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Seed as major gizmo in ret medicinal and aromatic plants conservation- a perspective

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Abstract

Seed quality comprises of genetic and physiological characters that govern successful reproducibility by conserving genetic information from one generation to other. Seed is the pivotal input for the conservation of Rare, Endangered and Threatened medicinal and aromatic plants. The fundamental objective of genetic resources conservation is the maintenance of broad-based genetic diversity within each of the species. Further, genetic diversity and attainment of homozygosity of the individual or the population of plants selected for conservation lay the ultimate footstone. Selection of suitable strategies keeping the seed developmental biology offers a great means for conserving RET species. Prominent among these are provenance, genetic diversity, appropriate seed bearing in self and cross-pollinated species, seed polymorphism, the presence of inhibitors is also a primary source of seed dormancy and germination, and tissue culture methods are the key features of employing seed as the chief source to accomplish successful conservation.

Keywords: Conservation; Provenance, Genetic Diversity; Homozygosity; Seed Polymorphism, Dormancy, Germination and seed quality

Introduction

On a global basis, it has been estimated that about 10 (%) of world's vascular plant species totaling to about 20,000 to 25,000 species are under varying degrees of threat. The flora of India is very rich with an estimated 50,000 plant species, of which 15,000 are flowering plants. Of these 5,000 species are endemic to India, while about 1500 species i.e. about 10 (%) of the flowering plants are under varying degrees of extinction. Some 75 species of vascular plants are thought to be endemic to the Eastern Ghats region of India spread across the states of Orissa, Andhra Pradesh, Tamilnadu, and Karnataka. In a recent census, 17 endemic, 12 apparent endemic and 56 rare/endangered plant species have been reported from Orissa state alone. The Western Ghats, one of the global biodiversity hotspot comprising of approximately 5000 species of flowering plants, of which 70 (%) are claimed to be medicinal and about 1700 species (34 %) are endemic.

Medicinal and aromatic plants are the main ingredients of local medicines and are of vital importance in traditional health care. People use medicinal plants species for the sustenance of their traditional health care system both logistically as well economically. But due to more inclination towards modern technology and over-extraction of many of these plants has resulted in considerable depletion of the population of such species and some have become extinct. The natural biosphere reserve is a useful solution for species that are endangered and nearly on the point of extinction. The fundamental objective of genetic resources conservation is the maintenance of broad-based genetic diversity within each of the species (*i.e.* intra-specific genetic diversity) with a known or potential value in order to ensure availability for exploitation by present and future generations. Future progress in crop improvement and our food security depends, to a great extent, on the immediate conservation of the rapidly vanishing crop gene resources and their effective utilization by plant breeders. In this context, a great deal has been accomplished in the last decade to safeguard the plant genetic wealth which constitutes the natural heritage. However, much still remains to be done in improving the conservation strategies and upgrading the collections, which encompass a wide range of diversity comprising wild relatives, primitive cultivars and landraces, weedy forms, unimproved and modern cultivars, and genetic testers (Khanna and Neeta Singh) ^[1].

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In Karnataka, according to the study of the Botanical Survey of India there are 3924 species belonging to 1323 genera and 199 families in the forests, of which 1493 species are of medicinal value. These belong to 808 genera and 108 families. They occur in different vegetation types across the Western Ghats. What has come as alarming news is the inclusion of 81 medicinal and aromatic plants in this list of Rare, Endangered and Threatened species nearing to extinction. The loss of these plants will not only jeopardize the environment but pose difficulties to many ayurvedic medicine manufacturing companies and practitioners of this ancient form. Some of the threatened plant species that have been marked 'red' by the forest officials are, Arisina Balli, Sambar Balli Twak, Maakali Beeru Fly Catcher, Tandavari, Sampige, Bobbi Mara, Tarakasheera, Seethe Ashoka, Kadu Pindi. Most of these species collection of quality seed material alone makes the immediate essence, a chief source as well as means of conservation. An attempt has been made to underline this major gizmo and other available and suitable concepts to be emphasized during the conservation.

According to FAO, Plant Genetic Resources for Food and Agriculture (PGRFA) conservation focuses explicitly on maintaining the diversity of the full range of genetic variation within a particular species or taxa. The main reasons for conserving PGRFA are to ensure the future adaptability of cultivars and wild populations; to preserve data and traits that ensure sustainable agriculture; to promote the use of genetic resources in commerce and biotechnology; to conserve genetic diversity for cultural reasons. *Ex-situ* conservation entails conservation of biological diversity components outside their natural habitats. The main storage infrastructures for such conservation techniques are genebanks; millions of accessions are now stored in hundreds of genebanks around the world for conservation and utilization purposes. Whereas, *in-situ* conservation means the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties. Common approaches for *in-situ* conservation are Genetic reserve conservation and On-farm conservation.

Seed and its quality in conservation

Seed is a fertilized matured ovule together covered with seed coat is called seed or it is a propagating material *i.e.* part of agriculture, sericulture, silviculture and horticultural plants used for sowing or planting purpose. "Structurally a true seed is a fertilized matured ovule, consisting of an embryonic plant, a store of food and a protective seed coat, a store of food consists of cotyledons and endosperm". However, from the seed technological point of view seed may be sexually produced matured ovule consisting of an intact embryo, endosperm and or cotyledon with a protective covering (seed coat). It also refers to propagating materials of healthy seedlings, tuber, bulbs, rhizome, roots, cuttings, setts, slips, all types of grafts and vegetative propagating materials used for production purpose.

Seed develops according to genetic specification for each species, which are coded in the chromosomes of each cell. Seed quality and performance are greatly influenced by molecular, biochemical, hormonal and environmental changes during the seed development, which is basically the embryo that derives from a fusion of the nuclei of male and female gametes and it is surrounded by integument's which encloses the food reserves in endosperm or cotyledon. Thus, the seed is the most vital and crucial input for the conservation of Rare,

Endangered and Threatened (RET) medicinal and aromatic plants. The sexual cycle of reproduction assures mechanism of reproduction and multiplication of these species by the formation of seeds. If a RET species offers such seed setting cycle it is really advantageous so that the conservation ends up in collecting true seeds and their further multiplication by the process of seed germination and seed establishment and further reproduction cycles through gamete formation and fertilization. If the set of seeds have problems associated with seed germination, there are available means and ways to enhance the germination which are associated with the metabolites these RET plants produce.

In any case, if the RET species lacks the sexual cycle of reproduction through forming seeds one has to look upon for the vegetative means of reproduction by employing the totipotent principles. In such cases tuber, bulbs, rhizome, roots, cuttings, setts, slips, all types of grafts and tissue culture can be effectively used as a means of propagation keeping the view of somatic variations to obtain true to type individuals for conservation. Often the effort of planning, exploration, collection and conservation becomes a myth the following points are envisaged. Most of the times the collected seed of planting material if has any genetic variation within the individual plants identified for collection makes it difficult to obtain the true to the type of individuals collected.

Provenance and subsequent area adoption of quality seed material in conservation

The provenance of seed material used in ecological conservation or habitat creation is a prominent source and means for a success (Hamilton, 2001)^[2] and (Hufford and Mazer, 2003)^[3]. Native provenances are often suggested as they are normal to be better adapted to the site environment suitable for plant establishment. Apart from the insufficient establishment, the use of non-local seed provenances might involve environmental risks such as cryptic invasions of superior genotypes (Saltonstall, 2002)^[4] or outbreeding depression, *i.e.* a reduction in hybrid fitness following gene flow from an alien into local populations. Indeed, plant populations often exhibit pronounced genetic differentiation in fitness-related traits resulting from habitat-specific selection or genetic drift (Linhart and Grant, 1996)^[5], but spatial scales and degree of differentiation at which negative consequences of genotype introduction can be expected are less known. Selection is expected to favour appropriate responses to local environmental cues that synchronize germination with periods that are optimal for seedling survival. Such a selection may result in site-specific adaptation of germination (Armin Bischoff *et al.*, 2005)^[6].

Provenance and area of subsequent adoption or plays important role in further multiplication collected RET medicinal plants. If the adoption is a viable option without any change in the genetic constitution of plant species, then the any suitable are can be employed for further conservation like *ex-situ* methods. If the plant species is not adopted and upon conservation, if the area chosen for adoption influences the genetic character over a period of time the then goal of conserving it by *ex-situ* methods invariably converts in to *in-situ* method.

More off then it is evident that the seeds set on an *ex-situ* conservation area or plot if evaluated for occurrence of genetic variation over a period of successive reproduction year after year or season after season. If the polymorphism exists between the seeds of the individual plant across the season or within the season, then naturally the conservator has

to think of non-adoption. In most of the cases, adoption is said to be a failure only upon non-establishment of a conserved plant or its inability to produce the seeds by the formation of sex gametes or their effective fusion. If the above criterions are not noticed the plant tend to be adopted however once has to keep a check on the genetic and physiological morphs to achieve a successful conservation. Thus, a conservator of RET species has the two choice of either *ex-situ* or *in-situ* conservation techniques that has to employed shall look in to the above aspects keenly before taking up the task.

As a species maintaining significant genetic variability through sexual reproduction is an important evolutionary tool for its successful establishment and perpetuation in nature. The factors that impact genetic variability and population structure can be broadly divided into (1) general (direct) factors which relate to a species (or groups of species), *e.g.* breeding system, life form, pollen and seed dispersal (Vekemans and Hardy, 2004)^[7] and (2) specific (indirect) factors which affect variability and structure indirectly through gene flow, *e.g.* presence/absence of gene flow vectors such as wind, waterways, animals and management system (Ducci and Santi, 1997)^[8]; (Oddou Muratorio *et al.*, 2004)^[9] and (Ganopoulos *et al.*, 2013)^[10]. Hence, in conservation of rare, endangered and threatened species of medicinal and aromatic plants the conservator must have the in-depth perceptive of the biology of the plant in question. In addition to the fact, the principal means of its reproduction either by sexual seed cycle or by vegetative means of propagation must be emphasized. In this context, the following are the key facts conservator must envisage and practice for a successful conservation.

Genetic diversity and seed polymorphism

i) Genetic diversity

The total number of genetic characteristics in the genetic makeup of a species constitutes its diversity genetically. It is distinguished from genetic variability, which describes the tendency of genetic characteristics to vary. Genetic diversity serves as a way for populations to adapt to changing environments. Further, in simple terms "the variation in the amount of genetic information within and among individuals of a population, a species, an assemblage, or a community" UN (1992)^[11]. Keeping above in the view the conservation of RET medicinal and aromatic plants assumes a greater importance of understating the concepts before a group or individual plants of such species available in the wild or a place where it is found to native makes a greater sense in achieving the goal of conservation.

A conservator must be well aware of the facts that the goal or success of conservation ends only upon success full identification of the chief source or true to type of a species in question. For that, a detailed genetic diversity study has to be undertaken before venturing into the collection by primary explorations. The fowling is two key concerns one has to relay before attempting for final collection of considered RET species for conservation. Genetic diversity plays an important role in the survival and adaptability of a species. When a population's habitat changes, the population may have to adapt to survive; the ability of the population to adapt to the changing environment will determine their ability to cope with an environmental challenge. Further, Genetic diversity is the term for the diversity of the genetic material in a population or species. More different genes inside the population mean a higher genetic diversity. The gene pool is bigger. If a species has two populations, each of them can be

genetically similar within its own population but genetically very dissimilar between each population. The total genetic diversity of the species is high, but the genetic diversity of the populations is low. Genetic diversity can be measured as; Percentage of loci with different alleles Amount of heterozygosity, the number of alleles for one locus present in the population.

The best method is to determine the number of alleles per locus, but this is not always feasible to use as the method is more precisely than determining heterozygosity. Keeping above points in view, a separate disciple called Conservation Genetics is on the emergence. Wherein, it is an interdisciplinary subfield of Population Genetics that aims to understand the dynamics of genes in populations principally to avoid extinction. Therefore, it applies genetic methods to the conservation and restoration of biodiversity. Researchers involved in conservation genetics come from a variety of fields including population genetics, molecular ecology, seed biology, evolutionary biology, and systematics. Genetic diversity is one of the three fundamental levels of biodiversity, so it is directly important in conservation. Genetic variability influences both the health and long-term survival of populations because decreased genetic diversity has been associated with reduced fitness, such as high juvenile mortality, diminished population growth, reduced immunity, and ultimately, higher extinction risk. (Leberg, 1990).^[12] Genetic diversity determines the potential fitness of a population and ultimately its long-term persistence because genes encode phenotypic information. Extinction risk has been associated with low genetic diversity because several researchers have documented reduced fitness in populations with low genetic diversity. For example, low genetic diversity as low heterozygosity has been associated with low juvenile survival, reduced population growth, low body size, diminished adult lifespan (Lande, 1988)^[13].

The importance of genetic diversity has many reasons; for example, one can consider the probability of within any given population that at least one individual or a potential reproductive pair have the genetic composition encoding a phenotype capable of survival in an environment event. If one of the individuals are nearly identical, if new pressures (such as environmental disasters) occur, a population with high genetic diversity has a greater chance of having at least some individuals with a genetic makeup that allows them to survive, or if genetic diversity is very low, none of the individuals in a population may have the characteristics needed to cope with the new environmental conditions. Such a population could be suddenly wiped out. Besides, the genetic diversity of a species is always open to change. No matter how many variants of a gene are present in a population today, only the variants that survive in the next generation can contribute to species diversity in the future. Once gene variants are lost, they cannot be recovered. Thus, keeping diversity the conservation efforts are to be concentrated on selecting individual plant morphs by determining the level of homozygosity attained for otherwise the selection of stable genotype for effective conservation cannot be achieved. The variability existing is of considerable importance for the constitution of individual plant genetic resources of a species which offers a potential tool for crop breeding in terms of its gene pool. However, in terms of conservation, a stable genotype selection among the population of a species under conservation upon the existence of variability is the key that has to be emphasized in RET medicinal plants.

a) Concerns in the conservation of self-pollinated species

Often collected RET medicinal and aromatic plants are assumed to be true to type i.e. they have attained homozygosity. The ability of a self-pollinated individual to reproduce genetically upon successive progenies as a pure line. The extent of homozygosity attained in a population on n number of RET plants identified on wild plant depends largely on type, extent, and mechanism of self or cross-pollination. One has to obtain the true to type of plants by either selling the conserved plant upon attainment of reproductive phase and evaluating the next generation progenies for the segregation or occurrence of the difference between the individual progenies through morphological characters expressed as a result of the true genetic constitution of the plant. However, the genetic purity of the obtained lots is in question. The collection and simple reproducing didn't address the whole part of conservation. Until and unless the genetic purity in terms of attainment of homozygosity is attained the conservation of a particular RET plant will be questionable.

b) Concerns in the conservation of cross-pollinated species

If an individual RET plant having sexual reproduction through forming male and female gametes and cross-pollinated the strategies for selecting a homozygous plant for further conservation needs a special attention. The collected plant for conservation may be of either homozygous or heterozygous condition. If it's in the homozygous state (AA or aa) then the progeny obtained are said to near homozygous with a strict self-pollination ensuring no cross-pollination. However, during the next cycle of reproduction strict self-pollinating must be ensured. If so the collection and conservation efforts are fruitful.

Taking an example of one segregating loci in any self-pollinated RET medicinal and aromatic plant with sexual reproduction involving formation of male and female gamete and their further union through fertilization to set seeds and are employed as a chief source of progeny multiplication the following can be envisaged. In case the collected plants are in the heterozygous state (Aa); further, the individual plants produce two types of pollen grains say A and after reduction, division to form haploid gametes either in PMC or MMC. After fertilization, if such two haploid gametes unite to form zygote in random leads to the creation of genetic variation. Thus the recombination of these gametes will yield three definite and distinct individuals in various numbers as outlined by the Mendelian theories and the Hardy Weinberg principles of genetics. The foremost operating primarily leaves us with three distinct individuals with AA, as and Aa content of genetic information. Out of these, the two homozygous individuals upon successful self-pollination without the later effect of random mating are said to attain homozygosity in a generation. While the heterozygous individuals as the matter of Mendel's laws fifty percent will attain homozygosity innately.

However, the attained homozygous nature creates two distinct individuals each with 25 percent (AA and Aa). Unlike if the cross-pollination within the population only will have three distinct individuals among a group of the progeny over a period of time. If individuals express the attained genetic homozygosity and heterozygosity nature through evaluation of stable morphological characters of such collected RET plants. Selections can be further affected to choose the appropriate plant type with desirable characters for further multiplication. If the collected individuals are of different

genetic background, then the complexity of perpetuating them by the mechanism of sexual seed cycle tends to be highly complicated. As the individuals will undergo random mating leading to the extraneous generation of different individuals differing in genetic composition. Further, attainment of homozygosity is near myth in spite of several generations of strict selection and self-pollination.

The conservator must have set his mind to choose or select the area and individual's plants after careful examination and scrutiny by repeated visits if possible to the wild before thinking of selecting individuals for collection and further multiplication. If the variability exhibits in a natural population of individuals of particular locality single plant selections or section of few seeds from a single fruit is a viable option to reduce such complexity. If the of a RET plant is assessable in natural habitat, then planning for a self-pollination through bagging or hand pollination offered a great chance in collecting genetically pure accessions for further multiplication and conservation. If the species is under cross-pollination the diversity existing among population must be well thought and after assessment of such natural population, the only collection shall be planned.

ii) Seed polymorphism: is a common phenomenon associated with discrete or continuous morphological or physiological variation among individual seeds produced by an individual or population (Venable, 1985) ^[14]. Seeds produced within a somatic polymorphism may vary in size (*Xanthium* sp.), color (*Atriplex heterosperma* Bunge) and/or external structure (*Chenopodium album* L.). These variations in morphology are frequently accompanied by differences in germination requirements with the consequence that germination of polymorphic seeds may be staggered in time. Such somatic channelling of progeny into distinct classes of behaviour may increase the fitness of individuals in environments that are temporally patchy, at the expense of the intrinsic rate of increase that could be attained by a unique seed type in a uniform environment (Silvertown, 1984) ^[15]. Thus seed polymorphism determining the seed quality of conserved species must be given prior importance to achieve a successful conservation. For stable homozygous plant selection, successive progenies or next cycles of seeds are thoroughly evaluated for various seed morphological character *viz.*, seed coat colour, size, scar of hilum *etc.* followed by the variation in seed germination can reduce the risk of selecting undesirable types for conservation. Thus having a prior thought of occurrence of seed polymorphism and its evaluation offers a main source of collection homozygous true breeding individuals for conservation.

Seed dormancy and germination

Seed dormancy is defined as a state in which often seeds are prevented from germinating even under environmental conditions normally favourable for seed germination. These conditions are a complex combination of water, light, temperature, gasses, mechanical restrictions, seed coats, and hormone structures. The significance of the seedling in plant population ecology has long been recognized (Baskin and Baskin, 1998) ^[16]. The germination response pattern of seeds is also regarded as a key characteristic of plant life history strategy (Baskin *et al.* 1993) ^[17]; (Baskin and Baskin, 1972) ^[18]. The variation in seed dormancy and the subsequent patterns of seedling emergence are largely known to be controlled by environmental conditions. Important factors controlling the variation seed dormancy within species

include the environment of the mother plant during the time of seed maturation and environmental conditions after the seeds have been released (Bewley, 1997) ^[18]. Certain environmental conditions are required to break dormancy, and other conditions are also required to permit germination after dormancy is completely broken (Bewley and Black, 1994) ^[20]. Seeds of many species require after-ripening period or to metabolically reduce the inhibitors (in days, weeks, or months at normal conditions or at low temperatures) to overcome dormancy (Bradbeer, 1992) ^[21] and (Chauhan & Johnson, 2008) ^[22].

Being medicinal and aromatic crops the RET plant species most offers a great challenge to conserve them even though they produce viable seeds. The main impairment being the various metabolites they produce of which are the important constituents employed in curing ailments of human and employed for aesthetic values as perfumeries. The germination response pattern of seeds is an important phenomenon in plant life history strategy (Mayer and Poljakoff-Mayber, 1989) ^[23]. Considerable attention has been given to study seed germination and reproductive biology on different groups of these medicinal and aromatic plants (Han and Long, 2010) ^[24]; (Kameneva and Koksheeva, 2013) ^[25]. However, very little is known about the seed germination pattern of medicinal plants (Liza *et al.*, 2010) ^[26] and (Rahman *et al.*, 2012) ^[27]. Since the RET medicinal plants have to be conserved and are employed for their medicinal values, greater emphasis to be given on seed germination patterns as to successfully carry them into cultivation and to avoid exploitation in the wild. In any such cases, different dormancy and germination mechanism have to validate and ware to be well studied. To overcome and to have success full seed germination offers a major requirement so that they are conserved and to multiply them for use commercially.

Metabolites as seed inhibitors as a primary source of seed dormancy and germination

Further, most of these medicinal and aromatic plant species produce metabolites which impart their medicinal and aromatic values are known to be the derivatives of Benzoic Acid, Cinnamic Acid, Coumarin, Naringenin, Jasmonic and Abscisic Acid (ABA) through various biosynthetic pathways. And in turn, these compounds directly or indirectly inhibit the process of germination. The presence of such medicinally important derivatives in seeds at high concentrations is under consideration for inducing dormancy directly. While also by inhibiting the production of the primary enzyme required for seed germination GA₃ or subsequent inhibition of alpha-amylase enzyme which is the source for conversion of complex sugars into simple sugar for catering the seeds of energy requirements during the process of germination. Besides several other inhibitors of proteases are also being noticed in these medicinal and aromatic plant as major or secondary metabolites. Seed germination is a complex physiological process that responds to environmental signals such as water potential, light, and other factors. A major limiting factor for propagation and conservation of threatened medicinal plant species is their unsatisfactory seed germination. Seed germination in most of these medicinal and aromatic plants is thought to be controlled by many factors like natural growth and germination inhibitors (growth). (Angevine and Chabot, 1979) ^[28].

In some temperate species, dormancy is broken by a period of warm temperatures followed by cold stratification. This response is most often associated with morphophysiological

dormancy; however, seeds with morphophysiological dormancy have underdeveloped embryos (Durrani *et al.* 1997) ^[29]. In order to accelerate this method, it can be combined with some treatments such as chemical applications or mechanical seed coat removal (El-Barghathi & El-Bakkosh, 2005) ^[30]. Many investigators have studied the effects of exogenous growth regulators on seed germination. While, Gibberellins eliminated the chilling requirements of peach and apple seeds and increased their germination (El-Barghathi & El-Bakkosh, 2005) ^[30]. Recent studies have revealed that cold stratification has a direct effect on the production of gibberellins (GAs) in seeds of *Arabidopsis thaliana* (Fernandez *et al.* 2002) ^[31] and (Hartmann *et al.* 1997) ^[32]. Exogenously applied GA overcomes seed dormancy in several species (Hassan & Fardous, 2003) ^[33] and (Hradilik & Cisarova, 1975) ^[34] and promotes germination in some species that normally require cold stratification, light, or after-ripening (Kandari *et al.* 2012) ^[35]. Pre-chilling, scarification, and treatments with gibberellic acid (GA₃) or nitric acid (KNO₃) are the standard procedures used to enhance seed germination of dormant seeds.

However, many attempts have been made to investigate seed germination and seedling emergence of different annual and perennial species including medicinal plants (Bewley, 1997) ^[19]; (Liebst & Scheneller, 2008) ^[36]; (Liza *et al.* 2010) ^[26]; (Martinez-Gomez & Dicenta, 2001) ^[37]; (Mayer & Poljakoff-mayber, 1989) ^[23] and (Mehanna *et al.* 1985) ^[38]. However, no study has surveyed germination patterns in medicinal plants from all over of India. Until and unless these metabolites are quenched or removed safely without damaging the essential structures and apparatus in the seed successful germination cannot be achieved to effectively conserve them.

***In vitro*/ Tissue Culture method**

This method offers a potential tool for conservation of RET medicinal and aromatic plants where the sexual seed cycle is absent or hampered by various mechanisms hindering successful fertilization. However, for success full regeneration and conservation through tissue culture, each species one needs to standardize the nutrient medium required for culturing the callus by existing protocols for other related species. In such success, full regeneration plants obtained in large numbers (minimum 400) have to thoroughly inspect for an occurrence of somatic hybrids or chimeras. Upon finding none of the regenerated plants are chimeric the multiplication can be envisaged.

Somatic mutations have also been found to affect the genetic variability of woody species which often propagate vegetatively in nature, *e.g.* *Populus tremuloides*, *Populus nigra*, and *Robinia pseudoacacia* or in *ex-situ* conditions, *e.g.* *Vitis vinifera*, *Olea* spp., *Pinus pinaster* and *Picea abies* (Kristjan *et al.*, 2015) ^[39]. The occurrence of somatic mutations-permanent inclusion of random errors in DNA-lead to differences between original and copied DNA sequences and per se represent a basic source of genetic variability in vegetative means of reproduction (Hamilton, 2009) ^[40]. Thus, they can be passed on to subsequent ramet generations. Furthermore, because plants do not sequester their germline, these mutations can be transmitted to reproductive organs and subsequently to sexual progeny. As such, somatic mutations are an important factor of 'clonal evolution' in plants, which depends on (1) the age of the clone (the longer it propagates vegetatively, the longer it is subject to stress conditions and the more mutations can accumulate), (2) various environmental stresses and (3) genotype, since some

genotypes are more susceptible to mutations than others. In addition, this difference in susceptibility is also present at the level of individual loci and alleles (Ellegren, 2004) ^[41].

Seed quality comprises of both genetic and physiological characters that govern successful reproducibility to carry conserved genetic information from one generation to other. However, it is the at most important to the conservator to confirm that the reproduced seeds are true to type and carry desired genetic constitution of the species in need. Upon ensuring the above mentioned key features governing a seed quality characters' conservation of Ret medicinal and aromatic species can be achieved successfully.

Conclusion

Seed and its quality play a crucial role in the conservation of RET medicinal and aromatic plants. Detailed studies on suitability of Agroclimatic conditions for collection and conservation of quality seed material lays a fundamental prerequisite for the success of conservation endeavour. Further, genetic diversity and attainment of homozygosity of the individual or the population of plants selected for conservation lay the ultimate footstone in planning, executing, exploration and collection of rare nature conserved plant genetic resource in RET medicinal and aromatic plants species.

References

1. Khanna PP, Neeta Singh. www.biodiversityinternational.org
2. Hamilton NR. Is local provenance important in habitat creation? A reply. *Journal of Applied Ecology*. 2001; 38:1374-1376.
3. Hufford KM, Mazer SJ. Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology and Evolution*. 2003; 18:147-155.
4. Saltonstall K. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences*. 2002; 99:2445-2449.
5. Linhart YB, Grant MC. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics*. 1996; 27:237-277.
6. Armin Bischoffa, Beatrice Vonlanthenb, Thomas Steingera, Heinz Muller-Scharera. Seed provenance Matters-Effects on germination of four plant species used for ecological restoration. *Basic and Applied Ecology*. 2006; 7:347-359.
7. Vekemans X, Hardy OJ. New insights from fine-scale spatial genetic structure analyses in plant populations. *Mol. Ecol*. 2004; 13:921-935.
8. Ducci F, Santi F. The distribution of clones in managed and unmanaged populations of wild cherry (*Prunus avium*). *Can. J Forest Res*. 1997; 27:1998-2004.
9. Oddou Muratorio S, Demesure Musch B, Pelissier R, Gouyon PH. Impacts of gene flow and logging history on the local genetic structure of a scattered tree species, *Sorbus torminalis* L. Crantz. *Mol. Ecol*. 2004; 13:3689-3702.
10. Ganopoulos IV, Aravanopoulos FA, Tsiftaris A. Genetic differentiation and gene flow between wild and cultivated *Prunus avium*: An analysis of molecular genetic evidence at a regional scale. *Plant Biosyst*. 2013; 147:678-685.
11. UN. Environment and Development (Terminology bulletin: 344). United Nations, New York, USA, 1992.
12. Leberg PL. Influence of genetic variability on population growth: implications for conservation. *Journal of Fish Biology*. 1990; 37:193-195.
13. Lande R. Genetics and Demography in Biological Conservation. *Science*. 1988; 241:1455-1460.
14. Venable DL. The evolutionary ecology of seed heteromorphism. *Am. Nat*. 1985; 126:577-595.
15. Silvertown JW. Phenotypic variety in seed germination behaviour: The ontogeny and evolution of somatic polymorphism in seeds. *Am. Nat*. 1984; 124:1-16.
16. Baskin CC, Baskin JM. Seeds, ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, 1998.
17. Baskin CC, Chesson PL, Baskin JM. Annual seed dormancy cycles in two desert winter annuals. *Journal of Ecology*. 1993; 81:551-556.
18. Baskin JM, Baskin CC. Ecological life cycle and physiological ecology of seed germination of *Arabidopsis thaliana*. *Canadian Journal of Botany*. 1972; 50:353-360.
19. Bewley JD. Seed germination and dormancy. *Plant Cell*. 1997; 9:1055-1066.
20. Bewley JD, Black M. *Seeds Physiology of Development and Germination*. Plenum Press, New York, USA, 1994.
21. Bradbeer LB. *Vegetable production and their uses*. Africana publication limited Lagos, Nigeria, 1992, 67-70.
22. Chauhan BS, Johnson DE. Influence of environmental factors on seed germination and seedling emergence of *Eclipta (Eclipta prostrata)* in a tropical environment. *Weed Science*. 2008; 56:383-388.
23. Mayer AM, Poljakoff Mayber A. *The germination of seeds*. Pergamon Press, New York, NY, 1989.
24. Han CY, Long CL. Seed dormancy, germination and storage behavior of *Magnolia wilsonii* (Magnoliaceae), an endangered plant in China. *Acta Bot. Yun*. 2010; 32(1):47-52.
25. Kameneva LA, Koksheeva IM. Reproductive biology of seven taxa of *Magnolia* L. in the south of Russian Far East. *Bangladesh J Plant Taxon*. 2013; 20(2):163-170.
26. Liza SA, Rahman MO, Uddin MZ, Hassan MA, Begum M. Reproductive biology of three medicinal plants. *Bangladesh J Plant Taxon*. 2010; 17(1):69-78.
27. Rahma M, Rahman MO, Hassan MA. Seed germination of two medicinal plants: *Desmodium pulchellum* (L.) Benth. and *D. triflorum* (L.) DC. *Bangladesh J Plant Taxon*. 2012; 19(2):209-212.
28. Angevine R, Chabot BF. Seed germination syndromes in higher plants In: Solbrig OT, Jain S, Johnson GB & Raven PH (eds) *Topics in Plant Population Biology*. Columbia University Press, New York, 1979, 188-206.
29. Durrani MJ, Qadir SA, Farrulch H, Hussain F. Germination ecology of *Bunium persicum* (Boiss) Fedtsch and *Ferula oopoda* (Boiss and Bulse) Boiss. *Hamdard Medicus*. 1997; 40:86-90.
30. El-Barghathi MF, El-Bakkosh A. Effect of some mechanical and chemical pre-treatments on seed germination and seedling growth of *Quercus coccifera* (Kemes Oaks). *Jerash Private University*. 2005.
31. Fernandez H, Perez C, Revilla MA, Perez Garcia F. The levels of GA3 and GA20 may be associated with dormancy release in *Onopordum nervosum* seeds. *Plant Growth Regulation*. 2002; 38(2):141-143.
32. Hartmann HT, Kester DE, Davies Jr F, Geneve RL. *Plant Propagation Principles and Practices*, Sixth Edition. New Jersey, Prentice Hall. 1997.
33. Hassan MA, Fardous Z. Seed germination, pollination and phenology of *Gloriosa superba* L. (Liliaceae). *Bangladesh Journal of Plant taxonomy*. 2003; 10(1):95-

97.

34. Hradilik J, Cisarova H. Studies on the dormancy of caraway (*Carum carvi*) achenes. *Rostlinna. Vyroba.* 1975; 21:351-364.
35. Kandari LS, Rao KS, Payal KC, Maikhuri RK, Chandra A, Vanstaden JV. Conservation of aromatic medicinal plant *Rheum emodi* Wall ex Messl. Through improved seed germination. *Seed Science & Technology.* 2012; 40:95-101.
36. Liebst B, Schneller JS. Seed dormancy and germination behavior in two *Euphrasia* species (*Orobanchaceae*) occurring in the Swiss Alps. *Botanical Journal of the Linnean Society.* 2008; 156:649-656.
37. Martinez Gomez P, Dicenta F. Mechanisms of dormancy in seeds of peach (*Prunus persica* (L.) Batsch) cv. GF 305. *Scientia Horticulturae.* 2001; 91:51-58.
38. Mehanna HT, Martin GC, Nishijuma C. Effects of temperature, chemical treatments and endogenous hormone content on peach seed germination and subsequent seedling growth. *Scientia Horticulturae.* 1985; 27:63-73.
39. Kristjan Jarni, Jernej Jaks, Robert Brus. Vegetative propagation: linear barriers and somatic mutation affect the genetic structure of a *Prunus avium* L. *Stand. Forestry.* 2015; 88:612-621.
40. Hamilton MB, Wiley Blackwell, O'Connell LM, Ritland K. Population Genetics: Somatic mutations at microsatellite loci in western red cedar (*Thuja plicata*: Cupressaceae). *J Hered.* 2004-2009; 95:172-176.
41. Ellegren H. Microsatellites: simple sequences with complex evolution. *Genetics.* 2004; 5:435-445.