] described three inter-specific hybrids with novel variants in flower colours: *P. blissii* × (*P. geminiflora* × *P. tuberose*) with orange red flowers; *P. bundrantii* 'Mexican firecracker' like a modern hybrid between *P. howardii* and *P. tuberose* with flowers marked internally in shades of wine or purple and externally in red or pink and green and the hybrid *P.* 'Sunset'. *P.* sp. # 2 × *P. tuberose* with pinkish or reddish exteriors and yellow interiors. There is some inter-specific and inter-generic breeding research being conducted in Japan, Taiwan and the US to develop orange-, yellow-, pink-, and lavender-flowered tuberose for the cut flower market as well as dwarf types for garden use. The use of wild *Polianthes* species in tuberose breeding programmes was previously reported by Shen *et al.*[35,38], who made inter-specific crosses mainly using single and double cultivars of *P. tuberose* and *P. howardii* (reddish-purple flowers) in order to bring the flower colour of *P. howardii* into tuberose. Several hybrids have been reported showing pink, reddish-purple, purple, orange and yellow suitable only for cut flowers. Huang *et al.*[49] analysed the pigments in the hybrids obtained by Shen, and reported the presence of either carotenoids or anthocyanins in the tepals of some hybrids and both pigments in others. They concluded that the use of *P. howardii* in tuberose breeding can contribute to the extension of the diversity of flower colours. Huang *et al.*[49] developed and analysed flower pigments of nine hybrids (purple, orange and yellow flowers). *Polianthes tuberose* 'single' and 'double' and *Polianthes howardii* were used to develop hybrids through crossing and back-crossing. Wide range of variations in pigments were observed among the parents and hybrids. Two white cultivars of *P. tuberosa* had neither carotenoids nor anthocyanins. Only anthocyanins were recorded in four hybrids and only carotenoids in one hybrid. *P. howardii* and another four hybrids had both carotenoids and anthocyanins in their petals. Cyanidin was the main anthocyanidin in the petals of coloured tuberose, although delphinidin was present in the petals of four hybrids and *P. howardii*. The various flower colours in hybrids developed through crossings between two species were developed from various pigment combination like the yellow due to carotenoids, pink, reddish purple and purple colours by anthocyanin and orange flowers due to the coexistence of anthocyanins and carotenoids. *P. howardi.* contained all these pigments. Ratio of anthocyanins and carotenoids contents seems to play an important role in determining flower colours. This ratio can be changed through further crossing and selection and various shades of orange colours can be developed. Cyanidins contribute red appearance and delphinidin seems to contribute purple colour in *P. howardii* and the hybrids. Introduction of anthocyanins and carotenoids from *P. howardii* into *P. tuberosa* through further breeding created diversity of flower colours[49]. Progenies of these crosses contained both carotenoids and anthocyanins or only anthocyanins when *P. howardii* was used as either pollen parent or seed parent. Huang *et al.*[49] reported that 'double' is fertile in the early stage of flowers and can be used as both pollen and seed parents. It is also reported that *P. tuberosa* is self-incompatible[47]. Experiments proved that selfed progenies are obtainable, although they crossed the hybrids with *P. howardii*[49,53]. Huang *et al.*[54,55] studied the colour of tuberose by conducting cultural experiment of reddish-purple tuberose (*Polianthes tuberosa*) hybrid line '77A05' after cultivation in open fields at four different altitudes (25 (Pingtung), 75 (Chiayi), 500 (Hsinshe) and 1200 m (Tapan)) in Taiwan in 1999. Days to flowering from planting at Pingtung and Tapan were 80.3 and 89.5 respectively. The flower stalk was longer and floret size was increased at higher altitude, but number of florets was not affected. Flowers cultivated at Chiayi and Pingtung were pale purple but reddish-purple at Hsinshe and Tapan. The anthocyanin content of the flowers was approximately two and three times higher at Hsinshe and Tapan than that at Pingtung, respectively. Site selection is most important for cultivation of coloured tuberose when anthocyanin is the primary pigment. High elevation areas in Taiwan seem to be suitable for cultivation of anthocyanin-containing tuberoses. This is because low temperature favours higher accumulation of anthocyanin as reported in other crops. Pigmentation of petals is affected by two environmental factors, i.e. temperature and light intensity[56]. Anthocyanin concentration in roses is lower at higher temperatures and at 30°C pigment production is ceased[57]. Reduction of anthocyanins at high temperature has been reported in the petals of cherry and peach[58] and asters[59,60]. The cause of reduction of anthocyanin contents at high temperature is due to reduced supply of carbohydrate which is an important substance for the structure of anthocyanins[61–63]. There is no fruit set in single tuberose due to self-incompatibility but there is 63.78% fruit set when cross-pollinated with variegated cultivar. The variegated cultivar is both a male and female fertile variety with sufficient pollen production and when self- and cross-pollinated recorded a seed set of 12.13% and 28.84% respectively. But lower seed germination and viability were recorded after cross-pollination with variegated variety as a female parent, suggesting lower fertility and seedling vigour[64]. Solanco[48] reported characteristics of some varieties specially flower colours – *P. howardii* (reddish purple, red in the base and gradually green in the lobes); *P. bicolor* (orange-greenish, green lobes); *P. montana* (white, pink); *P. graminifolia* (red, orange, coral); *P. oaxacana* (pink outside, yellow inside); *P zapopanensis* (orange, pink); *P. multicolor* (almost white, pink, orange, orange-light yellow); *P. densiflora* (yellow); *P. platyphylla* (white tinged with red); *P. venustuliflora* (white tinged with pink); *P. palustris* (white); *P. tuberose* (white, buds may have a light pink); *P. longiflora* (white tinged with purple); *P. melsonii* (white, pink and sometimes red); *P. sensiflora* (white, pink and sometimes red). Muriithi *et al.*[65] examined changes in accumulation of anthocyanins and the resultant colour in tuberose (*Polianthes tuberosa* Linn.) after application of amendments to the soil. Magnesium was given as magnesium nitrate and nitrogen was given as calcium ammonium nitrate (CAN). CAN was neutral and ammonium sulphate (AS) was acidic. Results showed that supplying magnesium through fertilizer application to soil does not necessarily increase accumulation of Mg in tissues, and may ultimately not lead to accumulation of anthocyanins. Barba-Gonzalez *et al.*[26] did inter-specific and inter-generic crosses with *Polianthes* and found that *P. geminiflora* var. clivicola McVaugh was suitable both as pollen receiver as well as donor, with different species and with *Prochnyanthes. P. howardii* was identified as the better pollen receptor, because the pollen in these plants was sterile. Barbo-Gonzalez *et al.*[26] reported wild Polianthes species: *P. geminiflora* var. clivicola, *P. geminiflora* var. graminifolia, *P. graminifolia*, *P. howardii*, *P. palustris*, *P. platyphylla*, *P. pringlei*, *P. sessiliflora*, *P. Montana*; which were white, yellow, pink red, etc. Barba-Gonzalez *et al.*[66] reported 14 species of *Polianthes* from Mexico having coloured flowers ranging from scarlet red to yellow. They collected all accessions from the wild for the breeding programme and different genotypes were characterized by AFLP. They studied compatibility among species and were successful in inter-specific and inter-sectional hybridization. Results indicate molecular variability among species, creation of interspecific hybrids and the possibility to combine important horticultural traits from wild species into novel tuberose cultivars. Taipei University (Taiwan) was deeply engaged in developing more prolific and higher quality *Polianthes* varieties combining available vibrant colours, double flowers and disease resistance strains. A breeding breakthrough was development of pink tuberose 'pink sensation'. The

Dutch based breeding company, Ludwig and Co, licensee of new *Polianthes* breeding genetics, released the variety in 2014. The company has introduced a series of new *Polianthes*, both single and double flowering in soft yellow, dark yellow, soft pink and lavender pink. 'Pink Sensation' is single-flowered, soft pink, sweet-scented, shorter stem length and suitable for cut flower industry, pot culture and garden display. Their stunning fragrance though remains as good as ever (c.f. You Garden). From the above hybridization, it is clear that pigment composition plays an important role in developing new flower colour variety.

In ornamentals, flower pigment is an important factor which determines flower colour and its commercial value. Anthocyanins and carotenoids are the main pigments which differ qualitatively and quantitatively in flower colours. Carotenoids play an important role in determining flower colour. Knowledge on these pigment compositions is important for practical breeding programmes. Carotenoids and other pigments have been analysed in a number of original chrysanthemum and rose cultivars and their respective mutants/hybrids to understand their qualitative and quantitative changes due to mutation/hybridization[4]. Carotenoids estimation and thin layer chromatographic and spectrophotometric analysis of pigments have clearly shown that cultivars with the same flower colour may have different pigment composition, and cultivars with the same pigment composition may show different colour patterns. Different chemical groups attached at different locations on the basic molecule create different forms of anthocyanin which may further be modified due to acidity of the cell sap or presence of other pigments. For breeding purposes, it is important to understand the inheritance pattern of each pigment. Pigment analysis has indicated that mutation frequency is restricted in cultivars with limited pigment composition than in cultivars with high concentrations of all pigments. This has been confirmed from the fact that some cultivars with specific colour produced higher mutation rate and spectrum[4]. Such observations have been reported earlier[67–73].

Pigment analysis of a large number of mutant/hybrid ornamentals confirmed qualitative and/or quantitative differences between the pigments of original and mutants/hybrids. A schematic representation has been suggested which explains the probable manner in which differences in pigments of original and mutant cultivars may arise[4,74–80]. It has been clearly determined that new flower colour in ornamental plants will arise in four major directions, i.e. when there is any new flower colour variety (developed either through induced mutagenesis or hybridization) it has to follow one of the four paths (Figure 1):

(1) New mutant/hybrid flower colour may be due to either an increase or decrease, or both, in the concentration of one or more existing pigments.
(2) Mutant/hybrid colour may be due to blockage in synthesis of one or more pigments; this may also be associated with an increase or decrease in the concentration of one or more existing pigments.
(3) Mutant/hybrid colour may be due to the origin of new pigments, which may be associated with an increase or decrease in the concentration of one or more existing pigments.
(4) Mutant/hybrid colour may develop as a result of synthesis of a new pigment and the blockage in development of one or more existing pigments; this may be associated with either an increase or a decrease, or both, in the concentration of one or more existing pigments.

Self-incompatibility

This is another important aspect in hybridization. Tuberose is mostly propagated by vegetative means as there is no seed setting. Seed setting in tuberose is quite erratic in single-flowered cultivar and is not observed in the double flower. The variegated type however has a high degree of seed setting compared to others. The exact cause of sterility is not known. Joshi and Pantulu[81] reported that sterility is not due to any defects or deformation in the formation of the pollen grains. Uma and Gowda[82] tried to overcome self-incompatibility through some horticultural manipulations. They studied bud pollination, used IBA and IAA at the time of pollination to the varieties and used gamma-irradiated (0.5 kR) pollen. Bud pollination failed to induce fruiting. Irradiated pollen pollinated two days after anthesis resulted in fruit set up to 4.78% and

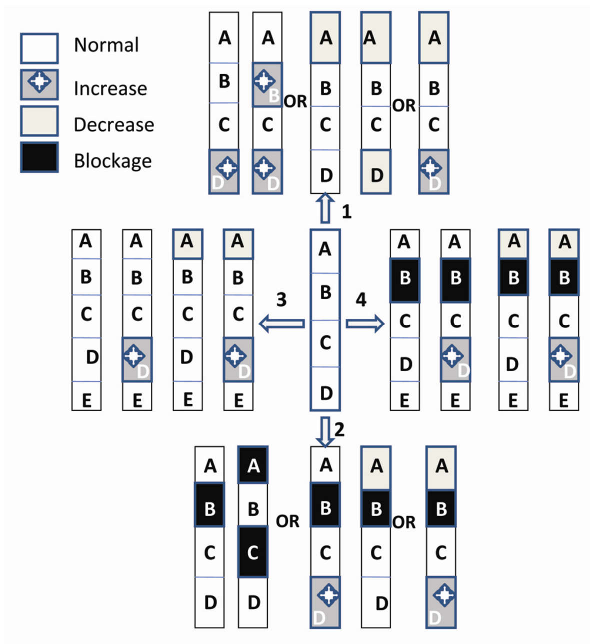

Figure 1. Schematic representation of role of pigments in original and mutants/hybrids.

seed viability was as high as 48.23%. Srivastava and Sridhara[83] studied floral biology of tuberose. Seetharamu *et al.*[84] studied pollen viability, pollen germination, seed setting behaviour, self- and cross-compatibility and percentage of seed germination in different hybrids and varieties of tuberose. Ranchana and Kannan[85] evaluated self- and cross-compatibility through selfing and crossing techniques in 10 single genotypes of tuberose. They concluded that the single genotypes of tuberose provide an evidence of a gametophytic self-incompatibility system and are of cross-compatible nature. Self- and cross-compatible within and between genotypes were studied for selection of superior plants from open-pollinated seedling populations of *P. tuberosa*[86,87]. They pointed out that controlled pollinations may be required between selected individuals especially for growth habit and pest resistance. Krishnamurthy and Srinivas[88] studied histological changes during ovule development after crossing Mexican single and Pearl double and reported that reproductive compatibility stimulates the metabolic activities in the associated tissues leading to the normal development of fruit seed formation.

Conclusion

The literature clearly indicates that the concept of hybridization work for developing further desirable genetic variability in tuberose dates back to 1899. Basic knowledge on cyto-genetics, pre-requisite for breeding, is available and there is every possibility to develop colour tuberoses through breeding. All combinations of agronomical trail using cultural practices, bulb size, time of planting and planting density, use of fertilizers, growth hormones, different chemicals have been done for optimization of production. Literature is also available for management of post-harvest life of cut spikes. From the earlier review[12] and the present review the following recommendations may be considered for further research on tuberose:

- No further agronomical trial experiment is necessary to increase the bulk of literature except multilocation trial of high yielding strains.
- Earlier recommendations on agro-technology and post-harvest management for optimum production and vase life should be commercially exploited.
- Mutation technique has played an important role in crop improvement and has developed many new flower colour/shape mutant varieties. No new flower colour/shape mutations could be induced in tuberose except chlorophyll variegations in leaves[4]. Selection of correct starting material is important for the success of the technique. All mutagenesis experiments were conducted with available existing materials with white flowers. In 'double' cultivar few bulbs which develop flowers are white but the lower portion of the flower tube and leaf base are tinged with pinkish red cells. The first attempt should be selection of lines with maximum pink red cells. These selected lines may be multiplied and used as starting materials for both conventional breeding and induced mutagenesis. Chances of induction of pink colour mutation will be more. Normally, physical and/or chemical mutagens induce mutation in vegetatively propagated plants as chimera. A novel technique has been standardized in chrysanthemum for management of such chimera. Direct shoot regeneration from chimeric mutant florets resulted in the development of new flower colour/shape mutants[4]. *In vitro* mutagenesis technique is now being applied to increase mutation frequency and solid mutants. The main advantage of this technique is to overcome chimera formation. *In vitro* mutagenesis experiments can be conducted with large population, within limited space at any time of the year. Here the explants should be the lower part of flower tube and leaf base with maximum pink cells.
- Molecular breeding is another option to develop new flower colour in tuberose using sense and anti-sense strategy or incorporation of new coloured genes in tuberose. Unfortunately, this is an untouched area on ornamentals in India. Tissue culture and molecular biology are being used in modern biotechnology for genetic improvement of plants. Quantitative trait loci analysis and marker assisted selection techniques are the present interest in genomics for crop improvement. Biotechnology has been successfully used in development of transgenic plants in more than 32 ornamentals[89–93].
- Scientists/breeders working on tuberose should collect available germplasm with different flower colour. This is because acquisition, propagation, preservation and utilization of coloured tuberose germplasm should be primary for creating further genetic variability. Germplasm is the main source of raw materials in tuberose representing genetic diversity in flower colour. Novel flower colour due to genetic diversity can be selected and introduced through germplasm. Germplasm can be used as base line material for further increase of genetic variability and improvement. The above observations may be utilized as guidelines for selective breeding for development of coloured tuberose.

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