Chapter 8

Opium Poppy: Genetic Upgradation Through Intervention of Plant Breeding Techniques

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Additional information is available at the end of the chapter

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1. Introduction

Opium poppy (*Papaver somniferum* L.) has its importance as a plant based natural pain reliever from the time dating back to early civilization till today. Its pain relieving properties had been described in various books of unani, allopathy and ayurvedic medication system. Today our pharmaceutical industries solely depend on opium poppy for their crude resources for manufacturing of pain killing drugs. The medical practitioners around the world routinely prescribe important life saving drugs, are the secondary metabolites produced as a result of complex plant metabolism. The important life saving drugs are mostly derived from five major alkaloids viz., morphine, codeine, thebaine, noscapine and papaverine which are present in opium latex in ample amount [1]. According to a report from an international organization i.e. WHO (World Health Organization), about 85% of the population in developing countries depend on herbal plants for curatives, medicinal and other medico related applications. India being one of the twelve mega biodiversity centers of the world is fully fledged with diverse array of herbal and medicinal plants which makes it "Botanical Garden of World". About 10,000 different medicinal plant species are found in India among which opium poppy occupies the highest place in terms of food (seeds) and pharmaceuticals (alkaloids). These valuable alkaloids are mainly extracted in India from green unripe capsules by making incision upto 1-2 mm in the epidermal wall of the capsule (Figure 1), but globally it is extracted from the dried capsule which is called CPS (Concentrated poppy straw) system. In CPS system, the dried capsules along with eight inches of peduncle are harvested and seeds are threshed. The remaining husk is used to extract various alkaloids. The whole plant parts of opium poppy are valuable in terms of food, medicine, vegetable and as brewages. The seeds of opium poppy are highly nutritious as it contains protein upto 24% and other vital nutrients beneficial for human health. The leaves of the plant are used as vegetable in some places in the world. The seed oil of poppy is also important for health point of view due to having high percentage of linoleic acid (68%) which helps in lowering blood cholesterol level in human body and is also used in the treatment of cardiovascular diseases in human system [2,3].

Red Latex

Pink Latex

Figure 1. Capsule having brown and pink latex in opium poppy.

2. Geographical distribution

In India, the main opium cultivating areas are divided into 12 divisions including Madhya Pradesh, Uttar Pradesh and Rajasthan while in other parts minor cultivation is also practiced (Figure 2). In Uttar Pradesh, the opium cultivation belt is around Barabanki, Shahjahanpur, Faizabad and Bareilly while Ratlam, Mandsaur and Neemuch in Madhya Pradesh are major opium producing areas. Kota, Chittorgarh and Jhalawar in Rajasthan are the areas producing opium. The opium poppy is distributed in the temperate and subtropical regions of the old world extending from 60° North West Soviet Union whereas the southern limit reach almost the tropics. Legally it is cultivated in India, China, USSR, Egypt, Yugoslavia, Czechoslovakia, Poland, Germany, Netherland, Japan, Argentina, Spain, Bulgaria, Hungary and Poland [4, 5]. India is the largest opium producing and exporting country in the world. Globally the licit opium poppy cultivation is under the strict control of Central Bureau of

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Narcotics with its headquarter at Vienna, Austria. But at some places illegal cultivation is also being practiced which include Golden Crescent (Iran, Afghanistan and Pakistan) and Golden Triangle (Thailand, Burma, Myanmar). In Afghanistan, illegal cultivation of opium poppy to a large extent is the reason for very high drug trafficking compared with other illegal cultivating areas. Eleven other countries i.e. Australia, Austria, France, China, Hungary, the Netherlands, Poland, Slovenia, Spain, Turkey and Czech Republic also cultivate opium poppy, but they do not extract gum. They cut the bulb with 8" of the stalk (CPS system) for processing to extract alkaloids (Described earlier).

Figure 2. Opium cultivating areas in India and different offices of Narcotics Deptt. Cited from: http://www.uwmc.uwc.edu/academics/departments/political_science/opiumprod.html#map

3. Economic importance of opium poppy and its derivatives

Opium poppy belongs to the family Papaveracae and has been attracting the interest of researchers because of its pharmaceutical, decorative and alimentary attributes. Scientists have been able to identify 2500 different compounds in opium poppy belonging to different biochemical groups used in pharmaceutical industries. Among the various drugs of medicinal importance, opioids are an important class of compounds produced by opium poppy which are used in medicine as a pain reliever. These opioids interact with the opioid receptor present in the central nervous system and gastro-intestinal tract [6]. However, several of these medicinal compounds can be made synthetically but alkaloids belonging to various groups viz., Phenanthredene (Morphine, Codeine, Thebaine), the true Benzylisoquinilone (Papaverine) and Phthalideisoquinilone (Narcotine) are only obtained from opium which place opium poppy at the highest place among the diverse array of medicinal plants [7]. The most important and potent alkaloid is morphine which can be used for both short term as well as long term pain control, is widely used in many prescriptions of pain medications. The drug occurs as a white crystalline powder or colorless crystals and is available for legal medical use. Recently, scientists at the University of Pennsylvania have noticed complication in patient with hepatitis C disease due to withdrawal of morphine as it suppresses IFN-alpha-mediated immunity and enhances virus replication. This disease is common among intravenous drug users. Due to the interactive role of morphine with hepatitis C disease, interest has been developed in determining the effect of drug abuse, especially morphine and heroin on progression of the disease. The discovery of the association between two would certainly help in the treatment of both HCV infection and drug abuse [8]. Morphine is also beneficial for immediate relief in reducing the symptoms of shortness of breath caused due to cancer and non-cancerous incident [9, 10]. Morphine is widely available in market as tablets, modified release-tablets, capsules, oral liquid and sachets of modified-release oral liquid, injections and suppository [11]. There are however, many serious side effects of morphine which includes shallow breathing, slow heartbeat, stiff muscles, seizure (convulsions), unusual thoughts or behavior, severe weakness, constipation etc.

Another important alkaloid is codeine which is considered as a prodrug because it is converted into morphine and codeine-6-glucuronide (C6G) in *in vivo* [12, 13]. Codeine is a natural isomer of morphine and is formulated as 3-methyl morphine. In *in vivo* system, 5-10% of codeine is metabolized into morphine, while remaining is left free or in conjugated system as codeine-6-glucuronide (~70%), or it is converted into norcodeine (~10%) and hydromorphone (~1%). Codeine is less effective and has lower dependence-liability than morphine [13]. Similar to all other opioids, continuous use of codeine induces physical dependence and it can be psychologically addictive. However, mild effects are caused due to its withdrawal, so is less addictive than other opiates. Codeine is also used as antitussive drug against coughing and widely used in the treatment of severe diarrhea and diarrhea predominant bowel syndrome. The most frequently used drug forms are "loperamide, diphenoxylate, paregoric and laudanum [14, 15]. In addition to analgesic and antitussive effect there

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are some side effects of codeine which includes euphoria, itching, drowsiness, vomiting, orthostatic hypotension, urinary retention, depression and constipation [16]. One of the most serious adverse effects includes respiratory depression [17]. Another alkaloid thebaine is also produced which is non-narcotic in nature can also be used as an analgesic. It is used for the production of oxycodone and other semi-synthetic analgesic opiates [18, 19]. Higher doses of thebaine cause convulsions similar to that of strychnine poisoning [20]. Another important constituent in opium latex is noscapine which is used in relieving cough and headache. Researchers are continuously investigating of its use in treatment of several cancers and hypoxic ischemia in stroke patients. In the treatment of cancers, noscapine appears to interfere with the functioning of microtubule and thus in division of cancer cells while in treatment of stroke patients, noscapine seems to block the bradykinine β-2 receptors which help in recovery from the disease. Early studies in the treatment of prostate cancer are very promising [21]. Scientists have found a noticeable decrease in mortality in patients treated with noscapine [22]. Noscapine is non-addictive, widely available, has low incidence of side effect and can be easily administered orally, prompting a huge potential for its use in developing countries. An important member of Benzylisoquinilone group 'Papaverine' is also an important alkaloid produced by opium poppy. Papaverine is used in the treatment of spasms of the gastrointestinal tract, bile ducts and ureter. It is also used as a cerebral and coronary vasodilator in subarachnoid hemorrhage (combined with balloon angioplasty) and coronary artery bypass surgery [21, 23-25]. Papaverine is also used as an erectile dysfunction drug alone or sometimes in combination with phentolamine [26, 27]. During microsurgery, papaverine is used as a smooth muscle relaxant and is directly applied to blood vessels [28, 29]. It is also applied in cryopreservation of blood vessels along with other glycosaminoglycans and protein suspensions [21, 30]. Papaverine also functions as a vasodilator during cryopreservation when used in conjunction with verapamil, phentolamine, nifedipine, tolazoline or nitroprusside [22, 31]. Scientists are continuously investigating for its use as a topical growth factor in tissue expansion with some success [23]. All these effects of papaverine are attributed to its inhibitory effect on phosphodiesterases [32]. Though papaverine has such extra ordinary attributes but has some common side effects which include polymorphic ventricular tachycardia, constipation, increased transaminase levels, increased alkaline phosphatase levels, somnolence and vertigo. The area under poppy cultivation varied according to the total demand of opium put through the United Nation. India is one of the largest producer and exporter of licit opium and produces about half the opium utilized by the world's pharmaceutical industries.

Keeping in mind, the enormous importance of opium poppy among the diverse array of medicinal plants, researchers were encouraged to work for its genetical improvement. Researchers engaged in opium poppy researches are continuously working to develop designer plants having all specific alkaloids in latex in large quantities. Previously, both conventional and molecular approaches have been applied to develop varieties rich in specific alkaloids. This chapter deals a detailed account (in different subheadings) of the conventional breeding techniques applied to upgrade the latex and alkaloid status along with its nutritional content in opium poppy.

4. Breeding objectives

Since, opium poppy is widely and commonly used for dual purpose i.e. food (seed) and pharmaceuticals (alkaloids) so the major emphasis has been given for its genetical upgradation on both these aspects. The different breeding objectives are depicted in following subheadings.

4.1. Breeding for modified opium yield, seed yield and specific alkaloid variety

Due to ever increasing global demand of opium latex raised by the pharmaceutical industries for manufacturing of life saving drugs, scientists/plant breeders took the challenge of developing high opium yielding varieties. However, they have been able to develop several high opium yielding varieties, but yet it is not able to fulfill the pressure created due to enhance global demand raised as a consequence of population growth. At present our scientists have been able to discover more than 80 alkaloids of immense medicinal importance. Despite of their best possible efforts to identify more and more alkaloids, the demand for five major alkaloids i.e. morphine, codeine, thebaine, narcotine and papaverine have elevated due to major application in medical field. The importance of these five major alkaloids has been discussed earlier. Previously, morphine being the main pain killer was in high demand, for which our scientist made great success in development of high morphine containing varieties. But now a days, the demand for specific alkaloids i.e. thebaine, codeine, narcotine and papaverine have arisen due to their specific use in different medical treatments. The scientists are now trying to develop varieties with specific alkaloid in opium latex through conventional and molecular techniques. Opium poppy is a narcotic crop, due to the presence of morphine (narcotic constituent) in major proportion of opium latex. In recent days, scientists are working to develop low morphine or morphine less varieties to check its illegal cultivation. The development of low morphine or morphine less varieties can also help Narcotics Department, as it will not require issuing license for growing opium poppy to the cultivators. Globally, different group of researchers are engaged in this direction using both conventional and molecular approaches.

Poppy seeds having high nutritive values are also in high demand and major emphasis has been given for the development of food grade poppy which can only be possible, if opiumless poppy varieties can be developed. Both conventional and molecular approaches are being applied aiming at this target, fortunately a variety “Sujata” has been developed by Central Institute of Medicinal and Aromatic Plants, Lucknow [33]. The development of such varieties can assist opium cultivators to grow food grade poppy without any restriction or permission in form of license. Seeds of opium poppy have high value in global market which puts a great pressure on plant breeders to develop high seed yielding varieties that can substantiate the ever increasing global demands. The importance of poppy seeds has been described earlier in details. However, many high seed yielding varieties have been developed but since global population is increasing at an enormous rate, plant breeders are continuously putting their best possible efforts to capture this ever increasing demand.

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4.2. Breeding for disease resistant variety, causal organism and their management

Diseases are major problem in cultivation of any crop. The development of multiple disease resistant varieties is in need from very long time in opium poppy. A number of diseases occur which ruins the entire crop and ultimately the opium products. Several researchers especially plant breeders have faced many challenges during specific breeding objectives due to severe disease in opium poppy. Our scientists have put their best possible efforts and continuously trying to develop such varieties resistant to major diseases through molecular and conventional tools. One of the major hindrances in any successful breeding program is the prevalence of certain fungal, bacterial, insect borne diseases etc., which cause an unexpected loss in terms of productivity. Opium poppy crop is highly susceptible to certain diseases but the most contagious diseases are caused by fungus results high losses in yield.

Some commonly found fungal, bacterial, viral and pest related diseases in opium poppy are summarized below:-

Downy Mildew: The causal agent for this most serious and widely spread disease of opium poppy is *Peronospora arborescens*. The symptoms include hypertrophy and curvature of the stem and flower stalks. The infection starts spreading upwards from the lower leaves and the entire leaf surface gets covered by brown powder. The plants dies prematurely as the stem, branches and even capsules are also attacked by this causal organism. In India, the disease appears annually on the crop from seedling to maturity stage mainly in the areas of Madhya Pradesh, Uttar Pradesh and Rajasthan. Capsule formation is also adversely affected due to infection causing significant reduction in opium yield. The primary inoculum of the pathogen is oospore which is present in infested soil and leaf debris introgresses through underground plant parts and infects the plant giving rise to stunting and chlorotic syndrome etc in the fields of opium poppy [34]. The major control measures of the disease include disinfection and spraying of the seed beds with 0.5% Bordeaux mixture and different copper containing fungicides. Some other control measures include use of Bisdithane (0.15%) followed by Benlate (0.05%), Gramisan, dusting with Thiram. **Powdery Mildew:** This disease is caused by *Erysiphae polygoni* and causes severe losses in opium production. It caused severe damage to poppy in Rajasthan in 1972. The symptoms appear in late stages of plant growth with white powder on the surface of leaves and capsules. The control measures include field sanitation along with spray of Spersul (0.5%) and seed disinfection. **Collar Rot disease:** This is one of the most severe fungal diseases of opium poppy caused by *Rhizoctonia solani* Kühn. Decline in seed yield, premature death of infected plant appears with the progress of disease in plants [35].

Seed borne diseases: Seed borne diseases are also a curse to opium poppy crop both in terms of production and yield. The major effect of seed borne disease is on capsules and seeds only, which results reduction in germination percentage and seedling delays. Some commonly spreading seed borne diseases have been discussed. **Leaf Blight** (causal agent - *Pleospora calvescens*): Symptoms include defused yellow spots followed by premature drying of infected leaves. During the course of pathogenesis, toxins are released by the parasites enabling it to assimilate the requisite nutrient. High temperature and heavy rainfall favors the disease. **Seedling Blight** (causal agents - *Phytium ultimum* and *Phytium mamimmatum*): Few studies undertaken on characterization of the disease revealed that the disease affects physiological process in poppy. However, no control measures could be found with total control effects. **Leaf Spots** (causal agent - *Helminthosporium sps*.): The main symptoms include dechlorosis of the leaves accompanied by curling. The disease is not of much importance, but due to correlation between opium alkaloids and leaf spot, it may be considered harmful. Several control measures to control the disease include seed disinfection or spraying of seed beds with 0.5% Bordeaux or any other copper fungicides, incorporation of lime as $CaCO_3$ at 285 kg/ha, Systox, Ogranol, borate and manganese superphosphate, germisan, Gramisan and spray of Bavistin. **Wilt & Root Rot** (causal agent - *Fusarium semitectum*): This is another major problem in poppy cultivation where plants in advance stage rapidly wilt due to desiccation. The infection appears at the stem base followed by damping of roots. The disease causes reduction in opium yield and can be controlled only by the removal of infected plants.

Diseases caused by bacteria: It would be worthwhile if there is a lack of description of bacterial disease in opium poppy. Since the bacteria are ubiquitous in nature, opium poppy is also not left by bacterial infection where heavy losses occur. Systemic infection prevails with the entry of bacterium through stomata and aquapores in later stage of growth. Multiplication of the bacterium starts in vascular system. Seeds are malformed and discolored as a result of infection. Plant parts are also damaged due to bacterial infection.

Diseases caused by viruses: There are certain viral diseases in opium poppy which are caused by Cabbage ring spot virus, beat yellow virus etc., which are transmitted through beans, sap, aphids etc. The symptoms include yellowing of plants, elongation of stem, irregular chlorotic bands along the veins, stunting etc. These viral disease cause heavy losses to poppy crop in terms of seed and opium yield and sometimes the whole plant dies results total loss of crop.

Diseases caused by insect and pests: Apart from different diseases caused by fungus, bacteria, viruses etc., some insects are also known to damage poppy crop. The most common among them are those damaging roots i.e. Root Weevil, damaging leaf and stem i.e. Aphids, floral damage i.e. thrips and sawfly, capsule damage by head gall fly, capsule weevil, capsule borer etc. A brief description of these are summarized here. **Root weevil** (causal agent - *Sternocarus fuliginosus*): This pest is known for maximum damage to poppy crop by boring into upper parts of the roots which ultimately turns blackish and leaves wither due to chlorosis while the larva mines the leaf lamina. The control measures include dusting of BHC (12%) along with superphosphate. However, the application of lindane 1.3D @10kg/acre in soil before sowing is beneficial. **Cutworm** (causal agent - *Agrotis spp*.): The larva of this pest is dark brown with red colour head, active at night and remains hidden in cracks in the ground. It mostly targets young plants destroying basal part of the stem while the adult, brown in color and dark color spots on wings also destroys the crop severely. The control measures include hand picking of the caterpillars and spraying of NSKE 5%. Additionally, poison bait with rice bran, jiggery and carbonyl can also be used as preventive measures. **Aphid** (causal agent - *Myzus persicae)*: This is also another major pest of opium poppy crop. The nymphs and adults suck the leaf sap results damage of leaf and consequently whole plant. The adults are yellowish green rarely reddish. The control measures include spray of neem oil 0.5% or NSKE 5%. However, natural enemies like coccinellid beetle can also be encouraged. **Capsule borer** (causal agent - *Helicoverpa armigera)*: The capsule borer is also a serious pest in opium poppy which harms capsule to a maximum extent. It destroys whole capsule eating up the floral head and seeds. The larva is greenish with dark grey lines along the sides of the body. The control measures include hand picking of the larvae along with pheromone traps is recommended while spray of NPV 250 lit/ha is also beneficial. The use of Bt spray formulation @ 2g/2ml per litre of water and use of egg parasitoids *Trichogramma chilonis* @ 5cc/ha is also found effective.

4.3. Breeding for growing conditions:

The opium poppy is an environmental sensitive crop. The temperature, photoperiod, rain, wind etc., majorly affects on its proper growth and ultimately yield. The poppy crop requires a maximum temperature upto 20°C at the time of germination while dry weather at the maturity. The humidity in the air is the major problem which posses maximum damage to crop by insect pests. Most of the fungal diseases also prevail in damp climatic conditions. The rains are also a big problem to poppy as heavy rains enhance the growth of plants and at the time capsules are ready to lance, the crops lay down resulting in heavy opium losses. Rains at the time of lancing also damages the yield as the latex is washed away by the rain water. Mist and frost increases the amount of latex and ease in collection. Since the poppy cultivation requires enormous irrigation, wet soil during the time of sowing can result in low germination percentage. The most preferred soil type for poppy is medium loamy textured sandy loam to loam with good aeration, soil conductivity, well drained and properly ploughed and pulverized. The best time for sowing is the first fortnight of November with temperature ranging from 20 to 25°C. However, the delay in sowing can cause poor germination and growth and hence poor yield. The quantity of seeds required for sowing depends on the mode of sowing with 6-7 kg/ha required for broadcasting and 5-6 kg/ha for row sowing with spacing 25-30cm apart. The plant density of 3.30 lakh plants/ha should be maintained. Recommended cultural practices should be followed for a good stand which include pre sowing addition of farmyard manure @ 10 t/ha, 5-6 t/ha neem cake and 30, 50, 40 kg /ha N, P, and K respectively as basal dressing. The recommended application of 60 kg/ha N in two split after 30 and 60 days after sowing as top dressing and spray of the fungicide diethelene biscarbamate (dithane M-45 0.2%) at 45 and 60 days after sowing [36] should be followed for obtaining maximum returns.

Germination in opium poppy requires optimal soil moisture which ensures good germination percentage. The first irrigation in given 20-25 days after sowing followed by frequent light irrigation at an interval of 15-20 days as the weather conditions prevail. A total of 6-8 irrigation is required for a good stand which includes last irrigation before the start of lancing. Weeding and hoeing are also necessary for providing poppy seedling a better chance to grow. The first weeding is done 20-25 days after sowing followed by 15-20 days interval. The optimum spacing between the plants should be maintained at a distance of 10cm apart. In India, lancing is done by cutting of the superficial layer of the capsule wall from which the latex oozes out. The mature green capsule is lanced with an instrument called "Nastar" having 3-4 small blades designed to ensure uniformity in depth of incisions. Generally 3-4 lancing is done in each capsule with parallel longitudinal cuts which is performed after mid day and allow the latex to remain overnight on the capsules for coagulation. In the following morning the latex is collected from the capsule walls with blunt edge of small iron scoop. The opium is kept in small plastic box or earthen pot or copper bowls. The latex colour varies from dark to light brown to pink based on the variety. The depth of incision should not be more than 1.2 mm. After collection of opium, lanced capsules are left to dry over plants for next 15-20 days for harvesting of seeds.

5. Conventional breeding strategies applied for genetic upgradation of opium poppy

The conventional breeding approaches are a step by step procedure to develop desired plant type. The important steps involved in opium poppy breeding program are described in following subheadings:-

5.1. Plant Introduction

Conventional plant breeding programs require distinct plant genotypes with specific characteristics to initiate any hybridization technique. The distinctness in the base material ensures higher percentage of success through breeding programme. The collection of diverse germplasm from different geographical regions can be the best approach for initiation of any breeding programme with specific objectives. The foremost step to initiate any crop breeding program is plant introduction. The procedure of growing a variety or a species into an area where it has not been grown earlier is termed as Plant Introduction. However, bringing plant material from one environmental condition to another within a country or continents is also called as plant introduction. Plant introduction and germplasm collection thus becomes one of the richest sources of creation of variability [37, 38].

In India, researches on opium poppy are confined at some agricultural and scientific institutes viz., Central Institute of Medicinal and Aromatic Plants, Lucknow, National Botanical Research Institute, Lucknow, Jahawarlal Nehru Krishi Vishwavidalaya-College of Agriculture, Jabalpur, Narendra Dev University of Agriculture and Technology, Faizabad, National Bureau for Plant Genetic Resources, New Delhi, Rajasthan Agricultural University, Udaipur. These centers have been working on genetic upgradation of opium poppy for the last four to five decades. Khanna and Singh [39] bought 190 strains from Russia, Hungary, Poland, U.K. and other temperate countries and evaluated these strains at NBRI, Lucknow. They noticed that most of the cultivars belonging to European countries require long photoperiod, hence were unsuitable in Indian climatic conditions. However, the cultivars of Iran were only possible to cultivate in India by introduction. Similarly, Prajapati et al. [40] screened capsule husk of a set of 115 Indian land races of opium poppy (*Papaver somniferum* L.) for papaverine, reticuline, narcotine, thebaine, codeinone, codeine, morphine and oripavine at CIMAP, Lucknow. These germplasms were grouped into four clusters on the basis of alkaloid profile. Based on the study of alkaloid profiles of these germplasm and correlations between alkaloids in all the four groups of accessions, they concluded that in Indian genetic resources of *P. somniferum* (a) morphine is synthesized from codeine rather than oripavine, (b) net alkaloid content was low under narcotine deficiency, and (c) accumulation of morphine and codeine was in limited upstream of codeinone and morphinone. It was also depicted from their study that the accessions identified based on alkaloid profiles, harboring genetic blocks in phenanthrene and benzylisoquinoline biosynthetic pathways can be useful for understanding the genetic control of secondary metabolism in opium poppy.

In continuation of plant introduction, Shukla et al. [41] studied alkaloid spectrum in 1470 individual plants belonging to 98 germplasm which has been collected from different sources and maintained at NBRI, Lucknow for several years. Based on alkaloid profiles, the content of different alkaloids were categorized into class interval exhibiting maximum number of plants and accessions for morphine fall in group of 10–15% followed by 15–20%, for codeine in group of 2–4% followed by 4–6%, for thebaine in 1–2% followed by 2–4%, for narcotine in 5–10% followed by 10–15% and for papaverine content 0–2%, while 24 germplasm lines had morphine content above 16.0%. Based on distinctness in morphological and agronomical characteristics, 1,000 distinct poppy germplasm lines were provided by Agriculture faculty, Ankara University from which 99 poppy lines were evaluated in terms of alkaloid analysis in *in vitro* [42]. They observed the range of different alkaloids in poppy husk (CPS) viz., morphine, thebaine, codeine, papaverine and noscapine from 0.110 to 1.140%, 0.005 to 0.134%, 0.005 to 0.27%, 0.001 to 0.440% and 0.006 to 0.418%, respectively. Dittbrenner et al. [43] evaluated 300 accessions of opium poppy for 35 morphological and agronomic traits collected from all over the world at IPK Gene Bank, Gatersleben, Germany. Based on their study on five major alkaloids taken for two years, they concluded highly significant correlation between total alkaloid content and morphine. However, four other major alkaloids i.e. codeine, thebaine, noscapine and papaverine did not show any correlation between them or with total alkaloid content. Additionally they also noticed that there is no important correlation between morphological traits and alkaloid content. They also determined the chromosome number in each accession and found that the subspecies *setigerum* was natural tetraploid while the rest of the subspecies were diploid. They finally concluded that none of the studied morphological traits could be used for prediction of alkaloid content which may give erroneous information in breeding programmes.

6. Diversity analysis through conventional tools

One of the foremost steps in the genetical improvement of any crop through conventional breeding program is to study the genetic diversity available in the introduced plant/crop material. To conduct any breeding program judiciously, diversity analysis based on morphological and biochemical traits is prerequisite. In opium poppy, several of the exotic collections at different research institutes have been evaluated for genetic diversity. Few studies on genetic diversity undertaken so far in opium poppy are summarized here. Singh et al. [44] studied genetic divergence using 101 germplasm lines of different ecogeographical origin for seed and opium yield per plant and its 8 component traits following multivariate and canonical analysis. They grouped the germplasm into 13 clusters on the basis of multivariate analysis which was also confirmed by canonical analysis. 68% genotypes were found genetically close to each other and grouped in 6 clusters while apparent diversity was noticed for 32 percent of the genotypes who diverged into rest 7 clusters. They concluded that the genotypes in clusters IX, X, XI and XII had greater potential as breeding stock by virtue of high mean values of one or more component characters and high statistical distances among them. Yadav et al. [45] made an effort to study the genetic divergence in a genetically distinct new stock of opium poppy using cluster and principle component analysis. They found that a large amount of variability exists among the accessions and formed 8 clusters from which some accessions were recommended which can be used in hybridization programme to get desirable transgressive segregants. Similarly, Yadav et al. [46] assessed genetic divergence in 110 population (20 parents and 90 F_1 hybrids) using multivariate analysis. All the entries were grouped into 14 clusters which indicated substantial diversity among parental genotypes which had potential to release considerable variation in their crosses. Similarly, Brezinova et al. [47] evaluated 404 genotypes of poppy from world collection to assess genetic diversity over the selected traits based on their morphological characteristic to create a digitalized visual documentation. On the basis of morphometric analysis, the important diversity in observed traits were recognized in agro-climatic conditions of Slovakia, documented by statistical characteristics and by digitalized documentation of accessions. Diversity based on alkaloid spectrum in 122 accessions of indigenous opium poppy was undertaken by Shukla et al. [48]. They obtained 11 clusters based on extent of correlation between five major alkaloids i.e. morphine, codeine, thebaine, narcotine and papaverine. Mostly the clusters comprised of accessions with different possible combinations of alkaloids comprising high in one alkaloid with high or low of another. Generally the percentage of morphine content was higher than the sum of four other alkaloids except in one cluster where narcotine content was slightly higher than morphine. Based on their study they concluded that successful breeding for specific alkaloids or a combination of alkaloids could be achieved by using these accessions in hybridization programme.

7. Creation of variability through hybridization

A breeding programme focused to develop improved varieties requires knowledge about the genetic variability that exists for the concern trait. It is documented that sufficient variation for composition and content of secondary metabolites occurs in a number of medicinal plant. Several studies have been carried out in opium poppy to study the existing variability in different set of materials which showed varying results for composition of secondary metabolites and other chemical compounds along with morphological variations. Singh et al. [49] found that F_8 genotypes obtained through interspecific cross between *Papaver somniferum* and *Papaver setigerum* had higher oil (>40%) and fatty acid concentration than respective parental species. They also obtained varying results for linoleic (68%-74.4%) and oleic acid

(13.6%-20.3%) content in F_8 genotypes. High oleic desaturation ratio and C18 polyunsaturated fatty acid with very low linolenic (18:3) acid (0.37%) indicated the possibility of using poppy oil for edible purposes. However, oleic (18:1) acid was not correlated with other fatty acids, except for significant negative correlation with linoleic (C18:2) acid. Ozturk and Gunlu [50] conducted correlation and path coefficient analysis for qualitative and quantitative traits in four poppy cultivars in Central Anatolia. They found statistically significant differences for all the studied traits among all the four genotypes. Positive and significant correlation of morphine yield with morphine content, seed yield, capsule yield, oil yield; capsule yield with oil yield; seed yield with capsule yield, oil yield were noticed. Through path analysis, it was noticed that morphine content, capsule yield, seed yield and oil yield had positive direct effect on morphine yield. Yadav et al. [51] analyzed F_1 and F_2 generations of a twenty parents fractional diallel cross in opium poppy (*P. somniferum* L.) to estimate the combining ability of the crosses based on ten quantitative and five qualitative (alkaloids) traits. The results indicated that significant differences exists among the parents for all the traits and GCA (General Combining Ability) and SCA (Specific Combining Ability) components of variances were also significant for all the traits. However, SCA component of variance (σ^2s) was predominant which indicated the preponderance of non-additive gene effect for all the traits except for leaves/plant and papaverine in F_1 hybrids. The average degree of dominance (σ^2s/ σ^2g) was more than unity indicated over dominance and also confirmed the non-additive mode of gene action. They suggested that the inclusion of good general combiners in a multiple crossing program or an intermating among the population involving all possible crosses subjected to biparental mating can be expected to offer maximum promise in breeding for higher opium and seed yield and alkaloid content. In an another study, Yadav et al. [52] examined combining ability for yield and its component traits along with morphine content to elucidate the inheritance pattern governing these traits and also to identify potential genotypes which could be further exploited in breeding programmes. They noticed that most of the traits were governed by non-additive gene action while additive gene action was also important for some other traits. They found three best parents viz., BR-232, BR-245 and BR-234 as good general combiners which could be used in hybridization programme aiming at maximum gain. Similarly, Kumar and Patra [53] also studied inheritance pattern for quantitative traits in four single crosses in opium poppy. They found that simple additive, dominance and epistatic genetic components were significant for inheritance of the traits under study. They also noticed differential gene actions with differential magnitude for different traits and concluded that following biparental mating followed by recurrent selection for desired recombinants may be utilized for genetic upgradation of opium poppy crop. Mishra et al. [54] evaluated progenies of randomly selected individuals from 14 promising hybrids over F_2 to F_6 generations for opium and seed yield and their contributing traits for the formulation of effective selection strategy in opium poppy (*P. somniferum* L.). They observed that in general heritability and genetic gain declined from generation to generation. They obtained a cross MOP541 x BR241 which showed similar pattern for genetic gain in all the traits. The values of broad sense heritability decreased from F_2 to F_6 generation for most of the traits. Matyasova et al. [55] evaluated 57 cultivars of opium poppy comparing the groups of values representing the indicators of production-significant morphologic and agricultural traits and morphine content in husk in relation to ideotype, which in these indicators represents 100% of the value. They observed lower values of morphine in husk of white coloured seeds while high morphine in blue to grey seeds. They observed that these cultivars achieved very good values in the morphological indicators and average value in the economic indicators. Based on their results they concluded that these results will be used in selection and classification of suitable genetic resources of poppy as industrial forms. Nemeth-Zambori et al. [56] conducted a hybridization experiment between five parents with different chemotypes namely Minoan, Medea, Korona, Przemko and Kozmosz and studied the alkaloid profile for F_1 to F_3 generations. They observed that in some cross combinations with high alkaloid containing parents, the content of total alkaloid, morphine and thebaine showed significant increase in hybrid generation which persisted upto F_3 generation. However, the concentration of narcotine was lesser than mid parent value and also showed decreasing trend over generations. As a matter of fact, homogenous strains started to accumulate at F_3 generation. In contrast to the high alkaloid parents, the cross combinations with low alkaloid parents exhibited considerable heterosis for total alkaloid content in F_1 while low alkaloid containing recessive individuals segregated in F_2 and stabilized in F_3 generation. They finally concluded that their experiment reflected well with the effects of genetic regulation at three levels of enzymatic processes during the alkaloid biosynthesis. The morphinans and narcotine was controlled by complex polygenic effects so, the selection for fixing of very low content of narcotine may be effective in early F_2 generation as narcotine was found lesser than mid parent value. However, selection for morphinane alkaloids which are in major proportion is not worthy before F_3 generation. Yadav et al. [57] investigated inheritance pattern for different quantitative traits through generation mean analysis using five parameter model on five cross combinations with five generations i.e. parents, F_1s, F_2s, and F_3s selected from an extensive hybridization programme carried out in partial mating design. They found that additive x additive and dominance x dominance was higher in magnitude than combined main effect of additive and dominance effect for all the traits in all five crosses. However, dominance x dominance effect was predominant over additive x additive for all the traits except for few. They also observed substantial amount of realized heterosis, residual heterosis and high broad sense heritability with moderate genetic advance and significant correlation among important traits in positive direction. Based on their study they finally advised selective diallel mating and biparental mating in early generations followed by recurrent selection which can be used for genetic upgradation of opium poppy. Kumar and Patra [58] undertook a study to understand the gene action involved in the inheritance of opium yield and its component traits (plant height, leaves per plant, peduncle length, capsule index, seed and straw yield per plant and morphine content) in two families viz., VG26 x VG20 and SG35II x VE01 of opium poppy. They found significant additive, dominance and epistatic genetic components for the inheritance of different traits and concluded that biparental mating followed by recurrent selection involving desired recombinants may be utilized for genetic upgradation of opium poppy through components traits.

8. Screening and evaluation for oil and fatty acids

One of the important aspects of breeding programmes is selection which is based on several factors and requires experience and command to observe. Selection can be based on maturity period, disease resistance, lodging, withering and yield etc. Investigation about oil yield, fatty acid compositions and total protein content of three varieties of Turkish poppy were done by Azcan et al. [59] who found that solvent extraction of yellow seed gave highest oil yield upto 49.2%, while white seed had 36.8% and blue seed 33.6% which was considerably low. Fatty acid compositions of oils were determined by GC/MS in which major components were of linoleic (56.4–69.2%), oleic (16.1–19.4%), and palmitic (10.6–16.3%) acid depending on the color of the seeds. Similar investigation on volatile compounds of several seed oil samples from *Papaver somniferum* L. using solid phase micro extraction (SPME) with DVB/Carboxen/PDMS Stable-Flex fiber was done by Krist et al. [60]. They identified 1-Pentanol (3.3-4.9%), 1-hexanal (10.9-30.9%), 1-hexanol (5.3-33.7%), 2-pentylfuran (7.2-10.0%), and caproic acid (2.9-11.5%) as the main volatile compounds in all examined poppy seed oil samples. Furthermore, the TAG (Triglyceride) composition of these oils was analyzed by MALDI-TOF and ESI-IT-MS/MS. The predominant TAG components were found to be composed of linoleic, oleic and palmitic acid, comprising 70% of the oil. Similarly, Ozcan and Atalay [61] investigated physical and chemical properties of seven poppy varieties. Weight of 1000 seeds, moisture, crude protein, crude ash, crude fibre, HCl-insoluble ash, crude energy and crude oil content of all seven varieties of poppy seeds were 0.29-0.429 g, 3.39-4.76 %, 11.94-13.58 %, 4.92-6.25 %, 22.63-30.08 %, 0.72-1.68 %, 6367.0-6740.5 kcal/100g and 32.43-45.52 % respectively. The poppy seed oil contained an appreciable amount of beta-tocopherol (309.5 ppm-567.3 ppm). Poppy seed oil also contained stearic, palmitic, oleic, linoleic and linolenic acid as the main constituent of fatty acids. Linoleic acid was established as the dominant fatty acid in all varieties. Similar investigation were also done by Hakan et al. [62] who investigated fatty acid, tocopherol and sterol content of the oil of several poppy seeds. They found that the main fatty acids in poppy seed oil were linoleic, oleic and palmitic acid while oil contained an appreciable amount of gamma-tocopherol and alpha-tocopherol. The concentrations of total sterol ranged from 1099.84 mg kg^{-1} to 4816.10 mg kg^{-1}. The major sterols were beta-sitosterol ranged from 663.91 to 3244.39 mg kg^{-1}; campesterol ranged from 228.59 to 736.50 mg kg^{-1} and delta (5)-avenasterol ranged from 103.90 to 425.02 mg kg^{-1}.

9. Stability analysis for identification of stable and adaptable varieties

The analysis of genotype x environmental interaction, which indicates the stability of genotypes has always been part of plant breeding programmes before release of any variety for commercial cultivation. To study the GxE interaction, several methods have been proposed to analyze it i.e. univariate methods such as Francis and Kannenberg's coefficient of variability [63], Plaisted and Peterson's mean variance component for pair-wise GE interactions [64], Wricke's ecovalence [65], Shukla's stability variance [66], Finlay and Wilkinson's regression coefficient [67], Perkins and Jinks's regression coefficient [68] and Eberhart and Russell's sum of square deviations from regression [69]. Simultaneously, two other stability models based on graphical representation of the genotypes in different environments are available i.e. Yan's GGE Biplot model and AMMI model. Yadav et al. [70] investigated stability for seed yield, opium yield and morphine content in 11 advanced breeding lines over five years in opium poppy. Combined ANOVA showed that both main effects and interactions were significant, indicating the presence of genotype x environment interactions. Yadav et al. [19, 36] studied phenotypic and genotypic variability, broad sense heritability, genetic advance under selection and interrelationship of traits in 74 and 122 accessions of opium poppy respectively. They found high variations among the accessions along with broad sense heritability and genetic advance. Genetic correlation analysis revealed negative correlation between opium yield and morphine and papaverine content while other alkaloids showed positive correlation. The 11 genotypes of opium poppy were evaluated on the basis of non-parametric model by Yadav et al. [71] for opium yield and morphine content over 5 environments to identify stable and promising genotypes which can sustain adverse environmental conditions. Several of the evaluated genotypes were found to be stable in all the environmental conditions and were stable for both the traits i.e. opium yield and morphine content. Yadav et al. [72] evaluated 22 strains of opium poppy to find out variability and suitable selection indices for opium and seed yield. The discriminant functions based on single character were less efficient while on the basis of combination it was in general more efficient. The comparison of different functions revealed that capsule weight/plant, capsule length, plant height were major yield components and thus practicing selection for attainment of high opium and seed yielding lines, maximum weightage should be given to these characters. The positive association of opium and seed yield suggested that by adopting suitable component breeding and selection, a dual-purpose variety (opium and seed yield) can be developed. Singh et al. [44] investigated the extent of genetic variability, heritability, correlation and path analysis for opium yield, seed yield and eight component traits in a group of 101 germplasm lines of different ecogeographical origins. They noticed high heritability coupled with high genetic advance and coefficient of variability for most of the traits. Path coefficient analysis indicated that capsule per plant had high direct path towards opium yield followed by four other traits.

10. Mutation breeding approaches

Besides, different hybridization programs, mutation breeding program was also flourished and encouraging results were obtained all over the world. An era of mutation breeding came into existence due to significant achievements obtained in many crops of pharmaceutical, industrial and food interest. In opium poppy also scientists obtained fascinating results. A mutation breeding experiment was carried out using physical and chemical mutagens to develop non-narcotic opium poppy from narcotic crop [73]. They isolated two families containing twenty latex less/opium-less and twelve 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 opiumless 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 proteinacious seeds along with healthy unsaturated seed oil. Similarly Chatterjee et al. [74] studied induced mutation through gamma rays, EMS and their combined doses in two varieties of opium poppy (NBRI-1 and NBRI-5) to create new genetic variability for isolation of high yielding genotypes along with specific alkaloids. The genetic coefficient of variability (GCV), heritability and genetic advance was noticed higher for opium and seed yield and capsule weight for all the doses in both the varieties with some exception. They finally concluded that the criteria for selection of plants should be based on capsule weight and capsule number which can provide ideal plant type with enhanced yield potential. Chatterjee et al. [75] also found a variant plant of opium poppy (*Papaver somniferum* L.) having high thebaine content. The M_2 seeds of variant plant were subjected to *in vitro* studies to investigate the prospects of thebaine production through tissue culture. Consequently, alkaloid profile of variants showed higher thebaine in stem followed by leaf callus, stem callus and cotyledons. From the same mutation breeding experiment Chatterjee et al. [76] made an effort of identify appropriate dose of the mutagens for the enhancement of specific alkaloid especially thebaine and also studied correlation between cytological aberrations and their effects on alkaloid quantity in two stable high yielding varieties of opium poppy i.e. NBRI-1 and NBRI-5. They found that NBRI-1 was more sensitive than NBRI-5 and that the mutagen EMS was most potent in creating chromosomal abnormalities. They concluded that two doses i.e. kR 10 + 0.2% EMS and 0.2% EMS was most effective for getting fruitful results. The dose 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. In continuation of their study Chatterjee et al. [77] also tried to broaden the genetic variability and to evaluate the advance generations for different agronomic and chemotypic traits in the experimental high yielding varieties i.e. NBRI-1 and NBRI-5 through induced mutations. Here, they noticed that 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. They further concluded that their study confirmed that the morphinan and phthalideisoquinilone pathway bifurcated at lower combined doses i.e. kR30 and kR10 + 0.4% EMS which was effective in causing micromutation in morphinan and phthalideisoquinilone pathways 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 [84]. This mutant was developed by a mutagen treatment to seeds of commercial poppy cultivar (*P. somniferum*). Phenotypically the mutation is visible in the form of pigmented latex than normal white. In TOP 1 mutant, one possibility is that the gene responsible for an enzyme 6-O demethylase which act on thebaine and oripavine might be affected at its transcriptional level or modified protein structure. It may be possible that there is an alteration occurs in transport component that blocks the entry of substrates (thebaine and oripavine) of the enzyme to the subcellular compartment for 6-O demethylation. These mutant plants are very important since the production of thebaine is only amenable which can help in checking of drug trafficking. However, identification of the candidate genes which has been blocked can be identified and characterized. The complex mechanism involved in morphinane biosynthesis can also be elucidated. These morphine free plants can be beneficial for the treatment of opioid addiction. But there is a slight risk with this mutant for licit to illicit uses (by conversion of non-narcotic alkaloids to narcotic alkaloids). The Tasmania drug industry has been using TOP 1 mutants since 1998 for production of various analgesic drugs viz. buprenorphine, oxycodone, naloxone and naltrexone.

11. Polyploidy approaches

The event of polyploidization has been observed long back as most of our cultivated and wild species are polyploids as a result of diploidization or cross pollination among various ploidy levels. This has been an important aspect in conventional breeding programs. Basically, polyploidy is of two types i.e. auto- and allo- ploidy, wherein the auto polyploids arises due to duplication in same genomic content in a species whereas in case of allopolyploidy, there is chromosome complementation i.e. two different chromosomal content from different species combine to form allopolyploid. Few studies on polyploidization in opium poppy have been undertaken so far. Polyploids are beneficial in many aspects viz., organism can resort to higher number of genes and higher number of allelic variants which may lead to substantial increase in the ultimate product. One of the recent study was undertaken on ploidy aspect of opium poppy [78]. They aimed to understand the phenotypic, genetic and genomic consequences of induced polyploidy and to enhance total alkaloid content along with specific alkaloid using colchicine. They observed that 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 autotetraploids 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.

12. Other conventional approaches

Apart from different conventional breeding strategies applied for genetic upgradation of opium poppy, several researchers with similar aim carried out several studies in opium poppy. A unique study was carried out on honey bees foraging on plant flowers [79]. They noticed significantly higher foraging response of honeybees (*Apis mellifera*) manifesting honeybee's preference towards specific plant morphotypes in genetically divergent plant of opium poppy (*Papaver somniferum*). Furthermore the genotype specific for foraging response of honeybees could be attributed to physico-chemical properties of opium poppy flowers. This could have implications for the development of opium alkaloid fortified honey for novel pharmaceuticals and isolation of natural spray compounds to attract honeybee pollinators for promoting crossing and sustainable hybridity in crops. Since the seed of opium poppy is widely used as food in almost all parts of the world, several researchers tried different ways to develop plants producing nutritionally rich seeds. Losak and Richter [80] studied the effect of nitrogen supplementation to cultivar 'Opal' of opium poppy plant in a pot experiment. They applied ammonium nitrate in single dose at two stages of plant growth i.e. at the beginning of growing season and at the stage of flowering. They observed that the increasing dose of nitrogen increased number of capsules per plant, morphine content and the capsule volume irregularly. However, an optimum dose of nitrogen i.e. 0.9 g N/pot showed statistically significant positive effect on seed yield. The effect of varying concentration of CO_2 (300, 400, 500, 600 μ mol mol^{-1}) was examined on various morphological traits such as number of capsules, capsule weight and latex-yield in *Papaver setigerum*. A significant positive effect of increasing CO_2 concentration on various morphological traits was noticed with an increase of 3.6, 3.0 and 3.7 times, respectively on per plant basis. Significant and positive response of secondary metabolites especially morphine, codeine, narcotine and papaverine was also noticed to CO_2 enrichment. However, the major alkaloid i.e. morphine was significantly increased by 10.4, 11.7, 12.9 and 12.4%, respectively at each dose (300, 400, 500 and 600 μmol mol^{-1}) of CO_2 [81]. Szabo et al. [82] investigated the effects of water stress on the alkaloid production and content at three different developmental stages i.e. Rosette, Flowering and Lancing in opium poppy. They used four types of water conditions i.e. control, withdrawal, 50% water supply and inundation and found that leaves responded significantly to water stress conditions. They further concluded that constant water supply is beneficial for the accumulation of alkaloids in poppy capsules. In many parts of the world, seeds of opium poppy are widely used as food and efforts are continuously made to develop nutritionally rich poppy seeds. In Central European countries, the content of selenium is very low in poppy seeds. Hence, with the aim of supplementing opium poppy plants with selenium (a trace element), Skarpa and Richter [83] tried to explore the effect of foliar application of this element on seed yield, selenium content in seeds and its uptake by the roots. They applied a single dose of selenium of 300 g/ha at two different stages i.e. during the stage of the end of elongation growth and after the fall of blossoms. They found that seed yield was reduced by 11.5% and 11.8% after both stages of application respectively but the content of selenium increased significantly from 139 μg/kg to 757 μg/kg of seeds. However, the uptake of selenium also increased significantly upto 4.8 times.

Since for the last few decades, scientific researchers have been continuously contributing for the genetic upgradation of opium poppy through various approaches including conventional breeding methodologies, mutation breeding and molecular techniques with breeding and it is a matter of high enthusiasm for the development of varieties, hybrids, synthetics and GMO in opium poppy till date. At present few varieties have been developed through conventional approaches that can be grouped as-

12.1. Varieties developed by National Botanical Research Institute, Lucknow

In due course of time many breeding approaches have been applied in opium poppy for the development of new high yielding and disease resistant varieties. These varieties are now stabilized and suited for different agro-climatic conditions. A brief characteristic description of the varieties are given below -

- **BROP-1**:- In this variety the plants are medium sized having 3-4 capsules/plant and capsules are of three types viz. oily, parrot coloured and black peduncle. Flowers are white. It is a synthetic variety stabilized after hybridization/intermating between three high yielding cultivars viz. kali dandi (black peduncle), suga pankhi (parrot color) and sufaid dandi (white peduncle) followed by selection. The average opium yield, seed yield and morphine content are up to 54kg/ha, 1000-1200kg/ha and 13% respectively. Geographically it can be cultivated mainly in Northern Indian plains.

- **NBRI-1**:- This variety is developed through selection. The plants are medium tall having large fringed leaves and white flowers. The average opium yield, seed yield and morphine content are up to 52kg/ha, 1000kg/ha and 12-13% respectively. Geographically it can be cultivated mainly in Northern Central India.

- **NBRI-2**:- This variety has intermediate tall plants, thick stem, broad leaves, long peduncle with big capsules and flowers are white. This variety is also developed through selection amongst local collection having above characters. Average opium yield, seed yield and morphine content are up to 52 kg/ha, 1200kg/ha and 15%respectively. Northern Central part of India is recommended for its cultivation.

- **NBRI-6**:- In this variety, plants are medium tall with narrow leaves and white flowers. It is developed by hybridization between two germplasm lines BR007 and BR008 (BR007 x BR008) followed by rigorous selection generation after generation up to eight generations. Average opium yield, seed yield and morphine content are up to 55kg/ha, 1200kg/ha and 13-14% respectively. Geographically Northern Indian plains are mainly recommended for its cultivation.

- **NBRI-9**:- In this variety, plants are intermediate sized with white flowers and large capsules. The variety is high yielding (seed yield) and is developed by hybridization between germplasm lines S-10 x S-18 followed by rigorous selection until the variety is stabilized (up to eight generations). It's average opium yield, seed yield and morphine content is upto 52kg/ha, 1400kg/ha and 12% respectively. For the cultivation of the variety, Northern Indian Plains are recommended as most suitable.

- **NBRI-10**:- The plants in this variety are medium tall, having dark green leaves and white flowers. The development of variety was done through hybridization germplasm lines (IC-30 x S-10) followed by rigorous selection up to eight generations. Average opium yield, seed yield and morphine content are up to 50kg/ha, 1200kg/ha and 12% respectively. Geographically Northern Central plains are recommended for its cultivation.

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- **Madakini**:- It is a high yielding variety for opium poppy, have multiple disease resistance and is granted US patent no.7,442,854B2 in 2009. The variety is developed by hybridization germplasm lines (BR007 x BR008) followed by rigorous selection. Plants of the variety are vigorous having dark green leaves, white flowers with blackish flowering stalk at the bottom of capsule at maturity. Average opium yield, seed yield and morphine content are up to 64kg/ha, 1200kg/ha and 15% respectively. Northern Central India is recommended for its cultivation (Figure 3).

- **High thebaine lines**:- As we know that thebaine is a non-narcotic alkaloid and can be used in making pain killing drugs. Thus for fulfilling the increasing worldwide demand of thebaine, with the help of interspecific hybridization (*P. somniferum* x *P. setigerum*) and mutation breeding experiments NBRI has succeeded in the development of few stable high thebaine lines. Thebaine content in these lines ranges 8-10% which is much higher than pre-existing varieties and germplasm (Figure 4).

Figure 3. Field view of developed high yielding variety "Madakini".

12.2. Varieties developed by Central Institute of Medicinal and Aromatic Plants, Lucknow

- **Rakshit**:-It is a disease resistant and morphine rich variety in CPS (concentrated poppy straw). The plants are 106-112 cm tall with 20-26 cm long green peduncle and oblong capsules with waxy surface. The variety is developed by hybridization and selection generation after generation up to eight generations. Average seed yield and straw yield of the variety are up to 1200-1400kg/ha and 900-1100kg/ha respectively.

- **Sanchita**:- In this high yielding variety, plants are 107 cm tall and have 2-3 capsules/plant. The average seed yield and straw yield are 840kg/ha and 640kg/ha respectively. Morphine content of this variety is very low in CPS (approx. 0.74%).

- **Vivek**:- The plants of this variety are 112 cm tall with 2-3 capsules/plant. It is also a high yielding variety having seed yield and straw yield up to 840kg/ha and 760kg/ha respectively. Morphine content of this variety is also very low in husk (approx. 0.73%).

- **Sweta**:- It is high yielding variety with 66.5kg/ha opium yield and about 18% morphine content in latex.

- **Subhra**:- In this variety plants are medium sized having 3-4 capsules/plant. The average seed yield and husk yield of the variety are approx. 910kg/ha and 790kg/ha respectively. The morphine percentage in husk is approx. 0.77%.

- **Shyama**:- In this variety plants are 105 cm tall with black peduncle and also has erect incised leaves. It is a high yielding variety having seed yield, husk yield and morphine content up to 720kg/ha, 650kg/ha and 0.75% respectively.

- **Sujata**:- In this variety plants are 80-100 cm tall having 3-4 flat glabrous capsules with 18-20 cm long erratic black peduncle. It is an opium less, alkaloid less and non narcotic variety.

Figure 4. Field view of developed high thebaine lines.

Narendra Dev University of Agriculture and Technology, Faizabad has developed a downy mildew resistant variety by selection and named Kirtiman (NOP-4). The plants of this variety are quite tall having white flowers and 1-2 oval capsules. Opium yield, seed yield and morphine content of the variety ranges 35-46kg/ha, 900-1100kg/ha and up to 12% respectively. Eastern U.P. region is best suited for its cultivation.

National Bureau of Plant Genetic Resources, New Delhi developed a variety Trishna (IC 42) for resistance to frost, root rot and downy mildew through inbreeding and selection. The plants of the variety are tall with 5-7 capsules/plant and pink flowers. Opium yield, seed yield and morphine content ranges up to 49-53kg/ha, 1000kg/ha and 12-14.78% respectively.

Rajasthan Agricultural University, Udaipur has developed a resistant variety to disease and lodging and named it as **Chetak (UO 285)**. The plants of this variety are average tall with big capsules. Flowers are white with smooth petals. Opium yield, seed yield and morphine content ranges up to 54kg/ha, 1000-1200kg/ha and 12% respectively. Geographically Rajasthan is most suitable for its cultivation.

Jawaharlal Nehru Krishi Vishwavidalaya, College of Agriculture, Jabalpur (M.P.) has also succeeded in developing a downy mildew resistant variety by pure line selection and named as Jawahar Aphim 16 (JA-16). Plants of this variety are tall having white flowers and 1-3 big capsules/plant. The variety has opium yield, seed yield and morphine content up to 45-54kg/ha, 900-1000kg/ha and 12% respectively. Madhya Pradesh is geographically recommended for its cultivation.

13. Future prospects

The medicinal uses of opium poppy are innumerable and also its value as food grade crop is significant. The genetic upgradation process in opium poppy cannot be ended until and unless it is able to meet the ever increasing global demand for opium alkaloids and nutritious seeds. The genetic upgradation process needs to be continued for the development of varieties rich in total alkaloid content. The conservation of germplasm and creation of genetic variability through the intervention of conventional, mutational, polyploidy and molecular approaches is essential to carry forward future breeding programmes aiming to develop designer plants in opium poppy. At present the indigenous poppy germplasm has very narrow genetic base, we need to concentrate on broadening of its genetic base through the intervention of above strategies. The prospects of mutation breeding and interspecific hybridization has proved useful in creation of genetic variability and development of varieties rich in specific alkaloid with high yield needs further efforts to enhance the total alkaloid content.

The opium poppy crop is highly sensitive to several diseases caused by biological agents. The development of multiple disease resistant varieties is another major challenge in opium poppy. Very few studies have been done on this aspect, so, further studies are required to develop resistant varieties in poppy against fungus, bacteria, viruses, insects, pests etc, which causes severe damage to the crop. The genes responsible for disease resistant can be identified and characterized through molecular techniques, so, efforts should be made in the direction of developing disease resistant transgenic plants, from which the candidate gene could be transferred through back crossing program into our high yielding varieties.

Another important aspect is that the opium poppy is highly sensitive to varied environmental conditions. Although a number of high yielding varieties have been developed, but the development of photoperiod insensitive, stable and adaptable varieties for different climatic conditions are still required. This can be achieved by transferring the genes of interest from the cultivars of different countries into our indigenous varieties in green houses. However, the development of morphine less or opium less varieties, which can check drug trafficking and allows the farmers to grow poppy without any restriction or necessity of obtaining license, is still a challenging task. Different molecular techniques such as virus induced gene silencing, RNA interference (RNAi) technology etc., can help in the development of opium less and morphine-less varieties. Till to date, 17 genes have been identified and characterized involved in alkaloid biosynthesis, but the genes involved in other benzylisoquinoline and pthalideisoquinoline pathways are still unknown. So, the efforts should be done to explore all the genes involved in alkaloid biosynthesis which may help in development of desired designer plants in opium poppy.

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