Asymmetric Somatic Plant Hybridization: Status and Applications

Lakshmanan Prabhu Shankar1,2, Eeckhaut Tom1, Deryckere Dieter1, Van Bockstaele Erik1,2, Van Huylenbroeck Johan1

1Institute for Agricultural and Fisheries Research (ILVO), Plant Sciences Unit, Applied Genetics and Breeding, Melle, Belgium; 2Department of Plant Production, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium.
Email: johan.vanhuylenbroeck@ilvo.vlaanderen.be

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ABSTRACT

To create asymmetric somatic hybrids, the genome of the so-called donor protoplast is fragmented prior to protoplast fusion. As a result, only a limited amount of the donor genome is transferred to the fusion product. This technique can circumvent some commonly observed problems related to symmetric fusion and offers a practical breeding tool for asexual hybridization. Genomes are typically fragmented by irradiation, microprotoplast production or application of metabolic inhibitors such as iodoacetamide. Irradiation and microprotoplast production fragment the nuclear genome, whereas iodoacetamide inactivates the cytoplasmic genome. It can therefore be used to introduce cytoplasmic male sterility, an important practical application. For hybrid verification and genome characterization, molecular markers and cytogenetic techniques are applied. This review highlights and discusses progress made during the last decade in spermatophytes asymmetric protoplast fusion.

Keywords: Asymmetric Somatic Fusion; Genome Fragmentation; Protoplast

1. Introduction

Plant cells from which the cell wall has been enzymatically or mechanically removed are called protoplasts. Theoretically, protoplasts are totipotent, meaning that after their isolation and subsequent culture they have the capability to dedifferentiate, re-start the cell cycle, go through repeated mitotic divisions and then proliferate or regenerate into various organs. Fusion of protoplasts from different species can therefore be a practical breeding tool [1] and circumvents sexual hybridization related prezygotic or postzygotic barriers [2]. Somatic hybridization differs from other techniques in many respects. When comparing somatic hybridization to transgenic approaches, somatic hybridization enables broadening of the germplasm base, allows the transfer of uncloned multiple genes and generates products that are not subjected to the same legal regulations as transgenic lines [3,4]. Also, it transfers both mono- and polygenic traits [5]. The first protoplast fusion was described more than a century ago by Küster [6]. Tobacco was the first crop in which successful interspecific somatic hybridization was reported [7]. Since then, improvements and somatic hybrids have been made in many species and complete plant regeneration was accomplished. A detailed historical overview of breakthroughs in protoplast related research is incorporated in Gamborg [8] and Davey et al. [9].

Protoplast fusion can be either symmetric or asymmetric depending on the nature of genetic contribution of the fusion partners. A somatic breeding protocol can typically be subdivided into the following steps: isolation, fragmentation (in case of asymmetric hybridization), fusion, regeneration and selection [10,11] (Figure 1). In symmetric fusion the complete genomes of both parent protoplasts are fused. However, when two complete genomes fuse, a phenomenon called “gene conflict” may arise, because certain chromosomes repel one another. Moreover, the technique introduces a significant amount of unwanted genetic material. These limitations result in abnormal growth, regeneration of hybrids with low fertility, non-rooted shoots, slow hybrid growth, and recalcitrant calli or microcalli [2].

In asymmetric fusion, after genome fragmentation only a limited amount of one genome is transferred to the
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Plasmolysed source tissue
Isolated protoplasts
Symmetric fusion
Asymmetric fusion
Donor genome fragmentation
Chemical or electrical impulse
Acceptor cell with donor DNA fragment
Hybrid cell with both parent genome
Callogenesis
Organogenesis
Somatic hybrid
Hybrid characterization

**Figure 1. Schematic representation of symmetric and asymmetric somatic hybridization techniques.**

fusion product [12,13] (Figure 1). Cytoplasmic genomes can also be recombined with nuclear genomes for applications such as cytoplasmic male sterility (CMS) introduction [11]. The technique is being applied to circumvent the above mentioned barriers in symmetric fusion. For example, symmetric hybrids between *Brassica napus* and *Lesquerella fendleri* are self-sterile, whereas asymmetric hybrids between the same fusion parents are self-fertile [14]. Similarly, symmetric fusion between *Orychophragmus violaceus* and *B. napus* yields sterile hybrids, whereas asymmetric hybrids are fertile and can set seeds [15]. By introgressing fewer genes than after sexual crossing or symmetric somatic fusion, the number of backcrosses can also be significantly reduced. Genome fragmentation of the donor parent encourages the elimination of much of its redundant genetic material in the somatic hybrid. Moreover, in asymmetric fusions, most karyotype instability causing donor genes are eliminated during the first post-fusion mitoses, as opposed to symmetrical fusions after which eliminations can occur up to the first sexually derived generation [16]. In other words, not only does asymmetric fusion introduce fewer genes in a recipient genome after fragmenting the donor genome, but elimination of disadvantageous genes or chromosomes also proceeds quicker. Nevertheless, chromosomes and chromosome fragments can still be lost during meiosis due to rearrangements [17].

The objective of this review is to provide an overview of the development of technologies for fragmentation and screening. Besides the progress made in spermatophytes asymmetric protoplast fusion related research during the last decade is highlighted and discussed.

2. Fragmentation Techniques

In order to obtain asymmetric fusions, only partial genomes are transferred. Several techniques can be used for genome fragmentation of the so-called “donor” genome: irradiation, microprotoplasts (MPPs), or metabolic inhibitors as iodoacetamide (IOA). In some cases asymmetric fusions were realized without fragmentation treatment. The production of these asymmetric hybrids decreased possible long term irradiation effects on hybrid growth and development [18].

2.1. Irradiation

Genome fragmentation by irradiating can be achieved by using either ionizing radiation techniques (X or gamma rays) or non ionizing radiation (UV rays). Irradiation often induces random chromosome breakage, but also gene deletion and rearrangement and can be responsible for hybrid sterility [19]. The first application of X-rays to obtain asymmetric hybrids was performed in parsley [20]. UV light used to create asymmetric hybrids was used for the first time on *Nicotiana* donor protoplasts [21]. Earlier, X or gamma rays were more frequently used for donor protoplast fragmentation, but nowadays UV treated protoplasts are more widely applied. Both irradiation types efficiently induce asymmetric somatic hybrids in a dose-dependent manner, e.g., between *Brassica napus* and *Arabidopsis thaliana* [22]. Nonetheless, within a single species susceptibility towards different irradiation types can vary strongly [23]. Hall et al. [24] investigated whether UV radiation could be used as an alternative for ionizing radiation techniques. They found that UV had a detrimental effect on sugar beet protoplasts: resynthesis of a cell wall, cell growth and cell division were partially or totally eliminated. However, protoplast viability had not decreased after 6 days culture, but after 14 days, the UV treated cells died. On the other hand, a significant advantage of UV radiation over ionizing radiation was its easy application and high reproducibility. Similar observations were made after exposure of *Cichorium* proto-
plasts to UV [25]. In cucumber, the negative effect of UV-C irradiation on cell wall regeneration, protoplast viability and the intensity of the nuclei after DAPI staining was also demonstrated [26].

A general problem is the quantification of DNA damage after an irradiation treatment. Abas et al. presented Comet assay single cell gel electrophoresis (SCGE) as a reliable tool to observe single and double strand breaks in mesophyll protoplasts of Nicotiana plumbaginifolia [27]. Xu et al. revealed extensive DNA fragmentation in UV irradiated Citrus unshiu protoplasts with the terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling assay [28]. However, both methods are not generally applicable.

2.2. Microprotoplast Mediated Chromosome Transfer

Next to donor protoplast irradiation, micronuclei and microprotoplasts mediated chromosome transfer (MMCT), which was originally developed for mammalian cells, has been considered as an alternative method for partial genome transfer [29].

Mass induction of micronucleation and efficient isolation of microcells are key steps in any MMCT for successful transfer of partial genomes [30]. Microtubules are involved in several processes such as chromosome migration, cell structure, cellulose microfibrils guidance and arrangement, cell wall formation, intracellular movement and cell differentiation. Toxic substances as anti-mitotic herbicides or colchicine prevent their normal polymerization [31]. Application of these spindle toxins to synchronized cells generally blocks cells in metaphase and cell division by irreversibly inactivating the cytoplasm. In red chicory mesophyll protoplasts division was totally inhibited after treatment with 2 - 4 mM iodoacetate [40]. When using IOA, a lower optimal concentration of 1.625 mM was found [25]. The lower optimal IOA concentrations compared to iodoacetate might be explained by better cell penetration of IOA. Similar optimal IOA concentration were found in Musa [41]. Lower IOA concentrations (0.5 mM) stopped growth of Gossypium hirsutum protoplasts, whereas 3 mM and 7.5 mM IOA were required to stop cell proliferation in Citrus [42] and Butpleurum scorzonerefolium [43], respectively.

Fusion of IOA-treated recipient parental protoplasts with irradiated donor protoplasts could produce cybrids. In Cichorium, successful asymmetric protoplast fusion has been performed between γ-rays-irradiated sunflower protoplasts and iodoacetate-treated red chicory protoplasts [40]. IOA treatments prevent cell division, but fusion with non-IOA treated protoplasts restores cell division ability, thus opening ways towards heterokaryon or cybrid selection.

3. Asymmetric Hybridization

In earlier protoplast reviews, the concept of asymmetric hybridization is well explained, but has not been the focus of the review [11,12]. Fragmentation techniques have been highlighted, however, [24,29] also in practical manuals [44]. In the review by Xia [13], wheat is used as an example to discuss chromosome engineering through asymmetric fusion.

Over the last decade, asymmetric fusion techniques have been widely applied and several new asymmetric hybrids were obtained (Table 1). The most studied families were Brassicaceae and Poaceae, followed by Rutaceae. The number of asymmetric hybridization realized through PEG fusion was 4-fold the number of fusions generated by electrical fusion.

Asymmetric hybridization has allowed new genome combinations that would be more difficult, if not impossible, to realize through classical symmetric fusion or sexual crossing. For the first time, an asymmetric hybrid
Table 1. Progress in asymmetric protoplast fusion in different plant families during last decade (2004-2013).

<table>
<thead>
<tr>
<th>Plant family and species (acceptor + donor)</th>
<th>Aim</th>
<th>Cell source</th>
<th>Fragmentation tool</th>
<th>Fusion tool</th>
<th>Characterization</th>
<th>Ref.</th>
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<tbody>
<tr>
<td><strong>Apiaceae + Gentianaceae</strong></td>
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<tr>
<td>Bupleurum scorzonerifolium + Swertia mussottii</td>
<td>Secondary metabolites</td>
<td>C</td>
<td>UV</td>
<td>PEG</td>
<td>GISH</td>
<td>RAPD, SQ RT-PCR</td>
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<tr>
<td>Bupleurum scorzonerifolium + Swertia tetrapera</td>
<td>Secondary metabolites</td>
<td>SC + C</td>
<td>UV</td>
<td>PEG</td>
<td>CC, GISH</td>
<td>RAPD, SSR(C)</td>
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<tr>
<td>B. scorzonerifolium + Gentianopsis paludosa</td>
<td>Secondary metabolites</td>
<td>SC</td>
<td>UV</td>
<td>PEG</td>
<td>CC</td>
<td>RAPD, RFLP</td>
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<td><strong>Asteraceae</strong></td>
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<tr>
<td>Helianthus annuus + H. maximiliani</td>
<td>Biotic resistance</td>
<td>H + M</td>
<td>UV</td>
<td>EF</td>
<td>RAPD</td>
<td>Isozyme analysis</td>
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<tr>
<td><strong>Brassicaceae</strong></td>
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<tr>
<td>Brassica napus + Isatis indigotica</td>
<td>Genetic variation, Secondary metabolites</td>
<td>M</td>
<td>IOA(A) + UV</td>
<td>PEG</td>
<td>CC, GISH</td>
<td>AFLP, CAPS(C)</td>
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<tr>
<td>Brassica napus + Ornithophragmus violaceus</td>
<td>Fragmentation tool</td>
<td>M</td>
<td>IOA(A) + UV</td>
<td>-</td>
<td>CC, GISH</td>
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<tr>
<td>Brassica oleracea + B. nigra</td>
<td>Genetic variation, Biotic resistance</td>
<td>H + M</td>
<td>UV</td>
<td>PEG</td>
<td>CC, FCM, GISH</td>
<td>AFLP, CAPS(C), CAPS(M)</td>
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<tr>
<td>Brassica oleracea botrytis + B. carinata + B. juncia + B. nigra + Sinapis alba</td>
<td>Biotic resistance</td>
<td>H + M</td>
<td>X</td>
<td>PEG</td>
<td>RAPD</td>
<td>Resistance screening</td>
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<tr>
<td>Brassica oleracea capitata + Barbarea vulgaris + Capsella bursapastoris + Diplotaxis tenuifolia + Hesperis matronalis + Matthiola incana + Raphanus sativus + Sinapis alba</td>
<td>Biotic resistance</td>
<td>H + M</td>
<td>X</td>
<td>PEG</td>
<td>RAPD</td>
<td>Resistance screening</td>
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<tr>
<td>Ornithophragmus violaceus + Lesquerella fendleri (GFP)</td>
<td>Plastome transfer</td>
<td>M + C</td>
<td>γ</td>
<td>PEG</td>
<td>ITS, CAPS(M)</td>
<td>Isozyme analysis, GFP</td>
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<td><strong>Brassicaceae + Apiaceae</strong></td>
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<tr>
<td>Arabidopsis thaliana + Bupleurum scorzonerifolium</td>
<td>Secondary metabolites</td>
<td>C</td>
<td>IOA(A) + UV</td>
<td>PEG</td>
<td>CC, GISH</td>
<td>RAPD</td>
</tr>
<tr>
<td>Arabidopsis thaliana + Bupleurum scorzonerifolium</td>
<td>Fragmentation tool</td>
<td>-</td>
<td>UV</td>
<td>PEG</td>
<td>CC,GISH</td>
<td>RAPD</td>
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<tr>
<td>Arabidopsis thaliana + Bupleurum scorzonerifolium</td>
<td>Hybrid analysis, fragmentation tool</td>
<td>C + SC</td>
<td>γ</td>
<td>PEG</td>
<td>CC</td>
<td>RAPD</td>
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<tr>
<td><strong>Brassicaceae + Poaceae</strong></td>
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<tr>
<td>Arabidopsis thaliana + Triticum aestivum</td>
<td>Hybrid analysis</td>
<td>SC + C</td>
<td>UV</td>
<td>PEG</td>
<td>CC, GISH</td>
<td>RAPD, SSR, CAPS(C)</td>
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<td><strong>Malvaceae</strong></td>
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<tr>
<td>Gossypium hirsutum + G. hirsutum</td>
<td>Alternative for symmetric somatic hybridisation</td>
<td>SC</td>
<td>UV</td>
<td>EF</td>
<td>CC</td>
<td>RAPD, SSR, CAPS(C)</td>
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<tr>
<td><strong>Musaceae</strong></td>
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<tr>
<td>Musa “Guoshanxiang” + M. acuminata</td>
<td>Biotic resistance</td>
<td>SC</td>
<td>IOA(A) + UV</td>
<td>PEG</td>
<td>CC</td>
<td>RAPD, ISSR</td>
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### Poaceae

<table>
<thead>
<tr>
<th>Hybridization System</th>
<th>Hybrid Analysis</th>
<th>SC</th>
<th>UV</th>
<th>PEG</th>
<th>CC</th>
<th>GISH</th>
<th>RAPD, SSR(C), MSAP</th>
<th>Isozyme analysis, mtDNA specific probes on Southern blots</th>
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<tbody>
<tr>
<td>Festuca arundinacea × Triticum aestivum</td>
<td>Hybrid analysis</td>
<td>SC</td>
<td>UV</td>
<td>PEG</td>
<td>CC</td>
<td>GISH</td>
<td>RAPD, SSR(C), MSAP</td>
<td>Isozyme analysis, mtDNA specific probes on Southern blots</td>
</tr>
<tr>
<td>Oryza sativa japonica × O. meyeriana</td>
<td>Biotic resistance</td>
<td>SC</td>
<td>IOA(A) + X</td>
<td>PEG</td>
<td>CC</td>
<td>RAPD</td>
<td>Resistance screening</td>
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<tr>
<td>Triticum aestivum × Avena sativa</td>
<td>Hybrid analysis</td>
<td>SC</td>
<td>UV</td>
<td>-</td>
<td>ISH</td>
<td>RAPD</td>
<td>Resistance screening</td>
<td></td>
</tr>
<tr>
<td>Triticum aestivum × Haynaldia villosa</td>
<td>Biotic resistance, protein content</td>
<td>SC + C</td>
<td>γ</td>
<td>PEG</td>
<td>CC, GISH</td>
<td>RFLP(C)</td>
<td>Isozyme analysis, 5S rDNA spacer sequence analysis</td>
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<tr>
<td>Triticum aestivum × Lolium multiflorum</td>
<td>Biotic resistance</td>
<td>SC</td>
<td>UV</td>
<td>-</td>
<td>CC</td>
<td>RAPD, SSR</td>
<td>Isozyme analysis, mtDNA specific probes on Southern blots</td>
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<tr>
<td>Triticum aestivum × Lolium multiflorum</td>
<td>Agronomic traits, biotic resistance</td>
<td>SC</td>
<td>IOA(A) + X</td>
<td>EF</td>
<td>CC</td>
<td>RFLP, AFLP</td>
<td>Isozyme analysis, mtDNA specific probes on Southern blots</td>
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<tr>
<td>Triticum aestivum × Setaria italica</td>
<td>Abiotic resistance</td>
<td>(SC + C) + C</td>
<td>UV</td>
<td>PEG</td>
<td>GISH, CC</td>
<td>RAPD, RFLP(C), RFLP(M)</td>
<td>Isozyme analysis, 5S rDNA spacer sequence analysis</td>
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### Poaceae + Apiaceae

<table>
<thead>
<tr>
<th>Hybridization System</th>
<th>Hybrid Analysis</th>
<th>SC</th>
<th>UV</th>
<th>PEG</th>
<th>CC</th>
<th>GISH</th>
<th>RAPD, SSAP</th>
<th>Isozyme analysis, mtDNA specific probes on Southern blots</th>
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<tbody>
<tr>
<td>Festuca arundinacea × Bupleurum scorzonerifolium</td>
<td>Hybrid analysis</td>
<td>SC</td>
<td>UV</td>
<td>PEG</td>
<td>CC</td>
<td>RAPD</td>
<td>Isozyme analysis, mtDNA specific probes on Southern blots</td>
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<tr>
<td>Triticum aestivum × Bupleurum scorzonerifolium</td>
<td>Genetic variation, genome mapping</td>
<td>C + SC</td>
<td>UV</td>
<td>PEG</td>
<td>GISH</td>
<td>CAPS, RAPD, SSR</td>
<td>Isozyme analysis</td>
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### Rutaceae

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<th>UV</th>
<th>PEG</th>
<th>CC</th>
<th>GISH</th>
<th>RAPD, SSAP</th>
<th>Isozyme analysis, mtDNA specific probes on Southern blots</th>
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</thead>
<tbody>
<tr>
<td>Citrus paradisi × C. sinensis</td>
<td>Genetic variation</td>
<td>SC</td>
<td>IOA(A) + γ</td>
<td>-</td>
<td>FCM</td>
<td>AFLP</td>
<td>Isozyme analysis</td>
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<tr>
<td>(Citrus reticulata x C. sinensis) × C.sinensis</td>
<td>Genetic variation</td>
<td>SC</td>
<td>IOA(A) + γ</td>
<td>-</td>
<td>FCM</td>
<td>AFLP</td>
<td>Isozyme analysis</td>
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<tr>
<td>Citrus sinensis × C. unshiu</td>
<td>Fragmentation tool</td>
<td>SC</td>
<td>UV</td>
<td>EF</td>
<td>CC, FCM</td>
<td>RAPD, AFLP, CAPS(C)</td>
<td>Isozyme analysis</td>
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### Solanaceae

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<th>Hybridization System</th>
<th>Hybrid Analysis</th>
<th>SC</th>
<th>UV</th>
<th>PEG</th>
<th>CC</th>
<th>GISH</th>
<th>RAPD, SSAP</th>
<th>Isozyme analysis, mtDNA specific probes on Southern blots</th>
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<tbody>
<tr>
<td>Nicotiana tabacum × N. repanda</td>
<td>CMS M Rhodamin 6G (A)</td>
<td>PEG</td>
<td>CC</td>
<td>RAPD, CAPS(M)</td>
<td>Isozyme analysis, mtDNA specific probes on Southern blots</td>
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<tr>
<td>Petunia hybrid × Nicotiana tabacum</td>
<td>Plastome transfer</td>
<td>M</td>
<td>UV</td>
<td>PEG</td>
<td>RAPD, CAPS(C)</td>
<td>MtDNA specific probes on Southern blots, chloroplast RNA specific probes on Northern blot</td>
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</table>

Species labeled with * were fused for the first time; C: callus; CO: cotyledon; H: in vitro hypocotyls; M: in vitro mesophyll cells; SC: suspension cells; -: described in earlier publications; (A): for acceptor; IOA: iodoacetamide; UC: ultracentrifugation; UV: UV ray irradiation; X: X ray irradiation; γ: gamma-ray irradiation; EF: electrofusion; PEG: chemical fusion with polyethylene glycol; -: described in earlier publications; CC: chromosome counting; FCM: flow cytometry; (G) ISH: (genomic) in situ hybridization; (C) on cpDNA, (M) on mtDNA.

was reported in banana [41]. Interfamilial asymmetric hybrids have been produced between the dicot Arabidopsis thaliana and the monocot common wheat [55]. Fusion between phylogenetically remote tall fescue, Italian ryegrass and common wheat was achieved [57,61,62]. In cotton, asymmetric hybrids were obtained as an alternative for symmetric hybrids [56]. New somatic hybrids were obtained between monocot Festuca arundinacea and dicot Bupleurum scorzonerifolium through symmetric as well as asymmetric fusions [65]. The first successful somatic hybrid regeneration between Oryza sativa L. ssp. japonica and O. meyerina L. was reported [58]. Scholze et al. produced the first Raphanus-Brassica somatic hybrids with fungal and virus disease resistance [52]. Cybrids were produced between chloroplast transformant tobacco and petunia [68]. Using UV irradiated asymmetric hybrids a radiation hybrid panel was established for Lolium multiflorum [63]. Taski-Adjukovic et al. [48] regenerated an asymmetric hybrid between sunflower and Helianthus maximiliani for the first time.

Acceptor protoplast sources for asymmetric hybridization existed mainly of suspension cell cultures, mesophyll, callus and hypocotyls. The donor protoplast source differed in about 30% of the cases from the one for the
acceptor. Brassicaceae and Asteraceae hypocotyl acceptor protoplasts were combined with mesophyll donor protoplasts [48,51,52].

Biotic resistance introduction, genetic variation, agronomic traits such as seedless fruits, hybrid analysis, fragmentation technology development and secondary metabolite production were the most important recent aims for asymmetric hybridization. Abiotic resistance introduction, hybridization, genome mapping and the establishment of chromosome addition lines were rare objectives (Table 1). In Bupleurum scorzonerifolium, asymmetric hybrids were obtained after protoplast fusion of UV treated B. scorzonerifolium and wheat protoplasts. However, instead of generating wheat carrying B. scorzonerifolium chromosome fragments, the reverse was found. This study can be of major interest for the construction of physical maps of the wheat genome [66]. The same was observed when untreated Arabidopsis thaliana protoplasts were fused with UV treated Bupleurum protoplasts [43].

Other motives were plastome and/or CMS transfer [67]. The latter is an important practical application of new genome/cytoplasmome combinations. Fitter et al. [69] demonstrated the possibility of introgressing CMS carried by mtDNA from a wild species into the cultivated crop. In Cichorium, CMS was introduced after asymmetric fusion with sunflower [40]. Sheahan et al. [70] reported the phenomenon of massive mitochondrial fusion (MMF) which leads to near-complete mixing of the mitochondrial population within 24 h. MMF appears specific to dedifferentiation, since it also occurs in mesophyll protoplasts of Arabidopsis and Medicago but not in protoplasts from already dedifferentiated cells such as tobacco BY-2 or callus cultures. These results allow a clearer interpretation of how novel mitochondrial genotypes develop following cell fusion. In other investigations, Sytnik et al. [71] demonstrated that also chloroplasts can be transferred to remote species by protoplast fusion.

4. Characterization of Asymmetric Somatic Hybrids and Genome Stability

Apart from morphological characterization, the majority of the publications reporting on complete plant regeneration describe the use of molecular tools to unravel the genomic constitution of the alleged hybrids (Table 1). DNA markers were sometimes complemented with isozyme analysis, sodium dodecyl sulphate polyacrylamide gel electrophoresis or sequence analysis. The most frequently employed molecular markers were Random Amplification of Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), Amplification Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP) and Cleaved Amplified Poly-
recombination was proven in *Triticum aestivum* + *Setaria italica* [64] and *Arabidopsis thaliana* + *Brassica oleracea* [76]. The latter authors proposed mitochondrial recombination as a tool for CMS introduction in cabbage. Although more rarely occurring, cpDNA recombination in hybrids has been demonstrated. In *Triticum aestivum* + *Setaria italica* hybrids, cpDNA coexistence as well as recombination occur [64]. It was also observed in *Bupleurum scorzonerifolium* + *Swertia mussottii* [46].

5. Conclusions

Recently, interest in protoplast related research has been renewed. Fusion of protoplasts allows us to move from traditional plant breeding methods to asexual methods. Compared to symmetric somatic hybridization, asymmetric fusion has some important advantages as it may limit genome conflicts. As shown in recent publications asymmetric fusion can create genomic variation for plant quality and yield improvement in agricultural or industrial crops. Especially the possibility to introduce CMS in important agricultural crops offers the breeders a tool towards hybrid seed production.

Interesting opportunities for further research may be found in the development of advanced methods for genome fragmentation including the combination of multiple techniques, such as the irradiation of MPPs, or the creation of MPPs from unreduced gametes formed by interspecific hybrids. The latter might open the possibility to transfer recombined chromosomes in a single step. Moreover, MPPs could be selected based on filtration properties, which would result in different genome types, enabling researchers to attribute plant traits to particular chromosomes and further utilize MPPs accordingly. Furthermore, the fast evolution in marker development will allow more profound studies on genome stability. Furthermore, the contribution of GISH to genome characterization studies after fusion will further increase and can be used as a tool for monitoring genomic stabilization.

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