International Archive of Applied Sciences and Technology
Int. Arch. App. Sci. Technol; Vol 9 [1] March 2018: 82-88
© 2018 Society of Education, India
[ISO9001: 2008 Certified Organization]
www.soeagra.com/iaast.html

IAAST
ONLINE ISSN 2277- 1565
PRINT ISSN 0976 - 4828

CODEN: IAASCA REVIEW ARTICLE

DOI: .10.15515/iaast.0976-4828.9.1.8288

Crop Germplasm Conservation and Propagation-A step towards sustainable agriculture- A Review

Ranju Gulati
Department of Botanys, DAV College Chandigarh.
Email: ranjugulatidav@gmail.com

ABSTRACT

The whole set of genetic material of a species of plants is known as germplasm of the organisms. Development of the human population accompanied by current climate change is in contrast to a relatively small spectrum of crops utilized for food. A small basic number of utilized crops are very disadvantageous from the ecological view and security of human food resources. Due to long-term genetic erosion, the increasing similarity of varieties etc., there is a necessity to use the opportunities offered by national parks and other localities, i.e., to search for new genotypes for plant breeding or as a new crop. From a historical view, the beginning of genetic erosion is an old affair, which happened in the Amazon region of South America. This occurred after 1492 when Europeans began to occupy the Amazon region. Indian populations used 138 or more species (crops) probably in a high state of domestication. The following decline of their populations has resulted in a decreasing number of crops used. The second unfavorable trend, the growth of cultivar similarity occurred mostly in the 20th century. Breeding to increase yield, quality and resistance to pests and diseases have led to the narrowing of the gene pool and genetic diversity. Cultivars are more similar from the morphological and physiological view. It is a disadvantageous process.

Key words: *Gene conservation; Genetic erosion; Genetic resources; New crops.*

Received 08/11/2017 Revised 20/12/2017 Accepted 15/02/2018

Citation of this article
Ranju Gulati. Crop Germplasm Conservation and Propagation-A step towards sustainable agriculture-A Review. Int. Arch. App. Sci. Technol; Vol 9 [1] March 2018. 82-88

INTRODUCTION

What are plant genetic resources? They are any plant materials such as seeds, plant cutting fruits, pollen, various organs, and tissues. They are the organs from which plants can be grown. Especially the breeders, genebank staff, researchers (also farmers), keep and utilize them [1-4]. Evolution, especially the future evolution of crop plants depends upon the availability of genetic diversity. The course of future crop evolution depends also upon current requirements and new demands (yield, nutritional quality, resistance to diseases and pests). The genetic diversity of crop plants has developed mainly by human conscious and unconscious selection. Currently, genetic diversity is still eroding, and the problems posed by this erosion on the robustness of production systems or the ability of agriculture production to adapt to climatic hazards are beginning to be seen. Movements are forming to save also old varieties. It becomes necessary to put tools and programs in place so as not to lose biodiversity. [1]

To make crops more resistant to pests and diseases and to improve food supply quality, quantity and variety, modern plant breeders continually seek genetic resources (germplasm) from outside the stocks with which they routinely work. Since no nation has within its borders the desired spectrum of genetic resources and because valuable genetic resources not yet collected and preserved may be endangered by land use changes in some countries, there is then the need for germplasm preservation and propagation since plant genetic resources are the basis of future agriculture. They include traditional and modern cultivars as well as their wild relatives.

Definition

Germplasm refers to the hereditary materials transmitted to the offspring through the germ cells (Wilkes, 1991). It is the total content of genes that serves as the raw material for the breeder to develop different crops [5]. In other words, germplasm can be defined as living tissue from which new plants can be grown. It can be a seed or another plants part; a leaf, a piece of stem, pollen or even just a few cells that can be turned into a whole plant [6]. Germplasm is the living genetic resources such as seeds or tissue that is maintained for the purpose of animal and plant breeding, preservation and other research uses [7]. These resources may take the form of seed collections such as seed banks, trees growing in nurseries, animal breeding lines maintained in animal breeding programmes or gene banks etc [8]. It contains the information for a species genetic makeup, a valuable natural resource of plant diversity. Germplasm collections can range from collections of wild species to elite, domesticated breeding lines that have undergone extensive human selection.The main objective of germplasm collection is preservation of genetic diversity of a particular plant or genetic stock for its use in the future [9].

Types Of Germplasm Germplasm can be organized in six different categories based on their station or advancement in the agroecosystem [9]. These include. Advanced (Or Elite) Germplasm:

(1) „Cultivars" or cultivated varieties, which are suitable for planting by farmers, either recently developed cultivars or „Obsolete" cultivars that are no longer grown.

(2) Advanced breeding materials that breeders combine to produce new cultivars (sometimes referred to as "breeding materials").

Improved Germplasm: This is any plant material containing one or more traits of interest that have been incorporated by scientific selection or planned crossing.

Landraces: These are varieties of crops improved by farmers over many generations without the use of modern breeding techniques. Within a modern breeding programme, landraces are sometimes used for resistance traits and generally required before their genes can be used in a final variety.

Wild Or Weedy Relatives: Are plants that share a common ancestry with a crop species but have not been domesticated. These plants can serve as another source of resistance traits, but these traits can be very difficult to incorporate on final varieties.

Genetic Stock: These are mutants or other germplasm with genetic abnormalities that may be used by plant breeders for specific purposes. Genetic stocks are often used for highly sophisticated breeding and basic research.

PLANT GENETIC RESOURCES

Crop wild relatives have been used to improve the quality and yield of known crops since thousands of years ago. They helped to improve resistance against pests and diseases and to improve crop tolerance to stressful abiotic conditions such as drought. Plants, micro-organisms, animals, and invertebrates used for Food, Agriculture, and Forestry that are called Genetic Resources, and are grouped under the concept of Agrobiodiversity Genetic Resources include both wild species and domesticated forms.

t is estimated that around 10,000 plant species have been used for human food since the origin of agriculture. Today, only about 150 plant species make up the diets of the majority of the world's population. Of these, only 30 crops provide 90 percent of the world's calorie intake, and just 12 species provide over 70 percent of food. And what is even more of interest, only four crops-rice, maize, wheat, and potatoes-represent over 50 percent of the food supply, but data from individual sources of information differ [5]. Genebanks in Europe maintain approximately one-third of the world's *ex-situ* crop germplasm collections. Very good is development conservation of genetic resources *et situ* in developing countries [10]. From this point of view, very important is also international agreements, the convention on biological diversity, to protect nature and conserve genetic resources [11]. Crops plants share a great deal of their genetic property with wild progenitors, but only part of the genetic diversity of the wild progenitors is present in their derivatives. Another source of diversity is the more distantly related species. Their exploitation is more laborious and time-consuming, but they may possess diversity which is absent in the primary gene pool.

Crop Wild Relatives (CWR) are species closely related to crops (including crop progenitors) and are defined by their potential ability to contribute beneficial traits to crops, such as pest or disease resistance, yield improvement or stability. They have also increased the nutritional contents of crops, including protein in durum wheat, calcium in potato, and Provitamin A in tomato. More than 1,700 new plants have been discovered including crop wild relatives in the past year, including species that could help provide food in the future. For example special species of five new types of Manihot, new species of climbing vine *Mucuna*, used in the treatment of Parkinson's disease. Seven new species of *Aspalathus*, etc., The discovery of wild crops relatives was important because contemporary crops had been bred for high yields and had often lost their genetic diversity and resilience to drought and pests. Crop wild relatives - their genomes have the genes that will enable resilience against biotic and abiotic stresses [8,12].

Roughly 50,000-60,000 species of CWR are known world wide, that is to say, they have the same genus as crops. 700 CWR is considered the highest priority from a global perspective, because of containing the primary and secondary gene pools of the world's most important food crops. Food and agriculture production is dependent on genetic resources originally domesticated elsewhere on the Earth and subsequently used and developed in other countries and regions.

Modern Plant Breeding Generally, plant breeders prefers to work with existing cultivars or advanced breeding materials (sometimes called elite materials), because these adapted sources of material are already highly productive and relatively easy to intimate. But because pests and diseases evolve over times, breeders continually need new and diverse germplasm from outside the standard gene pool to find specific traits to maintain or improve yields. Sometimes as a last resort, breeders rely on landraces and wild relatives of crops, but these generally carry unwanted traits that are linked with a desirable traits gene, making it difficult to incorporate the trait into highyielding cultivars. When used, however, genes from landraces or wild relatives often have had disproportionately large and beneficial impacts. Some breeders also seek and use traits and information from „genetic stock" which include mutants and other germpalsm with genetic abnormalities.

SHORT HISTORIC REVIEW

Centers of crop origin are also considered centers of plant diversity. A well-known scientist and traveler, initially identified 8 centers, later subdividing them into 12 in 1935. The designated centers of origin and their boundaries were re-revised subsequently by different authors in more detailed studies, and a greater number of crops/species was taken into account.

Sites of early farming which were discovered through efforts of archaeologists can definitely prove the presence of a cradle of agriculture on the site. Such sites of early farming have been discovered in Thailand (11,000 BC), Near East (9,000 BC) and Mexico (6,000 BC.) [13]. Suggested that generally, agriculture began not once but several times, more or less simultaneously and in different regions of the world. His concept envisaged centers of agricultural origin from which farming spread into one or more regions. According to him, the following basic centers and regions of diversity exist.

Nuclear centres and regions of diversity of domesticated plants after Northern China (China, India, South-East Asia), The Near East (Central Asia,The Near East, The Mediterranean;Ethiopia; West Africa),Southern Mexico (Meso America), Central to Southern Peru (Northern Andes,Venezuela, Bolivia) Analysis of the relationships among centers of biodiversity, centers of cultivation, breeding programs and gene reservoir spectrum of plant genetic resources states in his work [13,14].

Benefits Of Germplasm Collection Collection of germplasm has been found to yield many advantages and these benefits are outlined below.

1) Cell and tissue culture of many plants species can be cryopreserved and maintained in a viable state for several years and used when required [14]

2) Plant material from endangered species can be conserved using this method.

3) It is an ideal method for long term conservation of cell cultures producing secondary metabolites such as antibiotics

4) Recalcitrant seeds (seeds which loose their viability on storage) can be maintained for a long period of time.

5) Disease free plants material can be frozen and propagated whenever required.

6) Conservation of somaclonal variations in cultures.
7) Rare germplasms developed by using somatic hybridization and other genetic manipulation techniques can be stored.
8) Pollen conservation for enhancing longevity.
9) Germplasm banks facilitate the exchange of information at international level

Techniques of Germplasm Preservation And Propagation

Cryopreservation: The freeze preservation of cells or tissues in liquid nitrogen at -196oC is known as cryopreservation. This technique involves four steps.

a) Freezing: The procedure of freezing may be conducted slowly, rapidly or initial freezing by dropping temperature slowly and followed by a rapid decrease in temperature. In order that the plants are affected by the sudden decrease in temperature, treatment of cells with plant verification solution helps cells and tissue to overcome the harsh temperature. The medium was added with cry protectant like DMSO, glycerol and proline to the culture medium to protect cells from injury. The addition of cry protectant protects the cell by prevention of large crystals inside cell, protect from water loss from cell. The frozen cells are stored in a refrigerator containing liquid nitrogen. The temperature of such refrigerator is maintained at or below - 130^{o}C. Organized tissues like shoot tips, somatic and zygotic embryos are usually chosen for storage. Attentively cells can be immobilized in sodium alginate and then cryopreserved.

b) Thawing: Thawing of culture is done in a rapid process. The freeze preserved culture is dipped in a water bath containing water at about 37-40^{o}C for 90 seconds. This process is done rapidly so that no ice crystals are formed. The thawed culture is washed several times to remove cryoprotectant. In the recent times, the cryoprotectant is removed by diluting. This is done by fixing the culture along with a cryoprotectant onto a disk and is kept on a suitable medium. The disk is frequently transferred into a fresh medium. This frequent transfer dilutes out the cryoprotectant.

c) Reculture: The culture which is freeze preserved need to be thawed and cultured to bring it back to normal life. The optimum conditions of freeze preserved plants have to be determined for developing a successful reculture. After cryopreservation, some plants tend to show special requirement for growth which was not necessary under normal propagation of the corresponding plants. For example, tomato shoots tips when cryopreserved, thawed and recultured, the culture required some levels of abscisic acid in their medium in order to initiate and develop shoot tip from callus formed.

Slow growth cultures: This is another method that can be used in germplasm preservation and propagation. It involves limiting the conditions of growth so that the culture does not grow and propagate in ordinary pace. This can be achieved by limiting the factors affecting the growth. This provides an attractive alternative to cryopreservative as the procedure is cost effective and simply comparatively. There is also reduction of contamination and gene modification [15,16]. The various factors affecting the growth of cultures are temperature, nutrient restriction, growth regulation and osmotic concentration. Other factors that can affect growth of cultures include oxygen concentration, type of culture vessel used as well as restriction of illumination received by cultures.

DNA clones: The germplasm can also be preserved in DNA segments cloned into appropriate vectors but the process demands high expertise and is costly.

Artificial seeds: Another mechanism of germplasm preservation is by desiccating embryos and storing it as¬ artificial seeds. This has proved to be an effective technique, but was possible only with somatic embryo and in certain cases by shoot tips. This process of germplasm preservation offers several advantages like cost effective, availability of germplasm of specific plants to propagate, small storage space, and longer terms of storage. In addition, it reduces risks such as cell damage by cryopreservation, and does not involve high technology associated with other methods.

Cold storage: In this technique, bulbs, tubers and rhizome of certain crops can be stored at 0 -15oC under high humidity for several months or up to one year. However, it is also a type of short- term storage [17].

Super-cold storage: This technique allows the embryos, tissues and pollen grains to be stored for long¬ periods in liquid nitrogen at -1960C. However, its practical use is yet to be developed as crops require different cooling and thawing treatments and have different viability level.

In-vitro storage: This is another method of germplasm preservation which is now routinely used for germplasm of some crops like cassava. The in-vitro cassava gene bank at CIAT, Colombia, comprises nearly 5000 clones, in an area of 50 square meters, with transfer (subculture) intervals of 12 – 14 months [18]. While in-vitro storage thus offers some advantage over field genebanks, such as requiring less space and limited labour cost [18], however, the management of large collections remains problematical, due to the requirement for periodic subculture. The possible introduction of genetic variants during culture may be a risk with some types of culture [19].

Field conservation: Germplasm materials such as fruit trees, potatoes and grasses are grown in nurseries field for preservation. Field nurseries can be maintained at different elevations above sea level.

IMPORTANCE OF SPECIES REINTRODUCTION TO NATURE

Importance of reintroduction (*in situ* sourcing an *ex situ* sourcing) is great. Reintroduction may involve returning native species to localities where they had been extirpated and allows the creation of completely new genotypes due to epigenetic activities, which mean that genetic activity is very important. Sometimes we can read about "reestablishment" instead of "reintroduction". The practice of reintroduction for gene conservation is starting in the 20th century. More information can be found on Species reintroduction Species reintroduction is the release of a species into the wild nature, or to the other areas where the organism is capable of survival. The basic goal of species reintroduction is to establish a healthy, genetically diverse, self-sustaining population to an area where it already has been extirpated. The second option is the problem of augmenting of so far existing population. Species for reintroduction are often typically threatened or endangered in the wild nature [20]. National parks-their use in the search of genetic resources are known. For example, Ecuador, constitute one of the world's 5 megadiversity hotspots of vascular plants [21]. From the physiological view, there is also a possibility to find a new physiological model [21]. There is also option to use forgotten-crops as a-the-future crops-of-food [21-25]. The source of the new genotypes-genetic resources can be found not only in the world nature plant centers. (The Amazon, South America-Chile etc.,). There is a possibility to search for and use model plants with stress resistance from other different localities for physiological research. For example, there are no presented examples of crops, but examples of two interesting extremely dry-resistant plants. The first example is *boscia salicifolia* oliv[26-29]

CONCLUSION

Human interest in agriculture began about 10,000-14,000 years ago [30,31], and from this period there has been a change from the collection of plants for human food to their targeted cultivation. In this process, a wide variety of future crop variability was created through conscious or unconscious selection. The basis for the varieties emergence emerged in the current epoch of human development, when only 30 basic crops (and 12 major) have been used in many human generations [32].

The population growth, which is a matter of people's responsibility, climatic change, especially variability of weather, growth of greenhouse gases, directly forces to, apart from breeding [33], to seek out new crops and new genotypes of crops tolerant to abiotic and biotic stress, or to utilize to a greater extent some minor crops with a high level of resistance to environmental stress.

In the case of genetic resources, the search for new genotypes can be carried out not only in traditional sites where gene sources are located, but there is also a possibility for selection in nature reserves, where many plant species have adapted to the given conditions and can serve as new crops or genetic resources, and also as a physiological models for stress tolerant genotypes [34].

The greater use of crop wild relatives, which are species related to crops that have important potential ability to contribute to crops traits such as i.e., pest and disease resistance, yield stability, quality improvement etc., is also advisable. The utilization of natural resources is an advantage compared to genetically modified genotypes because we have a ready-made balanced polymorphism for the given environment, the nearly finished breeding material. Different crop wild relatives have been used for thousands of years ago [35]. They also help in process of breeding crops in the creation of stress -tolerant crops resistant against abiotic conditions such as drought. They have their use in crop improvement which should increase substantially.

Great attention is paid to the development of conservation of genetic resources and constantly improving this technology, but the growth of the human population accompanied by current climate change is in contrast to the relatively small spectrum of crops available for human food. The utilization of new crops is neglected, but a wide spectrum of crops would, in variable environmental conditions, be more advantageous in the case of yield failure of some crops according to environmental conditions.

We have almost exhausted the possibility to feed humanity with the so far utilized crops. One of the many ways how to solve this problem is not only genetic manipulation but also searching for new crops and finding crops better adapted to new conditions. Older Indian civilizations with their substantially less numerous populations had more crops not only for gurmanian reasons. And we? What are we waiting for?

REFERENCES

1. Alcorn, J. B. (1991). “Ethics, Economics and Conservation.” In M. Oldfield and J. Alcorn, eds., Biodiversity; Culture, Conservation and Ecodevelopment.Westview Press, Colo. Boulder
2. https://link.springer.com/chapter/10.1007/978-0-387-74907-5_7
3. Towill, L. E. (1988). Genetic considerations for germplasm preservation of clonal materials. Horticultural Sciences.23: 77-97.
4. Wilkes, G. (1977). “The World‟s Crop Plant Germplasm: An Endangered Resource.” Bulletin Atomic Scientist 38: 8-16.
5. Wilkes, G. (1991). “In Situ Conservation of Agricultural Systems.” In M. Oldfield and J. Alcorn, eds. Biodiversity; Culture, Conservation and Ecodevelopment. Westview Press, Colo. Boulder.
6. Williams, J. T. (1991). “Plant Genetic Resources: Some New Directions.” Advances in Agronomy 45: 61-91.
7. Duvick, D. N. (1982). Genetic diversity in major farm crops on the farm and in reserve. Australian Plant Breeding and Genetics Newsletter. 32: 1-40.
8. Stewart GR, Larher F (1980) Accumulation of amino acids and related compounds in relation to environmental stress. In: Miflin BJ (ed) The biochemistry of plants, Academic Press, New York, pp. 609-635.
9. Vandenbussche B, De Proft MP (1998) Cryopreservation of in vitro sugar beet shoot tips using the encapsulationdehydration technique: influence of abscisic acid and cold acclimation. Plant Cell Rep 17: 791-793.
10. Towill LE, Walters C (2000) Cryopreservation of pollen. In: Engelmann F, Takagi H (eds) Cryopreservation of tropical plant germplasm. Current Research Progress and Application,Ibarak, Japan, JIRCAS/IPGRI, pp. 115-129.
11. Zhao MA, Dhital SP, Fang YL, Khu DM, Song YS, Park EJ, Kang CW, Lim HT (2005) Application of slow-freezing cryopreservation method for the conservation of diverse potato (Solanum tuberosum L.) genotypes. J Plant Biotech 7 (3):183--186.
12. Zhang YL, Li BL, Wang H, Liu Y (2009) The changes of total soluble proteins and calcium in crypreservation pollen of Prunus mume. Paper presented at the 1 st International Symposium on Cryopreservation in Horticultural Species, Leuven, Belgium, 5-9 April 2009.
13. West TP, Ravindra MB, Preece JE (2006) Encapsulation, cold storage, and growth of Hibiscus moscheutos nodal segments. Plant Cell Tiss Organ Cult 87: 223-231.
14. Walters C, Volk GM, Towill LE, Forsline PhL (2009) Survival of cryogenically-stored dormant apple buds: a 20 year assessment. Paper presented at the 1 st International Symposium on Cryopreservation in Horticultural Species, Leuven, Belgium, 5-9 April 2009.
15. Tokatli YO, Akdemir H (2009) Droplet vitrification of shoot tips of fraser photinia (Photinia × fraseri Dress.). Paper presented at the 1 st International Symposium on Cryopreservation in Horticultural Species, Leuven, Belgium, 5-9 April 2009. Towill, L.E. 1991 Cryopreservation. In: Dodds JH (ed) In vitro methods for conservation of plant genetic resources, Chapman and Hall, London, pp. 41-70
16. Reed BM, Schumacher L, Dumet D, Benson EE (2005) Evaluation of a modified encapsulation-dehydration procedure in corporating sucrose pretreatments for the cryopreservation of Ribes germplasm. In Vitro Cell Dev Biol Plant 41: 431-436.
17. Rajasekharan PE (2006) Seed cryoservation: problems and prospects. Paper presented at the ICAR Short Course on In Vitro Conservation and Cryopreservation-New Options to Conserve Horticultural Genetic Resources, Banglore, India, 21-30 September 2006.
18. Rajasekharan PE (2006) In vitro conservation of horticultural crops. Paper presented at the ICAR Short Course on In Vitro Conservation and Cryopreservation-New Options to Conserve Horticultural Genetic Resources, Banglore, India, 21-30 September 2006

19. Pennycooke JC, Towill LE (2000) Cryopreservation of shoot tips from in vitro plants of sweet potato [Ipomoea batatas (L.) Lam] by vitrification. Plant Cell Rep 19: 733-737
20. Nkongolo KK, Nsapato L (2003) Genetic diversity in Sorghum bicolor (L.) Moench accessions from different ecogeographical regions in Malawi assessed with RAPDs. Genet Resour Crop Evol 50: 149-156.
21. McGregor CE, van Treuren R, Hoekstra R, van Hintum ThJL (2002) Analysis of the wild potato germplasm of the series Acaulia with AFLPs: implications for ex situ conservation. Theor Appl Genet 104: 146-156
22. Marassi MA, Scocchi A, Gonzalez AM (2006) Plant regeneration from rice anthers cryopreserved by an encapsulation/dehydration technique. In Vitro Cell Dev BiolPlant. 42: 31-36.
23. Kryszczuk A, Keller J, Grübe M, Zimnoch-Guzowska E (2006) Cryopreservation of potato (Solanum tuberosum L.) shoot tips using vitrification and droplet method. Food Agric Environ 4: 196-200.
24. Kobayashi T, Niino T, Kobayashi M (2006) Cryopreservation of tobacco BY-2 suspension cell cultures by vitrification with encapsulation. Plant Biotech 23: 333-337.
25. Panis B, Swennen R, Engelman F (2001) Cryopreservation of plant germplasm. Acta Hort 650: 79-86.
26. Morgante M, Hanafey M, Powell W (2002) Microsatellites are preferentially associated with non repetitive DNA in plant genome. Nat Genet 30: 194-200.
27. Hirai D, Sakai A (2003) Simplified cryopreservation of sweet potato (Ipomoea batatas Lam) by optimizing conditions for osmoprotection. Plant Cell Rep 21: 961- 966.
28. Harding K, Millam S (2000) Analysis of chromatin, nuclear DNA and organelle composition in somatic hybrids between Solanum tuberosum and Solanum sanctaerosae. Theor Appl Genet 101: 939-947
29. Singh S. Medicinal plants –A Natural gift to mankind. *Agriculture Today*. 2005;3(3):58–60.
30. Roberts EH. *Viability of Seeds*. London: Chapman and Hall; 1972.
31. Harrington JF. 'Seed and pollen storage for conservation of plant genetic resources'. In: *Genetic Resources in Plants*. Their Exploration and Conservation. Frankel OH, et al. editors. UK: Blackwell; 1970. p. 501–521.
32. Roberts EH. Problems of long–term storage and seed and pollen of genetic resources conservation'. In: *Crop Genetic Resources for Today and Tomorrow*. Frankel OH, et al. editors. Cambridge, UK: Cambridge University Press; 1975. p. 226–296.
33. Withers LA. Biotechnology and plant genetic resources conservation. In: *Plant Genetic Resources Conservation and Management*. Paroda RS, et al. editors. New Delhi: IBPGR Regional Office; 1991. p. 273–297.
34. Towill LE. Low temperature and freeze/vacuum–drying preservation of pollen. In: *Cryopreservation of Plant Cells and Organs*. Kartha KK, et al. editors. Florida, USA: CRC Press; 1985. p. 171–198.
35. Harding K, Benson EE (2001) The use of microsatellite analysis in Solanum tuberosum L. in vitro plantlets derived from cryopreserved germplasm. CryoLetters 22: 199-208.