Axillary Bud Proliferation Approach for Plant Biodiversity Conservation and Restoration

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Due to mainly human population pressure and activities, global biodiversity is getting reduced and particularly plant biodiversity is becoming at high risk of extinction. Consequently, many efforts have been deployed to develop conservation methods. Because it does not involve cell dedifferentiation of differentiated cells but rather the development and growth of new shoots from preexisting meristems, the axillary bud proliferation approach is the method offering least risk of genetic instability. Indeed, meristems are more resistant to genetic changes than disorganized tissues. The present review explored through the scientific literature the axillary bud proliferation approach and the possible somaclonal variation that could arise from it. Almost genetic stability or low level of genetic variation is often reported. On the contrary, in a few cases studied to date, DNA methylation alterations often appeared in the progenies, showing epigenetic variations in the regenerated plants from axillary bud culture. Fortunately, epigenetic changes are often temporary and plants may revert to the normal phenotype. Thus, in the absence of genetic variations and the existence of reverting epigenetic changes over time, axillary bud culture can be adopted as an alternative nonconventional way of conserving and restoring of plant biodiversity.

1. Introduction

Global biodiversity is defined as the variation of all life on earth and the ecological complexes in which it occurs [1]. Biodiversity refers to genetic diversity, species diversity, and ecosystem diversity [2, 3] and includes the forest and agricultural ecosystems and the wild animals [4].

Among the above components, plants represent a vital part of biodiversity and healthy ecosystems. They provide multiple ecosystem services including production of oxygen for the rest of living organisms [5, 6], removal of atmospheric carbon dioxide emissions in the photosynthesis process, creation and stabilization of soil, protection of watersheds, and provision of natural resources including food, fibre, fuel, shelter, and medicine [7]. They also play an important role in the water cycle and constitute habitat for a wide range of other living organisms. Thus, plants are the basis for life on earth and humans are quite dependent on them [8–10] given that they are fundamental structural and nutrient-sequestering components of most ecosystems.

Due to dependency on biodiversity, the number of threatened plant species has gradually increased during the last decade, the maximum being observed in 2011 [11].

The key factor in threats to earth's biodiversity is often cited as the human population size, density, and growth [12–15]. As reported by the United Nations Population Division, the world had 2.5 billion people in 1950. This number was almost tripled in 2005 by reaching 6.5 billion people, while it is projected to rise to more than 9 billion people by 2050 [16]. The less developed world is showed to have the highest rate of human population growth [17]. Thus, the needs for this growing population must have much impact on biodiversity on which it depends for its survival.

The world’s human population exponential growth and spatial expansion have been accompanied by changes in landuse, pollution, and overexploitation of natural resources [18],

First, human-driven land-use is among the greatest threats to terrestrial and aquatic biodiversity [36, 37] by causing habitat destruction in protected and nonprotected areas, which is motivated mostly by agricultural expansion [38–41].

Second, urbanization, another consequence of human population massive growth, is considered to be another major threat to biodiversity by a wide range of scientific literature [38, 42–53]. For instance, due to human population growth and migration, there will be nearly 2 billion new urban residents by 2030 [54], which means further habitat destruction accompanied by various sorts of pollution.

Third and corollary to the above impacts, human pressure is also responsible for different types of pollution, leading mainly to climate change, such as global warming [55]. The main consequences of climate change or global warming are (i) the extinction of plant pollinators [56, 57] which causes loss of genetic diversity and (ii) the habitat fragmentation resulting in loss of genetic diversity in local and global plant populations and bottleneck events in these populations [58].

These anthropogenic activities place global biodiversity and particularly plant species at risk of extinction even in the biodiversity hotspots [59, 60].

In order to reduce the growing extinction risk of plant biodiversity, its conservation and restoration was revealed to be more than a priority. Two conventional methods of conservation are used, *in situ* and *ex situ* conservation, which are complementary to each other. *In situ* methods allow conservation to occur with ongoing natural evolutionary processes [61, 62]. *Ex situ* strategies maintain the biological materials outside their natural habitats [63] and often use plant biotechnology such as plant cryopreservation or micropropagation. The most important technique in micropropagation is meristem proliferation in which apical buds or nodal segments harbouring an axillary bud are cultured to regenerate multiple shoots without intervention of callus phase [64]. Meristem culture is also an efficient tool for regeneration, elimination of viruses from infected plants, and then production of virus-free seed material of different plant species [65–67].

In the present review, we briefly present the axillary bud proliferation approach as an alternative way that can be used for plant biodiversity conservation and restoration.

2. Axillary Bud Culture and Mass Propagation of Plant Species

Among the methods developed for plant micropropagation, the axillary bud proliferation is the most used and is also considered the most suitable to guarantee genetic stability of the regenerated plants obtained. For rapid *in vitro* clonal propagation of plants, normally dormant axillary buds are induced to grow into multiple shoots by judicious use of growth regulators, cytokinins for activation and sprouting of dormant axillary bud and multiplication or cytokinin and auxin synergistic combinations for shoot multiplication.

In this way and because of its simplicity and reliability for clonal propagation, axillary bud culture was the method adopted for the mass micropropagation of various plants including almond [68], apple [69], *Acacia mangium* Willd [70], *Cedrus* [71], *Eucalyptus* [72], *Anoectochilus formosanus* Hayata [73], *Swertia chirayita* ( Roxb. ex Fleming) H. Karst. [74], *Curcuma longa* L. [76], *Rosa rugosa* Thunb. [77], hazelnut [78], marula tree [79], *Fagopyrum dibotrys* Hara mutant [80], *Curcuma amada* Roxb. [81], *Alpinia galanga* Linn [82], apple rootstock [83], apple rootstock Merton 793 [84], *Cannabis sativa* L. [85], hops [86], *Allium ampeloprasum* L. [87], squill [88], olive [89], *Pinus thunbergii* Parl. [90], *Piper longum* L. [91], *Prospis chilensis* ( Mol.) Stuntz [92], barley [93], rice [94], almond [95], *Dendrobium longicornu* Lindl. [96], and *Mahonia leschenaultii* Nutt. [97].

It is the stimulation of axillary buds to develop into a shoot [98] and this technique is comprised of the meristem and shoot tip culture and the bud culture [98]. It is a method exploiting the normal ontogenetic route for plant development by lateral meristems.

Since this technology does not involve cell dedifferentiation of differentiated cells but rather the development and growth of new shoots from preexisting meristems, it has been usually pointed out as the most faithful way of propagating plants *in vitro*. Indeed, it is thought to induce recovery of genetically stable and true-to-type progenies [99, 100], very little or no genetic variation [101], and epigenetic stability [102].

3. Axillary Bud Culture and Somaclonal Variation

Axillary bud culture is one of the multiple techniques of plant *in vitro* culture. A major problem associated with *in vitro* culture systems is the occurrence of somaclonal variation amongst subclones of one parental line [103, 104]. Somaclonal variation is manifested as cytological abnormalities, frequent qualitative and quantitative phenotypic mutations, sequence change, gene activation and silencing [105, 106], and transposon and retrotransposon activation [107–110]. Thus, somaclonal variation is considered at both genetic and epigenetic levels.

Although somaclonal variation provides a valuable source of genetic variation for the improvement of crops through the selection of novel variants, which may show resistance to disease, improved quality, or higher yield [111–114], it may result in off-types that reduce the commercial value of resultant plants [115] and then shall be an important obstacle for plant biodiversity conservation. Plant *in vitro* culture, being comprised of sequential dedifferentiation (formation of callus) and redifferentiation (regeneration into plants) stages [116, 117], represents traumatic stress to plant cells and organs and often engenders an array of genetic and epigenetic alterations [118].

It is generally assumed that axillary bud culture is the method offering least risk of genetic instability since meristems are more resistant to genetic changes than disorganized tissues [119, 120]. Though it is sometimes species-specific
Table 1: Clonally propagated plant species through axillary proliferation approach and the state of subsequent genetic somaclonal variation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Used marker</th>
<th>Genetic variation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cedrus atlantica L.</td>
<td>RAPD</td>
<td>Not significant</td>
<td>[71]</td>
</tr>
<tr>
<td>Cedrus libani L.</td>
<td>RAPD</td>
<td>Not significant</td>
<td>[71]</td>
</tr>
<tr>
<td>Eucalyptus tereticornis Smith</td>
<td>RFLP, RAPD</td>
<td>No</td>
<td>[72]</td>
</tr>
<tr>
<td>Eucalyptus camaldulensis Dehn.</td>
<td>RFLP, RAPD</td>
<td>No</td>
<td>[72]</td>
</tr>
<tr>
<td>Anoectochilus formosanus H.</td>
<td>ISSR</td>
<td>Low genetic instability</td>
<td>[73]</td>
</tr>
<tr>
<td>Swertia chirayita (Roxb. ex Fleming) H. Karst.</td>
<td>ISSR</td>
<td>No</td>
<td>[74]</td>
</tr>
<tr>
<td>Carcuna longa L.</td>
<td>Cytophotometry and RAPD</td>
<td>No</td>
<td>[76]</td>
</tr>
<tr>
<td>Prunus dulcis [Mill.] D. A. Webb</td>
<td>RAPD and ISSR</td>
<td>No</td>
<td>[95]</td>
</tr>
<tr>
<td>Alpinia galanga L.</td>
<td>RAPD and ISSR</td>
<td>No</td>
<td>[82]</td>
</tr>
<tr>
<td>Malus pumila Mill.</td>
<td>RAPD</td>
<td>Not significant</td>
<td>[83]</td>
</tr>
<tr>
<td>Apple rootstock Merton 1973</td>
<td>ISSR</td>
<td>No</td>
<td>[84]</td>
</tr>
<tr>
<td>Cannabis sativa L.</td>
<td>ISSR</td>
<td>No</td>
<td>[85]</td>
</tr>
<tr>
<td>Humulus lupulus L.</td>
<td>RAPD and REMAP</td>
<td>No</td>
<td>[86]</td>
</tr>
<tr>
<td>Allium amelooprasum L.</td>
<td>ISSR</td>
<td>No</td>
<td>[87]</td>
</tr>
<tr>
<td>Vitis spp.</td>
<td>RAPD and ISSR</td>
<td>No</td>
<td>[136]</td>
</tr>
<tr>
<td>Hybrid hazelnut</td>
<td>RAPD</td>
<td>No</td>
<td>[78]</td>
</tr>
<tr>
<td>Sclerocarya birrea subsp. caffra</td>
<td>RAPD</td>
<td>No</td>
<td>[79]</td>
</tr>
<tr>
<td>Ochreinauclea missionsis (Wall. ex G. Don) Ridsd.</td>
<td>ISSR</td>
<td>No</td>
<td>[108]</td>
</tr>
<tr>
<td>Fragaria x ananassa</td>
<td>RAPD-PCR</td>
<td>No</td>
<td>[137]</td>
</tr>
<tr>
<td>Doritaenopsis glenyle “Labios”</td>
<td>RAPD</td>
<td>No</td>
<td>[127]</td>
</tr>
<tr>
<td>Ocimum kilimandscharicum Guerke</td>
<td>RAPD</td>
<td>No</td>
<td>[138]</td>
</tr>
</tbody>
</table>

Table 2: Clonally propagated plant species through axillary proliferation approach and the state of subsequent epigenetic somaclonal variation.

<table>
<thead>
<tr>
<th>Species</th>
<th>Used marker</th>
<th>Epigenetic variation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bambusa balcooa Roxb.</td>
<td>MSAP</td>
<td>No</td>
<td>[121]</td>
</tr>
<tr>
<td>Cedrus atlantica L. and C. libani L.</td>
<td>HPLC</td>
<td>Yes</td>
<td>[71]</td>
</tr>
<tr>
<td>Corylus avellana L.</td>
<td>Isoschizomer restriction analysis</td>
<td>Yes</td>
<td>[123]</td>
</tr>
<tr>
<td>Malusdomestica</td>
<td>MS-AFLP</td>
<td>Yes</td>
<td>[124]</td>
</tr>
<tr>
<td>Myrtus communis L.</td>
<td>HPLC</td>
<td>No</td>
<td>[122]</td>
</tr>
<tr>
<td>Pisum sativum L.</td>
<td>MSAP, HPCE</td>
<td>Yes</td>
<td>[125]</td>
</tr>
<tr>
<td>Solanum tuberosum L.</td>
<td>MS-AFLP</td>
<td>No</td>
<td>[102]</td>
</tr>
<tr>
<td>Vitis vinifera L.</td>
<td>MSAP</td>
<td>Yes</td>
<td>[126]</td>
</tr>
<tr>
<td>Doritaenopsis glenyle “Labios”</td>
<td>MSAP</td>
<td>Yes</td>
<td>[127]</td>
</tr>
<tr>
<td>Humulus lupulus L.</td>
<td>MSAP</td>
<td>Yes</td>
<td>[86]</td>
</tr>
</tbody>
</table>

Contrary to a wide range of genetic investigations in regenerated plants from axillary bud culture, there is no much work in the epigenetic way. Nonetheless, the axillary bud culture system may cause epigenetic alteration according to few papers regarding the epigenetic aspect of somaclonal variation (Table 2). On the investigated cases, epigenetic stability was only observed in three plants including Bambusa balcooa Roxb. [121], Myrtus communis L. [122], and Solanum tuberosum L. [102]. The remaining show DNA methylation alterations in Cedrus atlantica L. and C. libani L. [71],
Genomic DNA

Propagation via axillary branching method

Time

No genetic alteration

No genetic alteration

(a)

Propagation in the field via seeds

Some epigenetic alteration

Less epigenetic alteration

(b)

Figure 1: Schematic representation of the genetic (a) and epigenetic (b) variations arising from the axillary bud proliferation approach and their status over time, that is, after plant propagation in the field via the seeds (adapted from [135]).

Corylus avellana L. [123], Malus domestica cv. "Gala" [124], Pum sativum L. [125], Vitis vinifera L. [126], Doritaenopsis glenyle “Labios” [127], and Humulus lupulus L. [86].

Interestingly in the same studied plant species, genetic variation is quite absent while epigenetic alterations occur [86, 127], showing that only epigenetics will impact on the phenotype of the progenies from axillary bud culture in such plants and will then alone govern the consequent variant phenotype.

4. Axillary Bud Culture in Plant Biodiversity Conservation

Biotechnology such as axillary bud culture is supposed to help in conservation and restoration of plants without affecting main features of the plants. It is known that phenotypic variations in living organisms are strongly governed by both genetic and epigenetic fluctuations [128–130]. Thus, conservation and restoration methods that could induce genetic and/or epigenetic variation such as somaclonal variations should produce mutations in plant progenies which may affect their stability and viability.

By using axillary bud culture for mass propagation of various plant species and particularly the endangered ones, it is evident through the present review that genetic variations are almost absent (Table 1). This confirms a clonal fidelity and then true-to-type progenies. The genetic stability will then remain through propagation in the field via seeds of the genetically stable progenies (Figure 1(a)) unless genetic variability is induced by sexual and natural selection processes.

The genetic stability explored throughout this review was depicted only by one DNA molecular marker in some cases (Table 1). Nonetheless, the use of one type of molecular marker to assess the stability of in vitro propagated plants may be insufficient. This is why different authors have recently exploited more than one molecular marker for studying of somaclonal variation in regenerants of several plant species. For instance, a relatively low level of polymorphism was detected with RAPD markers in Actinidia delicosa A. Chev. cultures, whereas the polymorphism was higher with SSR markers [131].

On the other hand, epigenetic changes are often observed even in the absence of genetic variation (Table 2). This may then produce off-to-types progenies at the epigenetic level. Epigenetics such as cytosine methylation has been proposed to have diverse cellular functions in eukaryotes, but its primary role was believed to serve as a genome surveillance and defense system such as taming of transposable elements [132, 133]. This should then provoke their mobility throughout the genome and genetic mutations. Thus, assessment of epigenetic alterations arising from axillary bud culture process should be accompanied by the analysis of the possible consequent mobility of transposable elements.

Fortunately, epigenetic changes are often temporary and plants may revert to the normal phenotype relatively easily though some can be long-lasting and may even be transferred during sexual propagation [134, 135]. By propagating the resulting progenies in the field via their seeds, there will be less epigenetic alteration in offspring (Figure 1(b)).

Thus, in the absence of genetic variations and the existence of reverting epigenetic changes over time, axillary bud culture can be adopted as an alternative way of conserving and restoring of endangered plant species. However, for a better planning of plant conservation and restoration by means of axillary bud proliferation approach, more than one genetic and epigenetic molecular marker should be used to rule out biased genetic and epigenetic results. This should be followed by the assessment of the status of transposable elements and the gene expression to assure the stability of the progenies.

5. Conclusion

The present review presented the benefits of the axillary bud culture as an alternative nonconventional way of conserving plant species which are facing extinction mainly due to human pressure. Evidence is increasing that axillary bud
culture generates clonal fidelity and true-to-parental type progenies. It should however be synergistically used with other confirmed biotechnological methods of plant conservation such as cryopreservation in addition to conventional techniques. Moreover, close attention should be paid to the genetic and epigenetic variation as well as karyological and morphological stability brought by axillary bud proliferation approach before any conservation plan.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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