Chapter 7
Breeding and Conservation of Medicinal Plants in India

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Abstract India is richly endowed with the plant wealth of medicinal plants, over 8500 species of ethno-botanical interest have been recorded. The medicinal plants are cultivated in commercial scale based on their demand in the industry. To meet demands of both industry and traditional usage, the development of resistant, high yielding, good quality varieties is of paramount importance. Breeding is the way to achieve the desirable traits in plants: to initiate any breeding or crop improvement programs, germplasm collection is essential. Prevailing conventional breeding methods in medicinal plants include selection, hybridization, induced mutation and polyploid breeding. Use of biotechnological tools and research on genes controlling the formation of secondary metabolites and on methods for their transmission are in fancy stage. Therefore, breeding can become one of the key factors for advancing the phytopharmaceutical sector in the future. Keeping this in view, the breeding methods and germplasm conservation details pertaining to commercially important medicinal crops *viz*, Aloe, Ashwagandha, Glory lily, Isabgol, Medicinal coleus, Medicinal yam, Medicinal solanum, Opium poppy, Periwinkle and Senna are discussed in this chapter.

Keywords Aloe · Ashwagandha · Glory lily · Isabgol · Medicinal coleus · Medicinal yam · Medicinal solanum · Opium poppy · Periwinkle and senna · Selection · Hybridization · Induced mutation and polyploid breeding · Germplasm conservation

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7.1 Introduction

India is one among the 12 mega-diversity countries of the world. As per the study made by Foundation for Revitalization of Local Health Traditions (FRLHT), of the 960 traded medicinal plant species, 178 species are consumed in volumes exceeding 100 MT per year, with their consolidated consumption accounting for about 80% of the total industrial demand of all botanicals in the country. An analysis of these species by their major source of supply revealed that 36 species (20%) from cultivation medicinal plants are the source of many important pharmaceutical preparations, especially plants rich in secondary metabolites are of interest. Considerable effort has been done to generate such metabolites in plant cell or tissue culture. Nevertheless, collection from wild and agricultural production of medical plants still remains the most important supply for plant-derived pharmaceuticals. However, harvesting from wild, especially in species with a high demand, can cause loss of genetic diversity and habitat destruction due to over harvesting.

In this context, breeding of new cultivars is a key factor allowing us for increased yield of valuable compounds, for elimination of unwanted compounds, for tolerance against abiotic and biotic stresses, and for better homogeneity of the cultivars. When compared with traditional food crops, breeding of medicinal plants is now in the initial stages, with the advantage that breeders can exploit a high available natural variability within one species. The generally high natural variability within medicinal plant species is one of the reasons that classical breeding approaches were mainly used till now. Knowledge of reproductive biology of medicinal plants is crucial for improvement, effective conservation and management plans to evolve genetically superior varieties.

7.2 Breeding Methods of Selected MAP Species

In this chapter, breeding of aloe, ashwagandha, glory lily, Isabgol, medicinal coleus, medicinal yam, medicinal solanum, opium poppy, periwinkle and senna, with their botanical description, floral biology and breeding methods, are discussed. Varieties developed in these crops are presented in Table 7.1.

7.2.1 Aloe

The genus Aloe is indigenous to African continent and Mediterranean countries. It is naturalized over arid tracts of all over India. Out of 275 species, 42 belongs to Madagascar region (Africa). 12–15 belong to Arabian Peninsula and rest of the species is distributed in South Africa**.** In India only four species are reported to be occurring and of these *Aloe barbadensis* is the most widely naturalized species**.**

Table 7.1 Varieties of medicinal plants

Crop/Variety	Character
Aloe	
CIM – Sheetal	Developed through clonal selection during 2005 High leaf and sap yielding suited to rainfed conditions
Aswagantha	
CIM – Poshita	Developed by CSIR-CIMAP during 2011 Developed through half-sib selection Dry root yield of 14 q/ha Alkaloids – 1.3 kg/ha Withaferin – 0.538% in dry leaves
CIM – Chetak	Developed through half-sib selection during 2011 Dry root yield of 11.77 q/ha Withanolide – 0.40% Withaferine – 1.22% in dry leaves
CIM – Pratap	Developed through half-sib selection during 2011 Long tap root with less fiber Suitable for drought Dry root yield of 34.95 q/ha
Rakshita	Developed from CIMAP – Lucknow 8–10 q/ha dry roots with alkaloid content High yielding variety
WS 20	Developed through selection High dry yield
Jawahar Asgandh-20	Single plant selection during 1989 High dry root yield
NMITLI-118	Developed jointly by CSIR-CIMAP and NBRI and was released in September 2009 The variety has uniform crop canopy, non-spreading plant architecture (more plant/unit area), withanolide A and withanone in roots and high content of withaferin A (up to 2) Dry root yield of about 15 q/ha.
NMITLI-101	Developed jointly by CSIR-CIMAP and NBRI Potential to yield up to 23 q dry roots under optimal agronomic conditions
Isabgol	
Niharika	Developed through mutation in 1998 Swelling index/mucilage: 442 Seed yield 10–11 q/ha
Mayuri	Developed by mutation breeding during 2003 Early maturing, higher seed and husk yielding variety Distinct pigment marker of the panicles relatable to the maturing Seed yield 11 q/ha
Nimisha	Developed by mutation breeding during 1999 Leaves dark green and medium, long panicles Seed yield 10 q/ha
Haryana Isabgol-5	Selection from GI-2 Duration 140–145 days Yield – 1000 -1200 kg/ha Husk seed ratio – 25:75

(continued)

Table 7.1 (continued)

Crop/Variety	Character
Jawahar Isabgol 4 (JI-4)	Selection from germplasm Boat shaped seeds Violet pink ovary Yield – 1300-1500 kg/ha
Gujarat Isabgol 1 (GI-1)	Introduction Dark green leaves Moderate tillers and medium spike length (4–4.5 cm)
Gujarat Isabgol 2 (GI-2)	Mutation (mutant from GI -1) Medium broad and pale green leaves Matured in 110–115 days
Gujarat Isabgol 3 (GI-3)	Selection from local germplasm collections Long, thin and dark green leaves Profuse tillers and long spike (4.5–5.1 cm)
DOP-14	Mutation breeding during 2010 Early maturing High harvesting index
Medicinal coleus	
Suphala	High yielding (15.93 t/ha) Year round cultivable variety
K-8	Selection from Karnataka (IIHR – Bangalore) 0.5% forskolin and a higher tuber yield
CO 1	Clonal selection from Theni local Moderately resistant to root rot and wilt diseases, field tolerance to nematode and mealy bug infestation. Duration 160–180 days Dry tuber yields 2 t/ha, 33% yield increase over local Forskolin content 23%
Medicinal solanum	
Arka Sanjeevani	Spineless variety
RRL-20-2	A reduced spine mutant developed by RRL (Jammu & Kashmir) through chemical mutagenesis of seeds.Occurrence of spines is reduced to 2–4 per leaf and it is absent on stem. It is photo insensitive Solasodine yield: 42–45 kg/ha
RRL-GL-6	A spineless mutant developed by RRL (Jammu & Kashmir) through chemical mutagenesis of seeds Spines are totally absent in stem, branches, leaf surfaces and pedicel. It is marginally poor in solasodine content.
BARC mutant	A curved spine mutant developed through irradiation of dry seeds with 10 Kr gamma rays High berry yield and glycoalkaloid content
GLAXO mutant	A less spiny mutant evolved by mutation breeding using wild *solanum* This mutant is charaterized by presence of well-developed straight spines on the laminar surface while the stem is devoid of spines Yield of fresh berries – 11.92 t/ha

(continued)

Table 7.1 (continued)

Crop/Variety	Character
Arka Sanjeevani	Inter-varietal hybrid between Glaxo mutant and BARC mutant developed from IIHR, Bangalore Least spiny hybrid with curved spines suitable for high density planting Three fold increases in berry yield and Solasodine content
ArkaMahima	Developed from IIHR, Bangalore Induced tetraploid recording higher solasodine content (2.88%) than diploid counterpart Arka Sanjeevani (1.99%) This variety is devoid of spines
IIHR 2n – 11	Completely devoid of spines Suitable for high density planting 2.5–3% solasodine content
Opium poppy	
Shweta	Developed through half-sib selection in 1984 Tall variety with fringed leaves, white peduncle and flowers Very big and bold capsules
Shyama	Developed through half-sib selection in 1984 Tall variety with black peduncle and white flowers Medium size capsules
Vivek	Developed through induced mutation breeding in 1984 Tall variety with white petal Big size capsule
Sapna	Developed through half-sib selection in 1987 Early maturing for extraction of latex White peduncle and flowers Small size capsules
Dola chota gotia	Dwarf cultivar 85–90 cm height
Sanchita	Developed through mass selection in 1990 Black peduncle and white flower
Rakshit	Developed through selection in intra-specific hybrids for diverse resistance Highly resistant to downy mildew disease caused by *Peronospora arborescens* High seed and straw yielding variety released in 2001
Shubhra	Developed through mass selection in 2001 Tall, medium fringed leaves, white peduncle and flower Concentrated poppy straw variety Medium size capsule
Sampada	Developed through induced mutation in 2002 Black peduncle
CIM – Ajay	Developed through hybridization followed by selection in 2010 White peduncle
Chetak	Developed through selection
Single dark red	Earliness (80–81 days)
Dhaturia	Individual plant selection
Soma	Spontaneous mutant variety in India

(continued)

Table 7.1 (continued)

Crop/Variety	Character
BROP 1	Crossing selections from Kali Dandi, Suryapankhi and Safed Dandi Highly adaptable to varied agro-climatic conditions Moderately resistant to diseases Morphine content – 13% A synthetic variety, with high morphine, opium yield and seed yield
Kek Duma	Hybrid between *P. somniferum* var. Havan and *P. orientale.* 0.65–0.75% morphine in the capsule as compared to low morphine content in *P. somniferum* var. Havan.
Sujata	Developed through mutation breeding in 2002 World's first opiumless and alkaloid free seed poppy cultivar
Jawahar Aphim 16 (JA-16)	Pure line selection from MOP 539 Flowers – White serrated petal Peduncle – Non hairy Capsule – Spherical, flat at the top and surface groved
MOP – 16	Beats white flowers Serrated petals and round flat topped capsules Lancing start 120 days after planting Drought tolerant
Periwinkle	
Dhawal	Developed from an induced mutant with high content of leaf alkaloids in 1997 Resistant to die-back Leaf yield 2.0 t/ha and total leaf alkaloids content: 1.3–1.7%
Prabhal	Developed through pure line selection in 2001 Winter hardiness
Nirmal	Developed as a pure line variety from a single plant in 1989 Possess high level of resistant in die-back and collar and root rot White colour flowers
Senna	
Sona	Dry leaves up to 8–10 q/ha and that of dry pods as 4–5 q/ha. Sennoside content – 3.51%
KKM 1	Selection from thenkalam local Sennoside – 2.5%
AFLT 2	Developed through selection Leaf purpose variety, late flowering

Aloes are in considerable demand because of laxative property of Aloin and its gel for cosmetic industry**.** Barbaloin is the main bitter components of aloe juice which on drying forms semi apaque, dark brown substance called Mussabar in trade. Pharmacopoeia recognized three grades of aloe drug

(a) Curacao aloe – obtained from *A. barbadensis*
(b) Socotrine aloe – from *A. perryi*
(c) Cape aloe – from *A. ferox*

Aloe barbadensis, a member of Lilliaceae, is a small, stemless, herbaceous perennial with shallow root system. It bears fleshy leaves in rosette to give a distinct

appearance. The fully grown mature leaves are greyish green, round at the base with broad flat upper surface. It bears bright yellow flowers which are arranged in axillary spike. The flower is actinomorphic, its perianth is arranged in two whorls of three tepals each. It has six stamens in two whorls, the outer whorl has longer filaments than the inner whorl. Ovary is superior. Anthesis period is of 5–10 days within raceme, start from 7.00 am and continued up to 3.00 pm. The receptivity of stigma was observed high at anthesis. The peak period of dehiscence observed from 10.00 to 12.00. The fruits Aloe vera matures within 60–67 days (Rathod et al. 2014).

Chromosome number of *A. barbadensis* is 2n = 14, a triploid plant (2n = 21) have been reported from monazite region and Kanyakumari (Abraham and Nagendra Prasad 1979). Morphometric analysis for different species of Aloe was carried out by Nayanakantha et al. (2010). As the species does not produce many viable seeds and plants are propagated by vegetative means like suckers. Anusree Das et al. (2015) evaluated morphological and genetic characterization of micro-propagated field grown plants of *Aloe vera* L. and identified three seed setting plants, designated as somaclones. Seeds were viable and germinated (70.58%) *in vitro*. Although chromosome number and morphology of somaclones were identical with the donor plants their RAPD profiles and ITS-1 sequences were different from donor plant. This study reports seed setting somaclones in *Aloe vera,* for the first time which may serve as new genetic resource for biotechnological improvement.

Selection is the method of breeding attempted in *Aloe vera*. CIMAP has released one variety CIM -Sheetal through clonal selection.

7.2.2 Ashwagandha

Ashwagandha or Asgandh (*Withania somnifera* Dunal) is an important medicinal plant belonging to Solanaceae family, mainly cultivated in Madhya Pradesh and Rajasthan. The total alkaloid content of the root varies between 0.13 and 0.31%. Roots are used in treating rheumatism, stomach and lung inflammation. It is an erect, stellate-tomentose, greyish, under shrub with 30–75 cm in height having long tuberous roots. Leaves are sub-opposite, broadly ovate to oblong, alternate, petiolate, entire and sub-acute with lamina (5–10 × 2.5–7) cm. Flowers are bisexual, greenish, small, solitary, axillary, or in few-flowered cymes. Fruit is a globose/berry, orange-red in colour when ripen and covered in the enlarged calyx. Seeds are many, discoid, yellow and reniform. The chromosome number is 2n = 48. Only two species, *Withania somnifera* Dunal) and *Withania coagulans* Dunal are found in India. *Withania coagulans*, a rigid, ashy-grey undershurb, 60–120 cm high is found occurring wild in Punjab.

Studies conducted at Indian Institute of Integrative Medicine, Jammu by Mira et al. (2012) revealed that flowering (peak) takes place during April–July and anthesis occurs between 08:00 and 11:00 h. The period of stigma receptivity coincides with anther dehiscence. Fruit set on pollination treatments ranged from 90.8% (passive autogamy), 72% (assisted autogamy), 30.30% (xenogamy), and 56.50%

(geitonogamy) through 50.40% (open pollination). Xenogamy brings about very low fruit set, seed-set and seed germination percentages. It is inferred that *W. ashwagandha* is predominantly an autogamous and self-compatible species. Self-compatibility is mainly accomplished due to close proximity of stigma and anthers.

Synchrony in the flowering periods of the wild and cultivated accessions, monoecious sex expression and an amenability to emasculation and crossing further enhance the possibility of genetic improvement of this amphimictic species through hybridization (Mira et al. 2012). Crossing is done during evening hours 4–6 p.m. Flowers are selected which will open in the next morning. Remove the rest of flowers and buds in the bunch. Petals along with the stamens of selected buds are removed and emasculated flowers are bagged. Pollination is done in morning. Opened or about to open flowers, anthers are collected. Corolla tube having attached anthers is separated by forceps from such flowers. Anthers are allowed to burst on stigma naturally. The flowers are bagged. Seed set is observed in 3–5 days.

Breeding objectives in ashwagandha includes increased root yield and alkaloid content besides resistant to biotic and abiotic factors. Half-Sib Selection with Progeny Testing is followed for development of varieties in ashwagandha. Selections are made based on progeny test performance instead of phenotypic appearance of the parental plants. Seed from selected half-sibs, which have been pollinated by random pollen from the population is grown in un replicated progeny rows for the purpose of selection. A part of the seed is planted to determine the yielding ability, or breeding value, for any character of each plant. The seed from the most productive rows or remnant seed from the outstanding half-sibs is bulked to complete one cycle of selection.

Pure lines form an important genetic resource for improvement of yield and quality. A set of 327 (DWS1-DWS327) pure lines were developed from JA134 out crossed population through individual plant selection, selfing and generation advancement for the first time in Ashwagandha. Variation for qualitative and quantitative traits was observed between pure lines and lines with distinct morphological traits were obtained. Heritability and genetic divergence among a set of 48 pure lines with JA134 and JA30 was assessed based on 20 root yield and its component traits. Based on the inter cluster distance and *per se* performance, the pure lines DWS84 and DWS85 were selected which could be intercrossed to obtain high heterosis and also to recover transgressive segregants for the improvement of root yield and its quality. Pure lines developed in the present study form important genetic resources for the improvement of yield and quality of Ashwagandha (Manivel et al. 2017).

Iqbal and Datta (2006) studied mutation breeding, mutagenic effectiveness and efficiency of gamma-rays, hydroxylamine (HA) and ethyl methane sulphonate (EMS) in *W. somnifera* based on M_1 biological damages (lethality, injury and sterility) and viable mutation frequency (M_2 generation) and suggested that HA and EMS were effective than gamma-rays; while lower doses of gamma-rays along with all the doses of chemical mutagens administered were found to be efficient.

Iqbal and Datta (2007) also reported a selection line possessing broad leaf trait (2n = 48) with high root and seed yield. Das et al. (2010) reported eight macro

mutants in 'Poshita' and 'Jawahar 22' of *W. somnifera* following EMS treatments (0.25, 0.50 and 1.00% for 2 h and 4 h durations) to dry seeds. Viable mutation frequency was recorded to be 0.00–3.00% in 'Poshita' and 0.00–2.31% in 'Jawahar 22'.

In Ashwagandha, availability of less variability in nature for total alkaloid content and root yield, it would be advantageous to develop a variety through ploidy breeding, having enhanced root alkaloid with higher root yield beyond the present existing level. In this regard, Chinapolaiah (2008) induced polyploidy in three genotypes of ashwagandha (Poshita, JA-20 and KRC-11) using different concentrations of colchicine solution (0.25, 0.5 and 0.75%) and found variety Poshita was more responsive for the induction of autotetraploids. Autotetraploids of ashwagandha has a potential in getting high alkaloid yield per unit area (Vidya et al. 2013).

7.2.3 Glory Lilly

Gloriosa superba L. (Colchicaceae) also known as Malabar glory lily is a perennial tuberous climbing herb, extensively scattered in the tropical and sub-tropical parts of the India, including the foothills of Himalayas. It is a beautiful perennial climber with hollow stem of about 6 m, which emerges per year from the tuberous underground stem in rainy season. The leaves are etiolated, alternate, sessile, lanceolate, and spear shaped with curved end, which helps them to climb and creep. Flowers are large, solitary at ends of branches, greenish at first, then yellow, passing through orange, and scarlet to crimpson. The peculiar structures of the large flowers with six perianth lobes bent backwards, six radiating anthers and the style bent almost 90° at the point of attachment to the ovary does not make them suitable for pollination by small insects. Fruits are oblong, ellipsoid capsule. Seeds are numerous and rounded.

Gloriosa superba is considered as a single highly variable species. *Gloriosa* is monobasic with a genetic base x = 11. Out of the 10 elemental species, *Gloriosa superba*, *G. lutea* and *G. plantii* are diploids (2n = 22), *G. carsonii*, *G. virescens* and *G. richmondensis* are tetraploids (2n = 44) and *G. rothschildiana*, *G. latifolia* and *G. magnifica* are octoploids (2n = 88). In general, octoploid species are comparatively short statured and constitute a medium group of plants.

Five stages of flower development *viz*., bud initiation, bud opening, pre-anthesis, anthesis, post pollination stage was reported. The flower colour changed during each stage of flower development. The perianth lobes at the bud opening stage were light greenish in colour followed by the stigma receptive stage which was characterized by perianth lobes that were scarlet red at the tip, yellow in the middle and greenish towards the base. Post pollination stage was characterized by the upper half of perianth lobes being scarlet red and the lower portion being yellow coloured. Lastly, the perianth lobes turned entirely into scarlet red.

Anthesis was observed to occur earlier than 7.30 am to 9.30 am with 40% of the flower opening by 7.30 am, 50% by 8.30 am and rest 10% by 9.30 am (Farooqi et al. 1993). According to Mamtha (1989), the peak period of anthesis in *Gloriosa superba* was between 8.30 and 10.30 A.M. It was observed that on the day of

anthesis, there was no anther dehiscence. One day after anthesis, the anther started dehiscing earlier than 7.30 am to 9.30 am. On an average, 5% of the anthers dehisced before 7.30 am, 70% before 8.30 am and another 25% by 9.30 am (Nagajothi 2008). According to Anandhi and Rajamani (2012), the anther dehiscence started from 6.30 am and reached the peak at 9.30 am and thereafter started declining and reached the minimum at 10.30 am. This indicated that glory lily is photosensitive and anthesis corresponded to the intensity of sunlight falling on the plants.

In *Gloriosa superba*, 97.50% pod set was observed in flowers which were pollinated on the day of anthesis, indicating the maximum receptiveness of stigma during anthesis. In general, the percentage of pod set was higher in the early morning hours (7.00–11.00 am) irrespective of the pollination done on different days. In general, the stigma remains receptive for 3 days *viz*., 1 day prior to anthesis, on the day of anthesis, 1 day after anthesis. These receptive periods coincided with preanthesis, anthesis and post pollination stage of flower development. The loss of stigma receptivity can be identified from the change in stigma colour from green to red. The mean percentage of fertile pollen in *G. superba* was maximum on the day of anther dehiscence and declined gradually as the age of pollen increased (Anandhi and Rajamani 2012). Vigneshwari and Renugadevi (2006) reported that flowering in *Gloriosa superba* occurs during the month of November to March, but peak flowering is in the month of December to January. The duration of flowering phase was 21.10 days in *G. superba.*

Gloriosa superba is both self- and cross-pollinated (Gupta and Raina 2001), seed set is dependent upon both pollinator activity and the time of pollination. Although there are no self or cross-incompatibility barriers, the hercogamous nature and attractively coloured flowers, favours cross pollination. Nagajothi (2008) stated that hand pollination recorded the highest pod set per cent of about 70.93% followed by air blowing pollination using power sprayer (65.52%). Maximum pod set was observed in artificial cross pollination within the species followed by self-pollination.

Breeding objective in glory lily includes increase in seed yield and alkaloid content with resistant to biotic and abiotic factors.

Mamtha et al. (1993) studied the relationship between vegetative growth and yield in glory lily who revealed that seed number was high in medium sized fruits and dry seed yield increased with increase in number of branches and leaf area. Seemanti Ghosh et al. (2007) attempted studies on polymorphism in five different populations (Amtala (AM), Baruipur (BR), Siliguri (SG), Darjeeling (DJ) and Sikkim (SK)) of *Gloriosa superba* L. The colchicine content in tubers ranged from 0.06% in AM to 0.37% in BR population. Chitra and Rajamani (2010) evaluated 18 ecotypes of glory lily at Coimbatore, Tamil Nadu. The ecotype GS15 exhibited superior performance for most of the morphological and yield characters, followed by GS06.

In *Gloriosa superba*, the genetic variability is low owing to the continued vegetative propagation through tubers. Wide hybridization enables the interspecific gene transfer, which may lead to the additional source of variation for desirable characters. Attempts were made by Anandhi et al. (2013) to investigate the possibilities for

developing variability in this species with varying flower colour, shelf life, high seed yield and improved colchicine content through interspecific hybridization involving *G. superba* with *G. rothschildiana*. Five ecotypes of *G. superba* were crossed with *G. rothschildiana* in both directions.

Varying percentage of pod set was observed with pods of 2.00 cm length within 25 days of pollination and thereafter shrinked and died irrespective of the cross combination under study. None of the pods reached the harvestable stage. Post fertilization barrier was observed in both direct and reciprocal crosses. This may be due to embryo abortion and degeneration during embryogenesis.

Induction of variability in glory lily through mutagenic treatments is of paramount importance for crop improvement. Chandra and Tarar (1991) worked on development of mutants using Co-60 gamma rays, EMS and DES on *G. superba* and obtained various morphological changes in height, structure of the plant, flower and capsules under gamma treatments. Multi armed tubers and furcated stem mutants under EMS and flower size mutants under DES treatment was obtained. Rajadurai and Vadivel (2001) concluded that colchicine content of leaves was higher on treatment with gamma rays @ 1.00 kR and also the yield attributes was greater in the treatment DES @ 0.75%. Anandhi et al. (2013) made an investigation to induce mutants in *G. superba* L. possessing high content of alkaloids *viz*., colchicine and colchicoside. VM_2 generation was raised from EMS, DES and gamma ray treated VM_1 tubers of glory lily. The maximum colchicine content of 0.70% was observed in T_7P_3 (2% EMS), followed by 0.702% in T_8P_2 (1.00% DES).

7.2.4 Isabgol

Plantago ovata is commonly called as Psyllium. India is the largest producer and exporter of this crop in the world. It is grown as a cash crop in Gujarat, Punjab and Uttar Pradesh. The seed husk is used to cure inflammation of the mucus membrane of gastrointestinal and genito-urinary tracts, chronic constipation, dysentery, gonorrhea and piles. It is native to Mediterranean region and distributed from Canary Islands across Southern Spain, North Africa, Middle East and North Western Asia. Although *P. ovate* is cultivated widely throughout the world, India dominates the world market in its production and export. In India, it is cultivated as a 'rabi' or post rainy season crop in the western regions. The main states in which it is cultivated on commercial scale are Gujarat, Rajasthan and Madhya Pradesh, more particularly in North Gujarat and its adjoining part in Madhya Pradesh and Rajasthan.

Plantago is a large genus of herbs of sub-shrubs distributed mostly in the temperate regions and a few in the tropics. The important species are *P. psyllium, P. indica, P. ovata* and *P. psyllium. P. psyllium* is glossy deep brown seeds with boat shape, outline elongated ovate, 2.0–3.0 mm in length with 100 seed weight of 0.09–0.10 g. *P. indica* has dull blackish brown with boat shaped seed, outline elliptical, 2.0–2.5 mm with 100 seed weight of 0.12–0.14 g *P. ovata* has dull pinkish

grey – brown with boat-shaped: outline ovate, 1.8–3.3 mm with 100 seed weight of 0.15–0.19 g.

Isabgol is a sub-caulescent or stem less, soft, soft, hairy annual herb which grows to a height of 30–45 cm. Leaves – 6.0–25.0 cm long, 0.5–1.0 cm broad, narrowly linear or linear lanceolate, strap like, recurved, finely acuminate, entire or distantly toothed, attenuated at the base, sessile Stem – pseudo petiole. Inflorescence-cylindrical terminal spikes (0.6–5.6 cm). Small, white or colourless, sessile, bisexual, tetramerous and actinomorphic flowers crowded on a main axis, sepals are 4, free, ovate, obtuse and glabrous. Petals are 4, fused, forming corolla tube, lobes ovate or orbicular and glabrous. Stamens are 4, epipetalous. Gynoecium is bicarpellary, syncarpous, ovary superior, bilocular, containing one ovule per locule. Style is filiform. Fruit is capsule covered by a persistant calyx. Seeds are hard, cymbiform (boat shaped), outline ovate, acute at one end, smooth surfaced and dull pinkish, pinkish grey or pinkish brown in colour.

The inflorescence spike with about 60 florets crowded at the top of the fragile peduncle in about 3 cm length. The flowers are protogynous with floral maturity occurring in acropetal succession. Thus, the gynoecium of the bottom most flower mature first protruding its stigma through the tip of the unopened flower studies on stigma-pollen maturation schedule and their interrelationship have revealed that anthesis occurred during early morning hours, but stigma maturity was distributed to both morning and evening hours with the lowest frequency at noon (Patel et al. 1980) also reported that 14 florets in a spike born matured stigmata before anthesis started in that spike. In a spike, minimum time gap between stigma receptivity and anthesis in the same floret was observed to be 14 h with variation up to 120 hours. Pawar (1981) concluded that stigma receptivity, as judged by stigma elongation, continued to occur in acropetal succession in florets of a spike throughout the day and probably at night. Pollen grains collected in the morning and stored at 0°c without silica gel till evening were most viable as judged by percent florets which set seed. Pollen grain could, however, remain viable for about 50 hours if stored at a temperature range of −3 to 7°c.The stigma remained highly receptive for 1 day (seed set 92.18%), moderately receptive for next 5 days and significantly lost the receptively in subsequent days. After the ninth day, the receptivity was completely lost.

Breeding objectives in Isabgol should be to increase in the seed size and yield, compact and non-shattering spikes, synchronous maturity, dwarf size, seeds with higher swelling factor and genotypes resistant to drought and frost.

Crop improvement in Isabgol is difficult as *P. ovata* has a narrow genetic base and lack of variability on account of low chromosome number (2n = 8), small chromosome size, presence of high heterochromatin in the chromosomes, low chiasma frequency and low recombination index and high selfing rate with the genome size of 500 Mb (Sareen and Koul 1999; Dhar et al. 2005).

The prospects of evolving better varieties through selection alone are limited since the species has a narrow genetic base and lacks variability.

Autotetraploid using colchicines @ 0.5% was found to be best for induction of tetraploid in the variety GI-2. Their autotetraploid status was confirmed by

conducting chromosome counts on dividing pollen mother cells (PMCs). Tetraploids showed superiority over their diploid counterparts except number of seeds per spike. Tetraploids were late in 50% flowering by 13.30% number of days than diploids. Pollen size was larger (31.55%) than that of diploids, but pollen viability decreased by 16.53%. Size of stomata was bigger in tetraploids.

Mutation breeding in Isabgol resulted in identification of an early mutant DOP 14. It has early maturing with desirable traits such as high seed yield, early & uniform seed maturation and high harvest index. Single plant with earliness (80 DAS) was identified and isolated in M2 generation of 0.4% DES treated Isabgol cultivar GI-2. It was self-pollinated and advanced to next generation. In M3 generation, the mutant bred true and all the plants showed the early maturity. DOP-14 mutant started flowering at 34 DAS and seed matured at 85 DAS at DMAPR, Anand, Gujarat which is 35 days early compared to its parent GI-2 (120 DAS). The harvest index was 22.8% which was 31% higher than parent GI-2. It can be an important source for developing early maturing high yielding Isabgol varieties with desirable quality (Manivel and Saravanan 2010).

7.2.5 Medicinal Coleus

Coleus forskohlii Briq. (syn. *C. barbatus* Benth.), an important indigenous medicinal plant in India is a member of the mint family, Lamiaceae. The diterpenoid, forskolin (syn. Coleonol), is very important among the secondary metabolites and is used for the treatment of various diseases *viz*., eczema, asthma, psoriasis, cardiovascular disorders and hypertension. It is a herbaceous, pubescent, aromatic species with annual stems and perennial rootstock, growing 45–60 cm tall. Tuberous roots are succulent but hard, tortuous or straight, short and stout or long and slender up to 56 cm in length. The cultivated types are fleshy and succulent, spindle shaped or fusiform, generally long, several and radically spread. Root tubers in both the kinds have white or orangish pink flesh with bitter after taste and are aromatic. Tuber skin is papery, yellowish brown, brownish or brownish black based on the soil substratum. Leaves are 7.5–12.5 cm in length and 3–5 cm in width *Coleus forskohlii Briq. (Syn. Plectranthus barbaturs* Andr.*)*. In India, the major medicinal *Coleus* plant species are the tuberous *C. forskohlii, C. amobonicus, C. blumei* and *C malabaricus*.

Reddy (1952) reported that *C. forskohlii* is diploid with n = 14. However, Riley and Hoff (1961) from their studies on chromosome numbers in South African dicotyledons reported that *C. forskohlii* is diploid with basic chromosome number n = 16. Bir and Saggoo (1982, 1985) reported that Central Indian collections have basic number of n = 17, while South Indian collections have n = 15 and concluded that variability in base number of various members of the family could be due to aneuploidy at generic level which ultimately leads to morphological variations. Shah (1989) reported that populations of *C. forskohlii* from different ecogeographic areas vary greatly in their morphology.

Breeding objectives of medicinal coleus include increase in tuber yield and forskolin content. To improve varieties for resistant to biotic and abiotic stress. Selection and mutation breeding were attempted in medicinal coleus and varieties were released.

7.2.6 Medicinal Solanum

The genus *Solanum* comprises of about 2000 species which can be broadly grouped as tuberous group and non-tuberous group. *Solanum viarum* belongs to the non-tuberous group.It occurs naturally in Sikkim, West Bengal and Orissa and in Western Ghats up to 1600 m MSL. *Solanum viarum* is a natural source of glycol alkaloid solasodine which is Nitrogen analogue of diosgenin. Solasodine content is extracted from berries of Medicinal solanum and the content is about 1.60 to 1.75%, Solasodine acts as a substitute for diosgenin in the synthesis of steroid hormones.

S. viarum is an erect perennial, 50–150 cm high, with shortly pubescent stems and branches with recurved prickles up to 5 mm long, pubescent at their base. There are also longer, straight spines up to 2 cm long on the petioles and the veins of upper and lower surfaces of the leaves. The leaves are broadly ovate up to 20 cm long and 15 cm wide, bluntly lobed with markedly undulate edges, generally dark green and glossy above, duller below. The flowers are white, borne in racemes with 1–4 flowers per cluster. The fruit is a globose in shape, berry is mottled green when young, maturing yellow, 2–3 cm across. It contains up to 400 brown, flattened, discoid seeds, 2–3 mm in diameter. Roots have buds which will regenerate new shoots. The root system can be extensive, with feeder roots 1–2 cm in diameter located a few cm below ground extending 1–2 m from the crown of the plant.

Flowers are hermaphrodite, white and appear in axillary racemes, 14–16 mm in diameter, complete, actinomorphic, bisexual, hypogynous, pentamerous in nature. Anthesis during early morning (4.00–6.00 h), anther dehiscence just after flower opening, mode of anther dehiscence is by apical pores. Pedicels are 6.8–7.5 mm in length. Sepals are five in number, spiny, persistent, green in colour. Petals are five in number, white in colour, recurved. Stamens are five in number, filament is short (1.0–1.5 mm), stout, slightly swollen at the base, whitish, anthers more or less oblong, 2-celled, basifixed, dehisces by apical pores, creamish white. Carpels are 2 in number, syncarpous in nature. in two different forms: long style (9.0–9.5 mm), and short style (2.0–2.5 mm), long style has high fruit setting percentage. Stigma is green in colour, wet, partially lobed (0.5–1.0 mm).Ovary is superior, 2 chambered with many ovules. Each berries are globose in shape with a persistent calyx, 20–30 mm wide, green with dark speckled, when immature, dull yellow when ripe. Somatic chromosome number is 2n = 24 (Krishnappa and Chennaveeraiah 1976).

Anthesis was found to be occur throughout the day with 84% flowers opening between 8.00 and 16.30 h. Anther dehiscence commenced an hour after anthesis. Stigma receptivity was noticed from 4 to 64 h after anthesis (Krishnan 1995).

Inter varietal hybridization was reported by Nandha Kumar (1983). Biparental matings were carried out adopting six parameter model using two morphologically distinguishable mutants-Glaxco and BARC varieties and the wild type. Differences in the shape and distribution of spines are the only characters that distinguished these three varieties. In the F2 generation of the cross involving BARC and Glaxo varieties, a new recombinant type for spine character was recovered. This recombinant was similar to the less spiny Glaxo parent in the restricted presence of spines in the lamina, but the spines are vestigial a curved resembling the BARC parent. Three advanced generation lines tested for 3 years were found to be on par with parents and other released varieties both for berry and solasodine yields (Krishnan et al. 1988). It indicates the narrow genetic variability and inadequacy of varietal hybridization for achieving enhancement of solasodine content and berry yield.

Interspecific hybridization of *Solanum viarum* was attempted with non-steroid bearing *Solanum* species but the crosses have resulted either in parthenocarpic fruits with aborted seeds and no fruit set (Krishnappa and Chennaveeraiah 1976).

As genetic variation in *S. viarum* is limited, mutation breeding is the option to achieve target characters for such as removal of spines, enhancement of berry yield and solasodine content. Bhatt (1972) reported the isolation of curved spine mutant (BARC variety) having higher berry yield and glycoalkaloid content following irradiation of dry seeds with 10 kr gamma ray dose. A less spiny mutant *viz*., Glaxo was evolved by mutation breeding using the wild type as the base material (Gadwal 1977). Two mutants viz., Glaxo and BARC were used as parents in the inter-varietal hybridization program which led to the development of IIHR-2n variety, Arka sanjeevini (Krishnan et al. 1988).

The seeds of diploid, Arka sanjeevini ($2n = 24$) and tetraploid, Arka mahima ($2n = 48$) varieties of *Solanum viarum* were used for obtaining explants for *in vitro* mutagenic study. Frequency of variant progenies in F2 (Tissue cultured and mutagen treated) was more than SC2 for many characters both quantitative and qualitative (Maruthi Kumar and Tejavathi 2011).

In-vitro mutagenesis using stem explants of diploid and tetraploid varieties of *S. viarum* with both chemical and physical mutagens and cultured on MS+IAA (17.13 μM)+BAP (8.87 μM) to obtain large number of multiple shoots. That obtained shoots were made to root on MS+IBA (4.90 μM) and transferred to land after a brief period of acclimatization. Berries were harvested and second generation progenies were raised from the seeds of untreated and treated first generation progenies. Harvested berries from eight samples including treated and untreated diploid and tetraploid varieties along with seed control progenies were subjected to HPLC analysis to evaluate the effect of mutagens on the solasodine content. Berries of tetraploid progenies obtained through physical mutagen treated cultures were found to contain more percent of solasodine compare to other progenies.

7.2.7 Medicinal Yam

Yam belongs to the genus *Dioscorea* of family Dioscoreaceae. The tubers of some species of Dioscorea are important sources of diosgenin, a chemical used for the commercial synthesis of sex hormones and corticosteroids, which are widely used for anti-inflammatory, androgenic and contraceptive drugs. Some specious twin clockwise and some anti-clockwise, all are dioecious and rhizomatous. Monocotyledonous, it shares several features with dicots. These include presence of cordate and acuminate leaves with 5–9 basal nerves and in some of its species, embryo with two cotyledons.

The genus contains some 600 species with more than 10 species cultivated for food and pharmaceutical use.

D. deltoidea Wall is an indigenous species to north western Himalayas, 1000–3000 msl, Chromosome number 2n = 20 m The petioles are 5–12 cm long, 4–12 cm wide cordate. It is a hairless vine, twinning clockwise. Flowers are borne on auxiliary spike, male spike 8–40 cm long and stamens 6, female spike 15c long 3.5 cm board, 4–6 seed, seeds are winged and round, rhizome is lodged in soil, superficial, horizontal, tuberous, digital and chestnut brown in colour. It produces very slender vines and is very weak. Rhizomes are ligneous, irregular. Regeneration of tuber is slow takes about 7–10 years – commercially not attractive.

D. composita Hemsl., is native to Mexico (Central America), Robust climber, right twining, nearly glaborous, alternate leaves, long petioles, memberanaceous or coriaceous lamina. The fasciculate, glomerate inflorescence, single or branched 2–3 sessile male flowering having fertile stamens. Female flowers bifid stigma tuber large, white, deep rooted upto 45 cm.

D. floribunda Mart. & Gal. is also a Central American introduction. Grown in Karnataka, Magalaya, Andhaman and Goa. Glaberous and left twining. Leaves alternate, boardly ovate or triangular ovate. Shallow or deeply cordate, coriaceous lamina with 9 nerves, petioles 5-7 cm long thick. Male flowers solitary rarely in pairs, female flowers divaricated stigma which is bifidat apex, capsule obovate, seed winged, tuners thick-yellow in colour and grows up to 35 cm.

The major breeding objectives in steroid bearing Dioscorea species are higher diosgenin content, high tuber yield, compact tuber growth, resistant to diseases and wide adaptability.

Under Banglore conditions, flowering in *D. composita, D. floribunda* occurred during March–October in both male and female parents and peak periods in March and April (Bammi et al. 1979). Anthesis occurred in the early morning hours between 4 and 6 am. Anther dehiscence occurred after or about an hour of anthesis. Stigma receptive for 12 h in *D. deltoidea*, 30–35 h in *D. floribunda* and *D. composita*. Under open pollinated conditions, fruits matured in 90, 110 and 120 days in *D. deltoidea*, *D. floribunda* and D. *composita* respectively. In *D. floribunda* and *D. composita*, physical proximity of male and female flowers (to lesser than 2 ft) was found to be necessary for pollination. Ants are pollinating agent. Intertwining of male and female inflorescence gives good seed set.

Selection in introduced materials of *D. floribunda* and *D. composita* was documented (Bammi and Randhawa 1975). Variability for tuber yield is 0.32–3.05 kg/plant and for diosgenin content it ranged from 1 to 3 kg/plant. In *D. deltoidea,* studies conducted at IIHR, Bangalore indicated wide clonal variations among collections drawn from Himachal Pradesh and Jammu & Kashmir states for diosgenin content. Possitive association was found between flesh colour of tuber and diosgenin content with white colour associated with high diosgenin content (Bammi et al. 1972).

Intra and inter specific hybridization was attempted in the three species. In *D. floribunda* 10 intra specific crosses were compared for germination percentage and seedling vigour. Character inter-relationships indicated that heavier seeds from large leaved vines with lesser number of fruits per panicle is likely to produce more vigorous seedlings in greater frequencies.

Inter specific hybridization was attempted in the steroid bearing Dioscorea species. Among the three species, the old world *D. deltoidea* was successful parent only in crosses with D. sylvatica but failed to cross with *D. composita*, *D. floribunda*, *D. alata* and *D. friedrichsthallii* (Rama Rao et al. 1973). In *D. deltoiea* and *D. sylvatica* cross both F1 and F2 generations were reported to combine the characters of parents *viz.*, high diosgenin content of *D. deltoidea* and compact tubers of *D. Sylvatica* parent.

D. composita and *D. floribunda* have been successfully crossed with each other (Martin and Cabanillas 1966; Rama Rao et al. 1973) and reciprocal crosses were also successful. The F1 hybrid of *D. floribunda* and *D. composita* cross manifested heterosis for leaf size, leaf number, stem diameter, side branching, flower size and seed size. The hybrid combined the early sprouting vigour and high tuber yield of *D. floribunda* parent. It had less marked dry season dormancy and high drought tolerance as the *D. composita* parent (Bammi and Randhawa 1975). Both *D. Composite* and *D. floribunda* have been successfully crossed with *D. friedrichsthallii* (Rama Rao et al. 1973). Reciprocal crosses of *D. floribunda* and D. *friedrichsthallii* resulted in the production of viable seeds while seed set and germination was obtained only in D. *friedrichsthallii* x *D. composita* but not receiprocol cross. The hybrids were low in tuber yields and not found promising.

Rama Rao et al. (1973) recognized four levels of crossability based on comprehensive study of hybridization involving *D. deltoidea, D. floribunda, D. composita, D. friedrichsthallii* and *D. alata*. In group A, represented by D. deltoidea as male parent in crosses with *D. floribunda*, *D. composita* and D. *friedrichsthallii* and *D. composita* and D. *friedrichsthallii*, hybrid embryo abortion occurred at four or eight celled stage resulting in post fertilization ovular breakdown. In group B, pre fertilization ovular breakdown following failure of fertilization ascribable to either non germination of pollen or slow pollen tube growth have been implicated for failure of crosses. The successful combinations falling in this group included reciprocal crosses of *D. alata* with *D. deltoidea*, *D. composita*, *D. floribunda* and *D. friedrichsthallii, D. deltoidea* as female parent in crosses with *D. floribunda, D. composita* and D. *friedrichsthallii.* In-group poor seed germination was noticed in *D. friedrichsthallii* x *D. composita* belonged to this group. The successful crosses with readily

produced interspecific hybrids were grouped in D: these consisted of four crosses involving three new world species, viz., *D. floribunda, D. composite, D. floribunda* x *D. friedrichsthallii, D. composite* x *D. floribunda* and D. *friedrichsthallii* x *D. floribunda.*

In mutation breeding of *Dioscorea*, chemical mutagens, especially (N-nitroso methyl urea-NMU and diethyl sulphate) stimulated germination and seedling vigour. Treatment with 0.05% EMS, resulted in higher tuber yielding mutant (88.63 t/ha as against 62.01 t/ha) with higher diosgenin content in 0.15% EMS treatment and compact tuber mutant (Sahoo et al. 1986).

Induction of autotetraploids have been reported in *D. deltoidea* (Janaki Ammal and Singh 1962) and *D. floribunda* (Murthy 1977). For induction of autotetraploids in *D. deltoidea*, aqueous colchicines solution (0.4–1%) was injected into dormant tubers, shoots with tetraploid chromosome number of 2n = 40 were identified and shoots of these autotetraploid were characterized by large and thicker leaves and bigger stomata than diploid. Rhizomes of tetraploid were found to be slow growing.

7.2.8 Opium Poppy

Opium poppy (*Papaver somniferum*) is an important medicinal plant belongs to family Papaveraceae. It is grown as a dual-purpose crop for both seeds and alkaloids. Its seeds are very nutritive and contain high percentage of linoleic acid which is lower blood cholesterol in human system. The opium latex is extracted from green but fully grown capsule which contains several alkaloids used as analgesic antitussive and antispasmodic in present day medicine. The opium is considered an oldest and perhaps the best known pain killer from times immemorial. Opium distributed in temperate and subtropical regions of world extending from 60°N in North West Soviet Union whereas the southern limit reaching almost the tropics. India is one of the largest producers of opium alkaloids in the world. To cultivate Opium poppy license has to be obtained from office of Narcotics department, Government of India. At present, cultivation is confined to UP, Rajasthan and MP. The major alkaloids present in *Opium poppy* are Morphine:7–17%, Codine: 2.1–4.4%, Thebaine: 1.0–3%, Nacrotics: 3–10%, Papverine: 0.5–3%.

The plant is an annual herb, erect, commonly 30–150 cm long with 0.5–1.5 cm thick stem.Root is much branched, tapering and yellow. Stem is glaborus with thick waxy coating.Leaves are numerous, alternate, sessile, spreading horizontally. In Indian poppywide variation of leaf serration was noticed by Nigam et al. (1989). Flowers few, solitary on 10–15 cm long peduncle. Buds are ovate – ovoid drooping hermaphrodite, regular with two caducous sepals, smooth. Petals are green, very large, poly petalous white in colour. “Malwa” forms like “Lukka” petals are large rose lilac or purple colour with fringed margin**.** Stamens are numerous, hypogynous arranged in several whorls. Anthers are linear attached with filament, cream coloured becoming pale brown and twisted after dehiscence. Ovary large depressed, globular, smooth pale green, one celled with large spongy parietal placenta. Stigma is sessile,

capitates. Fruit is a capsule which vary in colour, shape and stigmatic rays. Mature capsule may be globose or roundish. Seeds are numerous, very small, whitish grey in colour.

In India, six species of the genus Papaver are described by Husain and Sharma (1983) *viz*, *P. somniferum, P. orientale, P. nudicaule, P. rhoeas, P. argemone, P. dubium.* Among these, only *P. somniferum* is commercially cultivated and pharmaceutically useful due to the presence of rich sources of alkaloids. *P. somniferum* has two subspecies viz., *P. somniferum var. hartense* and *P. somniferum var. somniferum.* The subspecies *hartense* has dehiscent capsules with no opium but is useful for seed purpose. The subspecies *somniferum* bears indehiscent capsule containing latex (opium) when immature and white seeds are formed when matured. This is further divided into two groups viz., *var glabra* grown commercially for seeds and oil mostly in European countries. *var. album* which is cultivated mainly in India for opium production.

Chromosome number varies from diploid (2n) to octoploid (8n) in different species of *somniferum*. *P. somniferum* has chromosome number of 2n = 22. Self-pollinating crop, 9% cross pollination is reported and the percentage can be expected more due to major role of insect and wind in out crossing (between plants with low and normal alkaloid content). Anther dehiscence occurs before anthesis but stigma is not receptive and contribute for cross pollination.

Male sterility encourages out crossing in *Opium poppy*. As occurrence of spontaneous mutation is rare, induced mutation can be generated either by mutagenic treatment or due to distant hybridizations. Treatment of poppy seeds with10 and 20 kr gamma rays produce male sterility in M_1 generation (Khanna and Singh 1975). Male sterility was noticed in F_1 generation of Interspecific hybridization of *P. somniferum* and *P. setigerum* (Hrishi 1960). Khanna and Shukla (1989) reported triploid F_1 from *P. somniferum* and *P. setigerum* which was partially sterile and such type of sterility also promotes outcrossing. However, occurrence of spontaneous or induced male sterility is not enough for exploitation under heterosis breeding program. The nature and genetics of male sterility must be determined because only Cytoplasmic genetic male sterility is of commercial significance for hybrid seed production.

Breeding objective in Opium poppy includes development of variety with high seed yield and alkaloid content besides resistant to biotic and abiotic factors.

Introduction of exotics in Opium poppy revealed that cultivars introduced from European required long photoperiod and unsuitable for Indian condition, however some of them set seed, thus can be exploited in hybridization program to transfer specific genes to genetic background of Indian stocks. The Iranian race cultivated in India was by Introduction only (Khanna 1975).

Selection is a process by which individual plants or group of plants are sorted out from heterogenous population. The efficacy of selection is based upon the presence of variability in *Opium poppy*. Three different selection methods are followed in *Opium poppy.*

Mass selection is a simple method to improve the general level of local land races.A group of similarity appearing plant is selected and harvested seeds are mixed for commercial cultivation or for further selection. Khanna (1979) reported

indigenous land races manifested as high degree of intra population variation. He detected early types "Ornamental Red" and Aphundi" having 80–83 days flowering period. Similarly "MOP-2" and "Jawahar Ahim-16" from Madhya Pradesh and I.C.42 from New Delhi were isolated from open pollinated cultivars. Recently, the "Kirtiman" and "Chetak" were also developed through selections made in local races of Faziabad and Rajasthan, respectively (ICAR 1989).

Individual Plant Selection is practiced in populations where much variability is available in local land races. Generally, it is practiced where the particular character requires improvement. Khanna (1979) made individual selection for earliness (80–81 days) in variety 'Single Dark Red'. A high yielding from "Dhaturia" yielded 54 kg opium/ha on dry weight basis. Individual plant selection may also practice where spontaneous mutation occur. Nyman (1978) isolated and released mutant "Soma" from spontaneous mutation variety in India.

Pure line selection is also practiced in opium poppy. In self-pollinated crops, all the plants are homozygous because of continued self-fertilization. It consists of the progeny of self-fertilized plants and is used for developing variety. The variety developed is genetically pure and more stable. Khanna (Gupta et al. 1978) developed a number of pure line by several generations of selfing. Kumar (1981) and Shukla (1985) produced several pure lines and tested them for combining ability through line x tester and diallel analysis, respectively. Consequently, NBRI developed and released "BROP 1", a synthetic variety (ICAR 1989; Shukla et al. 1992) with high morphine, opium yield and seed yield. Earlier, Kopp et al. (1961), and Heltman and Silva (1978) also developed synthetics in opium poppy using suitable pure lines.

Pedigreee method of breeding was followed in Opium poppy. Taranich (1974) isolated high yielding recombinants with good morphine content lodging resistant from inter-varietal hybrids. Lőrincz (1978) developed 'Mayak' and 'Vaskhod' varieties of opium poppy and noticed 25–30% high morphine content than standards. Khanna and Shukla (1989) developed high opium and seed yielding genotypes from Interspecific crosses between *P. somniferum* and *P. setigerum.*

Hybridization is very easy in poppy as compared to other self-pollinated one. (Khanna and Shukla 1983), the handling of hybrids depend upon better recombinant/transgressive segregants. Extent of heterosis and combining effects of parents.

Inter-varietal/interspecific hybridization involving parents of same species/strains, varieties or races of the same species were attempted in Opium poppy. Earlier Singh and Khanna (1975) observed 37.33% heterosis for capsule/plant. 46.62% for opium yield and 10.26% for morphine content over superior parent. Khanna and Gupta (1981) studied heterosis for crosses between 'Aphuri' (not cultivated one), a grey seeded, violet flowered and dehiscent variety, and 15 major Indian cultivars and noticed 46.34% for opium yield and 37.14% for morphine.

Interspecific hybridization by crossing two different species of plant which leads to transfer of some genes from one species to another species was attempted in Opium poppy. Lorincz and Tetenyi (1970) developed 'Kek Duma' from the hybrid progenies of *P. somniferum* var. Havan and *P. orientale.* The new varieties had 0.65–0.75% morphine in the capsule as compared to low morphine content in

P. somniferum var. Havan. To explore *P. setigerum* for genes of economic values which may be transferred in *P. somniferum.* Khanna and Shukla (1989) crossed these species and produced triploid hybrids with viable seed. They investigated the potential of F_1 and subsequent generation. The F_1s are tall and bear 9–23 capsules/plant. The capsules are larger than *P. setigerum* and an average opium yield was 290 mg/plant.

A mutation breeding experiment was carried out using physical and chemical mutagens to develop non-narcotic opium poppy from narcotic crop. They isolated two families containing 12 latex less/opium-less and 12 partial latex bearing plants in M_1 generation which gave similar observations in M_2 generations also. The best mutant genotype, LL-34 of family C^{1}-Comb-113-2 with 5.66 g seeds/capsule had 52.6% oil was designated as cv. 'Sujata'. This was the world's first opium-less and alkaloid free seed poppy cultivar, offers a cheap and permanent (fundamental) solution to the global problem of opium-linked social abuse. Simultaneously, it serves as a food grade crop with proteinaceous seeds along with healthy unsaturated seed oil.

NBRI-1 was more sensitive than NBRI-5 and that the mutagen EMS was most potent in creating chromosomal abnormalities. Two doses i.e. kR 10 + 0.2% EMS possessed high chiasms frequency while 0.2% EMS in combinations with all doses of gamma was effective in enhancing the total alkaloid as well as specific alkaloids. The dose kR30 and kR10 + 0.4% EMS gave highest positive results for genotypic coefficient of variability, heritability and genetic advance (%) for seven traits in NBRI-1 and ten traits in NBRI-5 respectively.

A mutant variety known as 'TOP 1' ('thebaine oripavine poppy 1') in opium poppy (*Papaver somniferum*) was developed by Tasmania Company. In this mutant the morphinan pathway is blocked at thebaine results in absence of codeine and morphine. The major loss of this blockage is on the end product i.e. morphine which is absent in this mutant. The Tasmania drug industry has been using TOP 1 mutants since 1998 for production of various analgesic drugs viz. buprenorphine, oxycodone, naloxone and naltrexone.

Induced polyploidy to enhance total alkaloid content along with specific alkaloid using colchicines was attempted in opium poppy. The induced auto-tetraploidy did not show any significant differences in phenotypic level while stomatal and chromosomal studies confirmed the tetraploidy. They also noticed differential gene expression of the diploids and auto-tetraploids which led to the elucidation of dosage regulated gene expression leading significant enhancement in morphine content in tetraploid plants. Their study in auto-tetraploids opens avenues towards the development of hexaploids and amphidiploids which can give multifold increase in specific alkaloids. This study also opens a new vista towards understanding of ploidy level changes in term of phenotypic, genetic and genomic and a better understanding of the complex mechanism involved in polyploidization.

7.2.9 Periwinkle

Periwinkle (*Catharanthus roseus*) is considered to be a native ofthe West Indies but was originally described from Madagascar (Ross 1999). It is commonly grown as an ornamental plant throughout tropical and subtropical regions of the world by virtue of its wide adaptability, ever blooming nature and variously coloured flowers. The genus *Catharanthus* consists of eight species, seven of them viz., *C.roseus* (L.) G. Don, *C. ovalis* Markgraf, *C. trichophyllus* (Baker) Pichon, *C. longifolius* (Pichon) Pichon, *C. coriaceus* Markgraf, *C. lanceus* (Bojerex A. DC.) Pichon, *C. scitulus* (Pichon) Pichon, indigenous to Madagascar and one viz., *C. pusillus* (Murray) G. Don, indigenous to India (Stearn 1975).

Periwinkle is an annual or perennial semi-shrub growing to a height of 75 cm–1 m, sub-woody at the base and profusely branched. Stem colour – yellowish green, light pink or dark purple. Leaves, petioles and twigs contain milky latex. Leaves – oblong or ovate, opposite, short-petioled, smooth or pubescent with entire margin. Flowers, borne in pairs axils, pedecellate, bracteate, hermaphrodite, actinomorphic, complete, hypogynous and pentamarous. Calyx, five parted, the sepals free almost to the base. Corolla, five lobed, small to large, salver shaped, rose or white; tube cylindric, throat bearded, slender, externally swollen at the insertion of the stamens but contracted at the mouth; lobes free or overlapping, aestivation convolute. Stamens – five, attached to the middle of the corolla tube or just below the mouth, conniving over the stigma; filaments very short, not geniculate; anthers free from the stigma, dorsifixed, the connective not prolonged into an apical appendage, anther 2.5 mm long and the filament about 0.3 mm long, at anthesis. Carpels – two, distinct narrowly triangular glands present at the base of the carpels; ovules, numerous (about 10–30) in two series in each carpel; style long, slender; clavuncle shortly cylindric, truncate at base. Carpels united only by the style at the apex. Stigma capitate, bearded at the top and furnished with a cup-shaped/'skirt' like membrane below, which sheaths the upper part of the style. Fruit consists of two long cylindric pointed follicles (mericarps) diverging or parallel, containing 10–30 seeds, dehiscent at maturity along the length. Seeds – numerous, small (1.5–3.0 mm long), oblong, cylindrical, not arillate, with the hilum in a longitudinal depression on one side, blackish, muriculate, the surface minutely reticulate (Kulkarni 2016).

A knowledge of the morphology of the flower, its development, anthesis, pollination mechanism, mode of pollination, presence or absence of incompatibility, fruit and seed set of the plant is an essential prerequisite for understanding the breeding behaviour of the plant species. Information on these aspects is necessary for developing artificial selfing and hybridization techniques, maintenance of varietal purity and for choosing and executing appropriate breeding methodology. Flowering in periwinkle begins when plants are about 10–15 cm tall or about 10 weeks old and continue to flower as long as plants live. Flowers appear in pairs in the alternate leaf axils and the two flowers are never in the same stage of development, with one flower opening approximately 2–3 d before the other. Under South Indian conditions, anthesis generally starts from 15.00 to 16.00 h and continues until the next

day. Anther dehiscence occurs just before anthesis and the pollen is shed as a sticky mass The stigma was found to be receptive between 06.00 and 10.00 h, and between 15.00 and 17.00 h (Sreevalli 2002).

A characteristic feature of *Apocynaceae*, is the disc-like or otherwise shaped enlargement of the stigma-head with a sticky secretion and a brush of hairs on which pollen collects as it is shed. The receptive portion of the stigma is at the base of the stigma head, and owing to the position of the anthers, self-pollination is rendered almost impossible, and insect-visits are necessitated (Rendle 1971).

Experimental studies have also shown that automatic self-pollination does not occur in periwinkle and pollinators are necessary to bring about pollination (Kulkarni 1999; Sreevalli et al. 2000). Two pollinating butterflies *Pachlioptahector* and *Catopsilia pyranthae* have been found to exhibit flower colour constancy during their flower visits and cause about phenotypic assortative mating for flower colour resulting in greater number of intra-flower colour matings than inter flower colour matings (Kulkarni 1999). Some self-pollinating strains have also been found in periwinkle. In these strains self-pollination occurs due to continuous elongation of either ovaries or styles until pollination by overcoming spatial separation of stigma and stamen. Thus, both type of pollination occur in periwinkle (Kulkarni et al. 2001, 2005).

Periwinkle is a diploid plant species with a chromosome number of $2n = 16$ (Janaki Ammal and Bezbaruah 1963). Florry (1944) explained flower colour differences in three phenotypes. Genotypes carrying dominant alleles R and W bears pink flowers, only R pink eyed flowers and recessive alleles rr produce white flowers. Artificial hybridization and selfing techniques have been employed for carrying out genetic studies. Flower buds have been emasculated 1 d before anthesis (Kulkarni et al. 2001) or 2–3 d before anthesis (Levy et al. 1983; Sevestre-Rigouzzo et al. 1993) by making a partial cut, about 1 mm, above the base of the throat of the corolla tube. The top portion of the flower bud was then removed long with the undehisced anthers.(Kulkarni et al. 2001). The per cent fruit set ranged from 90 to 100% (Sevestre-Rigouzzo et al. 1993; Kulkarni 1999).

Levy et al. (1983) reported marked differences for yields of leaves and roots and for contents of ajmalicine in roots of three un related pure lines representing three flower colour types: pink corolla, white corolla and white corolla with red eye. The differences between lines varied according to developmental stage of the plant. They also observed 29 and 24% significant and positive heterosis over better parent for leaf and root dry yields per plant, respectively, in the F_1hy brid involving the parental lines, pink corolla, and white corolla with red eye. However, no heterosis was observed for ajmalicine content in roots.

Mishra et al. (2001) evaluated 32 accessions collected from wide geographical areas such as different regions of the Indian sub-continent, Sri Lanka, Madagascar, Singapore and Malaysia for 53 growth, development and alkaloid yield related characters over two seasons. Large differences were observed between accessions for six morphological and 14 agronomic traits; the differences were 3, 80 and 15-fold for the main alkaloid yield components viz., leaf dry matter yield, VLB and VCR contents, respectively.

Information on inter-trait correlations is essential to know the effect of selection for one trait of interest on other unselected traits, and to know the possibility of carrying out indirect selection for characters of interest which are difficult or time consuming to measure, or are less heritable. In periwinkle, estimation of contents of total alkaloids and specific alkaloids viz., VLB, VCR, vindoline, catharanthine and ajmalicine is time consuming and limits the number plants that can be evaluated in a breeding pro gramme. Any trait with high heritability and a strong correlation with contents of these alkaloids could be useful for preliminary screening for content of alkaloids as well as for indirect selection for these important traits.

Leaf yield and root yield, leaf yield and leaf alkaloid yield, root yield and root alkaloid yield were found to be positively correlated suggesting that simultaneous improvement for these pairs of traits should be possible through selection (Mishra et al. 2001; Sharma et al. 2012). Leaf yield and root yield were not correlated with leaf alkaloid concentration and root alkaloid concentration, respectively (Mishra et al. 2001). Therefore, it should be possible to combine high yield of these two plant parts with high concentrations of alkaloids in them.

No relationship was found between flower colour and contents of vindoline and atharanthinein 50 horticultural cultivars which had been bred for flower colour. However, one of the cultivars had low content of vidoline and ten times lower tabersonine-16-hydroxylase activity as compared with *C. roseus* cv. Little Delicata (Magnotta et al. 2006).

Catharanthus roseus is considered to be incompatible with other *Catharanthus* species. except *C. longifolius.* Reciprocal differences were observed in crossability between *C. roseus* and *C. trichophyllus. Catharanthus roseus* as female parent failed to form fruits and therefore, no introgressions were found from *C. trichophyllus* to *C. roseus* (Sevestre-Rigouzzo et al. 1993). In a reciprocal cross, however, upto100% seed set with good germinability was found. Alkaloid profiles of *C. trichophyllus* and *C. roseus* differed with absence of serpentine in leaves of *C. trichophyllus* and catharanthine in roots of *C. roseus*. Hybrids contained serpentine in leaves such as leaves of *C. roseus* and catharanthine in roots such as roots of *C. trichophyllus*. Further, significant heterosis was found for the contents of ajmalicine, catharanthine and serpentine both in leaves and roots and for the content of vindoline in leaves. The hybrids also had higher leaf and root yields than the parental species. Therefore, they suggested development of hybrids coupled with micro propagation to exploit observed heterosis for alkaloid production.

Induced auto polyploidy is a rapid method to increase the yield of vegetative parts in plant species and the method was experimented in periwinkle. Autotetraploids had significantly higher leaf yield, root yield and content of total alkaloids than diploids, while in other studies (Kulkarni et al. 1984; Krishnan et al. 1985) they were found to be on par with diploids for leaf yield and root yield but had lower ajmalicine con tent and harvest index. Goswami et al. (1996), The contents of vindoline, catharanthine and VLB were found to be higher in tetraploid lines than in diploids (Xing et al. 2011).

In industrial crops, such as medicinal plants, the content of the economically important metabolite is more important than the yield of the plant parts containing

the metabolite because it determines the cost of extraction of the metabolite. Mutation breeding is one of the most promising approaches for the development of 'ideochemovars' (Swaminathan 1972; Levy 1983).

Mutation breeding has been adopted more frequently in self-pollinating crops than in cross-pollinating ones, due to failure of recessive mutations to express in cross fertilizing systems without manual selfing or sib-mating. Periwinkle, although a herkogamous species, was earlier considered to be a self-pollinating species because of geitonogamy and the need for artificial selfing was not realized. Nevertheless, periwinkle has been subjected to induced mutagenesis and several mutants affecting different traits including contents of alkaloids, with direct or indirect utility through hybridization, have been isolated. Estimation of contents of alkaloids is time consuming.

In the absence of rapid methods for screening plants for their alkaloid contents, macro-mutants with altered morphology have been evaluated for identifying mutants for altered alkaloid contents. Induced macro-mutants in periwinkle include those with altered plant height, leaf morphology, floral traits, reproductive traits, and those with tolerance to salt, heat and water stress.

Plant height is an important trait which along with shoot branching and inflorescence morphology determines plant architecture and crop yield (Wang and Li 2006).Three distinct reduced plant height mutants, 'dwarf', 'semi-dwarf' and 'bushy', respectively, about 60, 40 and 30% shorter than their parental variety, Nirmal have been reported in periwinkle (Kulkarni et al. 1999a, b, 2009). The 'dwarf' and 'semi-dwarf' mutants were due to monogenic recessive genes (dw_1 and dw_2, respectively) which were allelic to each other and had significantly higher content of root alkaloids than parental variety. The 'bushy' mutant which was governed by an independently inherited non-allelic recessive gene (*by*), however, had similar contents of leaf as well as root alkaloids as the parental variety, Nirmal. The double-mutant recombinant ($bydw_1$) was 30% shorter than the shorter of the parental mutants and exhibited 20% higher content of root alkaloids than the better parent. All the three mutants and the double-mutant recombinant ($bydw_1$) had similar contents of leaf alkaloids. Higher root alkaloids content has been found to be related to thin root morphology in hairy root cultures of periwinkle (Palazon et al. 1998).

As economically important alkaloids are present in the leaves, altered leaf morphology may suggest altered alkaloid contents. Three leaf mutants, *viz.* wavy leaf margin, 'necroticleaf' (a lesion mimic mutant) and 'neriumleaf' (resembling leaf lamina of another Apocynaceous plant, *Nerium oleander*) exhibited higher contents of leaf alkaloids than their respective parents (Kulkarni et al. 1999a, b; Baskaran et al. 2013). Further, enhanced contents of leaf alkaloids of 'necrotic leaf' and 'nerium leaf' mutants over their parental variety were found to be due to recessive alleles at different loci, and 13 out 14 double mutant recombinants for parental mutant traits 'necroticleaf' and 'nerium leaf' developed by crossing the two mutants had significantly higher content of leaf alkaloids than parental mutants (Kulkarni and Baskaran 2014). No studies have been carried out on linkage between leaf alkaloids content and these morphologic al mutant traits. However, it appears that 'necrotic leaf' trait could be used as a seedling marker trait for enhanced content of

leaf alkaloids. Extract of *Pythium* (a soilborne pathogen of periwinkle) is well known to be an elicitor of alkaloid production in cell and tissue cultures of *C. roseus* (Nef et al. 1991). Therefore, constitutive expression of self-defence reactions in the 'necrotic leaf' mutant may have induced enhanced production of alkaloids similar to that elicited by *Pythium*. For commercial exploitation of het erosis, male sterility is required for large scale production of hybrid seeds. A patent has been granted for the method for developing hybrids in *Cathranthus* using male sterility (Bowman 2000).Streptomycin was used to develop genetic male sterile line '13,861–1' with msGS gene, which does not set selfed seed. The gene did not show any undesirable pleiotropic effect.

Two other types of male sterile mutants viz., indehiscent anthers (functional male sterility) and pollen-less anthers governed by a single recessive allele and duplicate recessive alleles, respectively, have also been reported (Sreevalli et al. 2003; Kulkarni and Baskaran 2008).The mutant with indehiscent anthers had relatively smaller anthers and about 30% lesser number of pollen grains but was otherwise phenotypically normal, had high pollen fertility and show ed. high seed set on artificial selfing.

Mutations affecting the gynoecium have also been reported. In periwinkle flower, the stigma is about 0.5 mm below the anthers, typical of reverse herkogamy. Mutants with short style (about one-third length of normal style) and long style with stigma 2.5 mm above the tip of the cone of anthers, with partial and high pollen sterility, respectively, have been reported (Mishra and Kumar 2003; Kulkarni and Baskaran 2008). The short-styled mutant trait was inherited as a recessive trait, while the long-styled mutant constituting 'pin' flower in contrast to 'thrum' flower in normal plants appeared to be under the control of inhibitory, epistatic interaction between two independently inherited genes *P* and *T*, with gene *T* being inhibitory to gene *P*. Accordingly, genotypes *P-T-, T-pp*, or *pptt* produce normal 'thrum' flowers whereas *P-tt* produce mutant 'pin' flowers.

A recessive mutant producing heterocarpous flowers, with one (3%), two (82%) and three (15%) carpels and high fertility has been reported with a possibility of genetic engineering for fruit size, carpel number and seed number per plant (Rai and Kumar 2001).

As periwinkle is also valued as an important garden plant because of its variously coloured flowers, mutants affecting flower colour, flower density, floral persistence etc., would also be of interest. A mutant described as 'leafless inflorescence', in which flowers are borne on nodes without leaves, has been reported and the locus '*lli*' has been mapped (Chaudhary et al. 2011). The mutant produced more number of flowers per plant than its parent and further enhancing its horticultural value. New improved horticultural genotypes were developed by crossing this mutant with other genotypes with different flower colours and plant habit (Kumar et al. 2012).

Another novel mutant with caduceus closed corolla corolla abscising before anthesis) inherited as a monogenic recessive, was isolated after mutagenesis with EMS. The trait was used for development of cleistogamy, a new trait, in periwinkle (Kulkarni and Baskaran 2013a, b).

Screening of plants treated with EMS 3600 resulted in identification one plant with high ajmalicine, and low catharanthine and vindoline contents was identified (Thamm 2014).

Eight mutants (*gsr1*to*gsr8*), tolerant to salinity (250 mM NaCl) or high-temperature (45 °C) stress have been isolated (Rai et al. 2001, 2003; Kumari et al. 2013). These mutants (*gsr 1* to *gsr 6*) accumulated more proline and glycine betaine constitutively as well as under water stress, and transpired lower amounts of water under water stress than their parental variety. The contents of catharanthine, VLB, VCR and serpentinein two of these *gsr* mutants (*gsr3* and *gsr6*) were found to be higher than those in their parental variety.

Detection of mutations is the first and most critical step in mutation breeding. Historically, mutants have been identified phenotypically, in large mutagenized populations, for easily recognizable characters such as, altered plant height and architecture, early or late flowering and maturity, altered flower, fruit and seed characteristics, resistance to diseases that can be screened easily in natural or artificial epiphytotics, and for biochemical quality traits for which low-cost, high-throughput and rapid evaluation methods are available. To increase efficiency of mutation breeding, high-through put DNA technologies for mutation screening such as TILLING (Targeting Induced Limited Lesions INGenomes), ECOTILLING, and high-resolution melt analysis (HRM), have been developed and used in crop plants; (Xin et al. 2008). These reverse genetics techniques can be used for discovering allelic variation in natural or mutagenized populations using large number of mutants isolated and TI A pathway genes already cloned in periwinkle.

7.2.10 Senna

Cassia angustifolia, a small leguminous shrub, is exclusively grown for its leaves and pods which contains glycosides having usefulness in a variety of ailments, such as liver complications and abdominal distress. It is also used in modern medicine as a laxative because of its glycosides-sennoside A and B. Leaves and pods from *Cassia angustifolia* Vahl and *Cassia acutifolia* Del are the commercial senna drug of the Unani system of medicine. Both species, *Cassia angustifolia* (native of South Arabia, West Asia) and *Cassia acutifolia* (Sudan, East Africa), are exotic to India. In their native lands, these species grow on arid tracts as perennial bushes.

Senna (*Cassia angustifolia* Vahl.) is a small perennial under shrub, native of Saudi Araia. It attains 0.7–1.0 m height under cultivation but grows taller (1.5 m) when left uncoppiced. It bears compound pinnate pale green leaves, having 5–8 pairs of shortly stalked leaflets. It bears axillary or subterminal racemes earing many large, brilliant, yellow coloured showy flowers. Pods are flat, thin, 3.5 × 6.5 cm × 1.5 cm, pale green in the beginning which change to greenish brown and dark brown on maturity and after drying. Each pod contains 5–8 obovate yellow to of cream coloured flat seeds. Alexandrian senna ears 4–5 pairs of leaflets which are shorter and narrow in dimension but dark green and thicker. Pods are short

(2.75–5.6 cm long and 1.5–2.5 cm broad) with 5–7 pairs of obovate seeds (Rajendra Gupta and Pareek 1995). Surface markings on the seed coat and stomatal index of leaves are distinct and remarkably consistent in the two species to maintain their separate identity (Fairbairn and Shesta 1967).

Senna (2n = 26,28) is predominantly a self-pollinated species but percentage of outcrossing is fairly large and pollination is done by beetles. Flowers are dichogamous with 7 stamens and 3 staminodes. Flowers open in the morning hour and anthers commence dehiscing after 3 h of opening of flowers, dehiscence is apical and the pollen is continued to be released for 24 h. Stigma become receptive soon after opening of flowers. Pollen grains are triangular, highly fertile (98%). The life of the flowering axis is around 35 days and flowering continues till the close of the season (Mohan Rao et al. 1976).

In India, 24 species of Cassia are distributed in different parts of India, Kapur and Atal (1982), tabulated the presence of various anthracene derivatives in different parts of 25 Cassia species from literatures.

Seeds of Tinnevelly senna were irradiated with gamma ray doses @ 350 Gy, 450 Gy, 550 Gy and 650 Gy. Single plant selection done on the basis of phenotypics from M1 were raised to get M2 generation. In both M1 and M2 there was in general reduction in percent germination with increase in the dose. Lowest germination percentage was observed for 650 Gy and highest for 650 Gy. Micromutants for quantitative traits *viz.,* days to 50% flowering, days to maturity, plant height, number of branches, pod length, pod width, number of seeds per pod, dry leaf weight/pod, pod weight/plant, seed yield/plant, 100 seed weight).Gamma rays affected the expression of quantitative traits except plant height and number of branches per plant. Gamma ray treatments 350 Gy and 450 Gy were found to be promising for inducing variability for yield and yield contributing characters.

Macro mutants for qualitative traits viz., leaf mutant, pod mutant, dwarf mutant, sterile mutant and synchronous maturity mutants were observed. Highest mutant frequency was deducted in 650 Gy gamma ray dose for all the macromutants. Among the leaf mutants, frequency of tiny leaf mutant was highest. Chlorophyll mutations were not observed for any of four doses. Among pod mutant's frequency of constricted pod mutants were highest. Sterile mutants and dwarf mutants were observed in all four doses but frequency was negligible. Synchronous maturity mutant was observed except 350 Gy (Yadawrao, S.V. 2012).

7.3 Conservation of Medicinal Plants

Loss of biodiversity of medicinal plants occur due to environmental factors, deforestration, developmental activities. Hence, conservation of medicinal plants is of very much important. The primary goals of biodiversity conservation is maintenance of essential ecological processes and life support systems on which human survival and economic activities. Preservation of species and genetic diversity and sustainable use of species and ecosystems which support millions of rural

communities as well as major industries. The conservation of the wild medicinal plants or any other such threatened species can be tackled by scientific techniques as well as social actions. Three methods of conservation are legislation, *in-situ* conservation and *ex-situ* conservation. The legislation is covered under existing laws pertaining to forestry which include Forest Act, 1927, Wildlife (Protection) Act 1972 and Wildlife (Protection) Amendment Act 1991. Forest (Conservation) Act, 1980, Environment Protection Act, 1986, National Forest Policy, 1988, National Biodiversity Act, 2002 and The scheduled tribes and other traditional forest dwellers act, 2006 De (2016).

7.3.1 In-Situ *Conservation*

Conservation of a given species in its natural habitat or in the area where it grows naturally is known as *in-situ* conservation. It includes Gene bank/Gene sanction, Biosphere reserves, national parks, sacred sites, Sacred grooves etc. It is only in nature that plant diversity at the genetic, species and eco-system level can be conserved on long-term basis. It is necessary to conserve in distinct, representative bio geographic zones inter and intra-specific genetic variation.

On-farm conservation: On-Farm Conservation involves the maintenance of traditional crop cultivars (land races) or farming systems by farmers within the traditional agricultural system. Traditional farmers use land races, which are developed by the farmer and well adapted to the local environment. This method of conservation has been gaining importance in recent years, though farmers have used it for centuries.

Home gardens: Home garden conservation is very similar to on-farm conservation; however scale is much smaller.

7.3.2 Ex-Situ *Conservation*

Conservation of medicinal plants can be accomplished by the *ex-situ* (i.e. outside natural habitat) by cultivating and maintaining plants in botanic gardens, parks, other suitable sites, and through long term preservation of plant propagules in gene banks (seed bank, pollen bank, DNA libraries, etc.) and in plant tissue culture repositories and by cryopreservation).

7.3.3 Field Gene Bank (Field Repository/Clonal Repository)

Gene Bank: Storage in the form of seed (Base collection at −20 °C; Active collection at +4 °C to l0 °C). The three national gene banks have been established in India for *ex situ* conservation of medicinal and aromatic plants are National Bureau of Plants Genetic Resources (NBPGR), New Delhi, Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow and Tropical Botanical Gardens Research Institute, (TBGRI), Palode, Thiruvananthapuram (Kerala).

7.3.4 Seed Gene Bank

Germplasm conservation in Seed Gene Bank is more economical. The NBPGR, New Delhi, houses National Gene Bank (NGB) which is primarily responsible for conservation of germplasm. These are referred to as "Base Collection" stored in modules maintained at −20 °C. The seeds are dried to attain 4–6% moisture content and hermetically sealed in moisture proof aluminium foil packets. These stored seeds remain viable for 50–100 years. In most crops, seeds samples with more than 85% seed viability are only processed. The seeds in gene bank are stored preferably as per the gene bank standards recommended by FAO/IPGRI.

7.3.5 National Active Germplasm Sites

The National Active Germplasm Sites (NAGS) are the integral component of the network. There are presently 40 NAGS, which are based at ICAR institutes, (crop-based institutes for a specific crop or a group of crops) and SAUs. These are integral part of national plant biodiversity conservation network. The NAGS are entrusted with the responsibility of multiplication, evaluation, maintenance and the conservation of active collection and their distribution to bonafide users both at the national and international levels. These active/ working collections are stored in modules maintained at +4 °C and 35–40% relative humidity (RH). Under these temperatures, seeds are expected to remain viable for 15–50 years. For medium term storage, seed moisture content is brought down to 8–10%.

The NBPGR has a network of II regional stations located in different agroclimatic zones of the country to support the active germplasm conservation activities of the regions.

7.3.6 Cryopreservation

The cryopreservation of *in-vitro* cultures of medicinal plants is a useful technique. Cryopreservation is long-term conservation method in liquid nitrogen (−196 °C) in which cell division and metabolic and biochemical processes are arrested. A large number of cultured materials can be stored in liquid nitrogen. Since whole plants can regenerate from frozen culture, cryopreservation provides an opportunity for conservation of endangered medicinal plants. For example, low temperature storage has been reported to be effective for cell cultures of medicinal and alkaloid- producing plants such as *Rauvolfia serpentina, Digitalis lanata, Atropa belladonna, Hyoscyamus* spp. When plants are regenerated and no abnormality is seen either in fertility or in alkaloid content, the materials can be stored using cryopreservation methods. Cryopreservation has been used successfully to store a range of tissue types, including meristems, anthers/pollens, embryos, calli and even protoplasts. However, the system will depend on the availability of liquid nitrogen methods (Tripathi and Tripathi 2005).

7.4 Conclusions

Breeding opens up the avenues to adapt plants to the particular demands of the stakeholders in the production chain. Conventional breeding methods that prevail in MAP breeding include selection in natural populations, combination and hybrid breeding, breeding synthetic varieties, induced mutation and clone breeding. Contemporary biotechnological breeding methods are highly expensive. Furthermore, consumers prefer natural products due to safety and reject herbal drugs that originate from genetically modified plants because they do not have their natural constitution. Nevertheless, the use of biotechnological tools and research on genes controlling the formation of secondary metabolites and on methods for their transmission are in fancy stage. At present, the exploitation of the genetic potential of medicinal plants by breeding is yet to be underutilized. Therefore, breeding can become one of the key factors for advancing the phytopharmaceutical sector in the future. Species specific characteristics influencing the success of postharvest processing should be well defined and considered in future improvement programs.

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