

## INTRODUCTION

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Ever since ancient times, the drugs derived from the plant kingdom have been used to alleviate or cure human diseases. The indigenous system of medicine is gradually gaining popularity mainly because of less or no toxic or side effects of herbal drugs. Simultaneously, there is growing interest among researchers for traditional remedies used by ancient tribes and old civilizations. It is believed that investigation of such ancient drugs on scientific lines with modern scientific appliances and methodology will unravel a vast number of effective remedies for the treatment of disease and alleviation of human sufferings. This infact, is gradually happening and more and more drugs of therapeutic usefulness are being worked out and brought into use in medical practice.

*Abelmoschus moschatus* (L.) Medik syn. *Hibiscus abelmoschus* L. of the family Malvaceae, popularly known as ambrette [commonly used names- Filipino: ambrette, kastuli; French: ketmie musquee; Indonesian: kasturi; Malay: kapas hutan; Thai: som-chaba; Vietnamese: b(us)p, v(af)ng (Widodo 1999); English name- Musk- mallow] is an important medicinal plant species and is native to India (Anonymous 1959). The generic name *Abelmoschus* is derived from Arabic ‘abu- l- mosk’ (father of musk) in allusion to the smell of the seeds; specific epithet means ‘musk smelling’ (Orwa *et al.* 2009). The plant species is also known by the following names- Arabic: hhabb el misk, anbar bul; Chinese: ye you ma, shan you ma, huang ku, huang kai; English: ornamental okra, musky- seeded hibiscus, musk okra, annual hibiscus, fautia, yorka okra; French: Ambrette, graine de musc, gombo mosque; German: bisamstrauch, bisameibisch; Italian: abelmosco, fior muschiato, ibisco muschiato, ambretta; Thai: chamot ton; Vietnamese: cay bong vang, bup vang- (Source: Agroforestry Database 4.0, Orwa *et al.* 2009); India: Hindi- mushkdana, kasture bhendi, Assamese- gukhia korai, Tamil- varttilai kasturi, Sanskrit- lalkasturika (Krishnamurty 1993); Bengali- lata kasturi, mushakdana. *A. moschatus* has been classified as “an herb of undefined safety” by the Food and Drug Administration (FDA). The roots, leaves rarely and seeds of the species are used in traditional system of medicine (Verma *et al.* 1993, Sala 1995, Widodo 1999). Herbal drugs containing ambrette are introduced by Indian drug manufacturers for medicinal uses (Duke 1985, Lawrence 1996).

*A. moschatus* is an annual or biennial, erect, hispid herb or undershrub (Widodo 1999, Sharma and Shahzad 2008, Ramu *et al.* 2010) or shrub (Nautiyal and Tiwari 2011) yielding ambrette oil (Orwa *et al.* 2009, Ramu *et al.* 2010, Nautiyal and Tiwari

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2011) of commerce (essential oil) which finds application in flavor and fragrance formulations (Arctander 1960, Rout *et al.* 2002). The ambrette oil obtained from seeds (possessing a characteristic musk like odor) is localized in the outer layers of the seed coat (Nee *et al.* 1986). The seed extract is widely used as a fixative in fragrance formulations (Bernard *et al.* 1988). The liquid oil is a valuable adjunct to high- grade perfume compositions to which it imparts a strong and characteristic musky note. It possesses a much smoother odor than synthetic musk compounds (Guenther 1948). The liquid oil of commerce is noted for its rich, sweet, floral- musky, distinctly wine like odor with a bouquet and “roundness” rarely found in any other perfumery material and with a tenacity of odor that is almost incredible (Arctander 1960).

Kerschbaum (1927) for the first time isolated a lactone from ambrette seed oil by fractionation (after removing fatty acids with dilute alkali) and called it ambrettolide. It has been identified as (Z) - 7 hexadecen - 16 - olide. Paredes (1973) reported that the ambrette seed essential oil contained farnesyl acetate as the major constituent; although, ambrettolide was responsible for the typical and characteristic musky odor. Maurer and Grieder (1977) reported isolation of (Z) - 5 - tetradecen - 14 - olide from ambrette seeds in addition to (Z) - 5 - dodecanyl acetate and (Z) - 5 - tetradecanyl acetate. Seed analysis reports the presence of 11.1% moisture, 31.5% crude fibre; 14.5% lipids, 13.4% starch, 2.3% protein, volatile oil (0.2 - 0.6%), calcium and resin (Srivastava 1995).

The bark of *A. moschatus* is processed into fibre and root mucilage used for sizing paper (Orwa *et al.* 2009). Sahoo *et al.* (2003) examined edibility of oil seeds. Further, ambrette seed oil is reported to be edible and therefore the species is under nontraditional oil seed crop of economic value under rainfed conditions (Sahoo *et al.* 2003, Rao *et al.* 2005) and may be considered as an alternative crop in comparison to the traditional oil crops.

The species is extremely important from therapeutic point of view. The aromatic seeds are valued medicinally as they are used as tonic and considered as aphrodisiac, ophthalmic, cardiogenic, digestive, pectoral, stomachic, constipating, carminative, diuretic and deodorant apart from possessing antiseptic, antispasmodic, and antiemetic properties (Cravo *et al.* 1992, Orwa *et al.* 2009, Ramu *et al.* 2010, Nautiyal and Tiwari 2011). The seeds are also found effective against *kapha* and *vata*, intestinal complaints and diseases of the heart (Orwa *et al.* 2009). The tincture of seeds

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is useful in nervous disability and hysteria (Sharma and Shahzad 2008). According to Unani system of medicine seeds allay dyspepsia, urinary discharge, leucoderma and itch (Ramu *et al.* 2010). Further, Ramu *et al.* (2010) used seed starch (16.0% w/w) of *A. moschatus* as disintegrant to paracetamol tablets at concentrations of 2.5 to 10.0% w/w. In the Philippines a decoction of the roots and leaves is taken as an emollient remedy for gonorrhoea and rheumatism (Agharkar 1991). In traditional Vietnamese medicine the plant is used as antivenom against venomous reptiles (Lindley 1985). In Indonesia pulverized seeds mixed with powder provide comfort against prickly heat. Liu *et al.* (2005) showed that myricetin (purified from aerial part of *A. moschatus*) enhance glucose utilization (effective dose 1.0 mg/kg in STZ - diabetic rats) to lower plasma glucose in diabetic rats lacking insulin. Liu *et al.* (2006) reported that plasma glucose lowering action of myricetin in insulin- deficient animals is mediated by activation of opioid mu- receptors of peripheral tissue in response to increased beta-endorphin secretion. Experimental studies on rats indicated that *A. moschatus* is potentially useful for adjuvant therapy for patient with insulin resistance and/or wishing to increase insulin sensitivity (Liu *et al.* 2007, Liu *et al.* 2010). Maheshwari and Kumar (2009) reported that hexane, ethyl acetate, methanol and aqueous extracts from the leaves of *A. moschatus* were with antibacterial activity against *Staphylococcus aureus*, *Bacillus megaterium*, *Shigella flexneri*, *Proteus mirabilis* and *Corynebacterium diphtheria*. Rival *et al.* (2009) reported that seed extract as a protective active ingredient to flavor FGF- 2 activity in skin reducing wrinkles. Gul *et al.* (2011) reported antioxidant, antimicrobial and anti- proliferative activities of *A. moschatus* leaf and seed extracts.

Ambrette seed oil is used in perfumery, cosmetic products and as an additive in the preparation of chewing tobacco, baked products, sweets, alcoholic and non-alcoholic drinks (Arctander 1960). The seeds are also used to flavor coffee in Arabia. In Malay the people uses leaves of *A. moschatus* to wrap parcels. The mucilage from the roots is used in China for sizing paper. Seeds are used to protect clothes against insects. The stem bark yields a good quality fibre rich in cellulose (78%) content (Sharma and Shahzad 2008). The flowers in the species are in demand for making zarda. Further, tender leaves, shoots and pods are edible. Dua *et al.* (2006) evaluated the aqueous extract of roots of the species against the larvicidal activity of mosquitoes

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(*Anopheles* and *Culex*) and noted positive response in the reduction of larva of *Anopheles stephensi* and *Culex quinquefasciatus* under field condition.

Apart from been native to India, the species is also native to Tropical Asia, Southern and some parts of Pacific Island, China, Cook Island, Fiji and Samoa (Du *et al.* 2008, Orwa *et al.* 2009). Widodo (1999) reported that the species is cultivated commercially in Java, India (mainly in the Deccan and Carnatic), Madagascar and in parts of Central and South America and to a small scale throughout the tropics and in warm temperate areas. *A. moschatus* is found to grow in variable places namely, roadsides, brushwood, fallow land and on the rice fields and occurs upto 1650 m altitude in Indonesia, and in India it is cultivated upto 1000 m (Widodo 1999).

Velayudhan *et al.* (2007) considered that the species is found rarely as a cultivated plant but occurs wild in India specifically all over the hilly regions. Considering the potential significance of ambrette seed oil it would be of utmost importance to keep the species under cultivation for sustainable use and to step the production of ambrette oil for commercial purposes. Being self-pollinated, the species offers little scope for improvement through conventional breeding techniques. It would be of paramount significance to raise desirable plant types (yield of essential oil content and value added plant products) exploring the existing germplasm(s). Induced mutation is of pivotal significance in creating genetic variations and widening the gene pool in a quick span of time.

Gustafsson (1947) advocated that mutation approach to be superior to other methods of crop improvement; while Brock (1977) was of the opinion that induced mutation may be exploited for manipulation of genetic variations. Swaminathan (1972) suggested that induced mutation provides an ample opportunity for reconstruction of plant ideotypes. Although induced mutation is of great relevance in modern plant breeding for the development of improved plant types (Kharkwal 2000), mostly drastic changes of the phenotype brought about by mutational events among the genes (Scossiroli 1965) due to random actions of the mutagen(s) employed, often causes low productivity and does not possess any practical value. Mutagenic responsiveness of the genotype concerned is therefore, considered to be a vital event for success of mutation breeding experiment (Hagberg *et al.* 1963). Thus, it is of utmost importance to gain basic information regarding mutagenic sensitivity, efficiency and effectiveness of the

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mutagens employed to monitor successful mutagenesis and to raise viable macromutants of interest.

Chemical mutagens played significant role for the artificial induction of mutation (Konzak *et al.* 1965) due to their mutagenic potentiality, relative ease of application and low cost and among them EMS (ethyl methane sulphonate), a mono-functional alkylating agent is considered to be most effective (Auerbach 1958, Gustafsson 1963, Rayan and Heslot 1963, Ehrenberg *et al.* 1966, Loveless 1966, Sato and Gaul 1967, Konzak *et al.* 1977).

Present investigation deals with morphological (including seed coat surface following SEM analysis), anatomical (root and stem anatomy), palynological (acetolysis technique), cytological (meiotic analysis), physiological (stomatal characteristics), biochemical (quantification of seed storage protein, soluble carbohydrate content and essential oil content; qualitative analysis of seed protein following SDS- PAGE) and molecular (using RAPD markers) characterization of two germplasms of *Abelmoschus moschatus* (L.) Medik with an objective of proper cataloguing of medicinal germplasm resources. Further, the methodology of induced mutagenesis has been adopted using the mutagens namely, Ethyl methane sulphonate (EMS) and Hydroxylamine (NH<sub>2</sub>OH) in two germplasms of *A. moschatus* to create genetic variations and to raise desirable 'plant type' mutants significant for economic benefits. Induced mutagenesis deals with the following aspects: 1, assessment of mutagenic sensitivity of the genotypes and mutagenic efficiency and effectiveness of the mutagens employed; 2, screening of 'plant type' mutations from M<sub>2</sub> population; 3, cytogenetic analysis of the morphological mutants; 4, study on the inheritance pattern of the mutant traits from M<sub>3</sub> population; 5, morphological (analysis of quantitative traits), cytogenetical and biochemical (quantitative analysis of seed storage protein, soluble sugar and essential oil contents; qualitative assessment of seed protein by SDS-PAGE) study of the mutants has been made at M<sub>4</sub> in relation to respective controls to assess viability and productivity of the mutants; 6, genetic analysis of different plant types of germplasm I and II has been made to determine selection criteria for improvement. Moreover, cytomorphological and molecular analysis of the raised F<sub>1</sub> hybrid (crossing performed between two germplasms) has also been performed.