

Review

Tissue Culture and Somatic Embryogenesis in Warm-Season Grasses—Current Status and Its Applications: A Review

Melody Ballitoc Mugerza ¹, Takahiro Gondo ^{2,*}, Genki Ishigaki ¹, Yasuyo Shimamoto ¹, Nafiatul Umami ³, Pattama Nitthaisong ⁴, Mohammad Mijanur Rahman ⁵ and Ryo Akashi ¹

¹ Faculty of Agriculture, University of Miyazaki, 1-1 Gakuenkibanadai-Nishi, Miyazaki 889-2192, Japan; melody.mugerza@gmail.com (M.B.M.); gishigaki@cc.miyazaki-u.ac.jp (G.I.); hi_tei05428@yahoo.co.jp (Y.S.); rakashi@cc.miyazaki-u.ac.jp (R.A.)

² Frontier Science Research Center, University of Miyazaki, 1-1 Gakuenkibanadai-Nishi, Miyazaki 889-2192, Japan

³ Faculty of Animal Science, Universitas Gadjah Mada, Jl Fauna 3, Yogyakarta 55281, Indonesia; nafiatul.umami@ugm.ac.id

⁴ Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand; pattama.ni@kmitl.ac.th

⁵ Faculty of Agro-Based Industry, Jeli Campus, Universiti Malaysia Kelantan, Jeli 17600, Kelantan, Malaysia; mijanur.r@umk.edu.my

* Correspondence: gondo@cc.miyazaki-u.ac.jp

Abstract: Warm-season grasses are C₄ plants and have a high capacity for biomass productivity. These grasses are utilized in many agricultural production systems with their greatest value as feeds for livestock, bioethanol, and turf. However, many important warm-season perennial grasses multiply either by vegetative propagation or form their seeds by an asexual mode of reproduction called apomixis. Therefore, the improvement of these grasses by conventional breeding is difficult and is dependent on the availability of natural genetic variation and its manipulation through breeding and selection. Recent studies have indicated that plant tissue culture system through somatic embryogenesis complements and could further develop conventional breeding programs by micropropagation, somaclonal variation, somatic hybridization, genetic transformation, and genome editing. This review summarizes the tissue culture and somatic embryogenesis in warm-season grasses and focus on current status and above applications including the author's progress.

Keywords: genetic transformation; genome editing; protoplast; somatic embryogenesis; warm-season grass



Citation: Mugerza, M.B.; Gondo, T.; Ishigaki, G.; Shimamoto, Y.; Umami, N.; Nitthaisong, P.; Rahman, M.M.; Akashi, R. Tissue Culture and Somatic Embryogenesis in Warm-Season Grasses—Current Status and Its Applications: A Review. *Plants* **2022**, *11*, 1263. <https://doi.org/10.3390/plants11091263>

Academic Editor: Małgorzata Kozieradzka-Kiszkurno

Received: 14 April 2022

Accepted: 4 May 2022

Published: 7 May 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Forage grass plays a vital role in the successful operation for livestock production since ruminants are heavily dependent on forage for their feed and production [1]. Grasses are the main feed for ruminants, accounting for 60–90% of ruminant feed requirements worldwide [2]. By 2050, the human population is projected to reach 10 billion [3], and meat and milk consumption in developing countries is expected to at least double [4]. Therefore, there is a concern to increase forage productivity and quality for efficient livestock production. Grasses can be grouped into two large categories; warm- and cool-season grasses. Warm-season grasses are C₄ plants and have a high capacity for biomass productivity. The grasses are cultivated mainly in the tropics, subtropics, and also in some warm temperate areas in the world. There are 4783 species of C₄ plants in the grass family worldwide [5], and they are highly diverse, some of which are economically important crops: maize (*Zea mays*), sorghum (*Sorghum bicolor*), and sugarcane (*Saccharum officinarum*). Bahiagrass (*Paspalum notatum*), Brachiaria grass (*Urochloa* spp.), Guinea grass (*Panicum maximum*), Napier grass (*Pennisetum purpureum*), Rhodes grass (*Chloris gayana*), etc. are used for grazing, forage and silage, and Bermuda grass (*Cynodon dactylon*), *Paspalum* grass (*Paspalum* spp.), *Zoysia* grass (*Zoysia* spp.) are used as turf and greening plants. Maize

and sugarcane are also used in bioethanol production. Recently, the high productivity of warm-season grasses has focused on the use of lignocellulosic biomass, and there have been studies on switchgrass (*Panicum virgatum*) and *Miscanthus* spp. as model bioenergy crops for sustainable energy production.

Many important warm-season perennial grasses multiply either by vegetative propagation or form their seeds by an asexual mode of reproduction called apomixis. The possibility of improving these plants by conventional breeding methods depends on the availability of natural genetic variation and its manipulation through breeding and selection. However, apomictic grasses have breeding barriers for hybridization and there are naturally not many genetic variations. Therefore, it is difficult to adapt to conventional breeding by crossbreeding in many warm-season grasses. Biotechnology involving plant tissue culture is a powerful complementary tool in conventional plant breeding programs [6]. Major categories of these methods can be summarized as induction and screening of desirable mutants at the cellular and tissue level, somatic hybridization between remotely related species, induction of haploid plants as breeding materials, and genetic transformation in protoplasts and plant tissues, as well as micropropagation of unique genotypes. However, in general, in vitro culture in warm-season grasses is not easy, and few grass species have established sufficient tissue culture systems [7].

There are two processes of plant regeneration, namely organogenesis and somatic embryogenesis. In general, organogenesis involves the sequential formation of shoots and roots from tissues, depending on the appropriate culture conditions. On the other hand, somatic embryogenesis is a developmental process in which plant somatic cells dedifferentiate to become totipotent embryonic stem cells with the ability to produce embryos. This new embryo can further develop into a whole plant [8]. Since the first description of somatic embryogenesis in the cell culture of carrots (*Daucus carota*) [9], this process has been reported in various plant species [10–14]. A unique characteristic of the somatic embryo is its continuous proliferation, as development is never arrested [15]. Therefore, somatic embryogenesis represents a powerful tool for mass production, germplasm conservation, protoplast culture and genetic improvement in plant species.

This review covers tissue culture and somatic embryogenesis including the influence of explants and culture condition. It also describes their application to breeding techniques such as protoplast culture, somaclonal variation, genetic transformation, and genome editing, including the authors' progress.

2. Tissue Culture and Somatic Embryogenesis

To date, an effective in vitro regeneration system in cool-season grasses has been reported in many species using various explants and culture conditions. On the other hand, a few grass species have established sufficient tissue culture systems in warm-season grasses [16]. Despite a number of in vitro regeneration systems having been reported in recent years, most of these regeneration systems are based on somatic embryogenesis (Table 1). In warm-season grasses, callus induction and somatic embryogenesis are important points for establishing efficient tissue culture systems. In the Gramineae, the first successful attempt was made in barley (*Hordeum vulgare*) [17] where somatic embryos were formed on the scutellum of cultured immature zygotic embryos. Somatic embryogenesis in warm-season grass was first reported in Guinea grass [18] and pearl millet (*Pennisetum glaucum*) [19], followed by reports of somatic embryogenesis and plant regeneration in most of the important species.

Table 1. Summary of tissue and protoplast cultures in warm-season grasses.

Plant Species	Explants Source ¹	Plant Regeneration ²	References
Callus Induction and Plant Regeneration			
<i>Bouteloua gracilis</i>	AM	SE	[20]
<i>Cenchrus ciliaris</i>	II	SE	[21]
	MS, AM, II	SE	[22]
<i>Chloris gayana</i>	SL	OR, SE	[23]
<i>Cynodon dactylon</i>	II	SE	[1,24–26]
<i>Digitaria sanguinalis</i>	AM	SE	[27]
<i>Eragrostis tef</i>	SL	SE	[28]
	MS	SE	[29]
<i>Imperata cylindrica</i>	AM, II	OR	[30]
	AM	OR	[31]
<i>Miscanthus sinensis</i>	NS	OR	[32]
	AM	OR, SE	[33]
<i>Panicum</i> spp.	AM, II, MS	OR	[34]
	MS	SE	[35,36]
<i>Panicum bisulcatum</i>	MS	OR, SE	[37]
<i>Panicum maximum</i>	L	SE	[18,38]
	II, IE, ME	OR, SE	[39]
<i>Panicum miliaceum</i>	IE	SE	[40]
	M	OR	[41]
<i>Panicum sumatrense</i>	II	SE	[42]
	II, L	SE	[43]
<i>Panicum virgatum</i>	II	SE	[44,45]
	II	OR	[46]
<i>Paspalum dilatatum</i>	II	SE	[47]
<i>Paspalum notatum</i>	IE, ME	SE	[48]
	MS	SE	[13,49,50]
<i>Paspalum scrobiculatum</i>	SL	SE	[51]
	M	OR	[52]
	IE	SE	[53]
	IE, ME	SE	[54]
	MS	OR	[55]
	SL	SE	[56]
<i>Paspalum vaginatum</i>	II	SE	[57]
<i>Pennisetum americanum</i>	II, IE	SE	[19,58]
	II	SE	[59]
<i>Pennisetum americanum</i> × <i>Pennisetum purpureum</i>	II	SE	[19]

Table 1. Cont.

Plant Species	Explants Source ¹	Plant Regeneration ²	References
Callus Induction and Plant Regeneration			
<i>Pennisetum glaucum</i>	AM	SE	[60]
	SL	OR	[61]
	IE	SE	[62]
	AM, II, MS	OR, SE	[63]
<i>Pennisetum purpureum</i>	L	SE	[64]
	II	SE	[65,66]
	AM	OR	[67]
	AM	SE	[68]
<i>Setaria italica</i>	II	SE	[69]
	MS	OR	[70]
<i>Urochloa brizantha</i>	NS, MS	OR, SE	[71]
	MS	OR, SE	[72]
<i>Urochloa ruziziensis</i>	SL	OR, SE	[73]
<i>Zoysia japonica</i>	MS	SE	[74–76]
<i>Zoysia matrella</i>	II, NS	SE	[77]
	NS	SR	[78]
Protoplast culture ³			
<i>Panicum maximum</i>		PL	[79]
		CL	[80]
<i>Panicum miliaceum</i>		PL	[81]
<i>Paspalum scrobiculatum</i>		PL	[82]
<i>Paspalum dilatatum</i>		PL	[83]
<i>Pennisetum americanum</i>		CL	[84]
		PL	[85]
<i>Pennisetum purpureum</i>		PL	[86]
<i>Zoysia japonica</i>		CL	[74]
		PL	[87]

¹ AM, apical meristem; IE, immature embryo; II, immature inflorescence; L, leaf; M, mesocotyl; MS, mature seed; NS, nodal segment; SL, seedling. ² OR, organogenesis; SE, somatic embryogenesis. ³ CL, callus; PL, plantlet.

There are two different modes of somatic embryogenesis: direct somatic embryogenesis and indirect somatic embryogenesis [88]. In warm-season grasses, indirect somatic embryogenesis is mostly observed, and somatic embryos are usually induced through callus formation. Therefore, the induction of callus forming somatic embryos (embryogenic callus) is the most important step to establish an efficient tissue culture system. At the stage of embryogenic callus formation, various factors affect its efficiency and quality, including the genotype of the donor plant, the explant type, the media, and plant growth regulators.

Cell totipotency is the most important characteristic of plant cell cultures, but not all cells are totipotent. For this reason, immature zygotic embryos and immature inflorescence that are capable of somatic embryogenesis with high potential for cell division are often used as explants in a wide range of cereal plants. Similar tissues are often used in warm-season grasses, and the authors have succeeded embryogenic callus induction and plant regeneration using immature zygotic embryos in Guinea grass [40] (Figure 1a) and immature inflorescences in dallisgrass (*Paspalum dilatatum*) [47] (Figure 1b). These tissues

have high potential for embryogenic callus formation and provide high quality material in tissue culture. However, immature and developing tissues have seasonal limitations as materials. Monocotyledonous plants have limited explant sources that can form somatic embryos compared to dicotyledons. In addition to the above tissues, apical meristems, mature seeds (mature zygotic embryos), axillary buds of internodes, and germinated plants are mainly used, and hypocotyls and young leaves are used for callus induction in some cases (Table 1). Among them, mature seeds are the preferred alternatives to immature embryos in warm-season grasses since they can be stored for a long time and could be used any time without seasonal limitation. However, the frequency of embryogenic callus formation in warm-season grasses is low, mainly due to the fact that most seeds are of the outcrossing mode of reproduction and genetic heterogeneity is strong which consequently prevents the induction of uniform, high-quality embryogenic callus. Therefore, the authors have devised a two-step callus induction method in which a large number of seeds are sown on filter paper soaked with liquid medium to induce primary callus and then sub-cultured on solid medium to produce embryogenic callus (Figure 1c). By using this method, it is possible to select high quality embryogenic callus lines from a large number of seeds, and to provide materials with high regeneration capacity for genetic transformation [89].

Plant propagation and regeneration by in vitro culture is driven by the assimilation of ions such as nitrogen, phosphate, magnesium, and calcium. The Murashige and Skoog (MS) medium is now widely used for plant tissue culture in most warm-season grasses [90]. MS medium provides the basic nutrients, with the addition of maltose and sorbitol to regulate osmotic pressure, thiamine, L-glutamine, nicotinic acid, casein, proline as amino acids, and AgNO_3 as ethylene inhibitor, and CuSO_4 as useful microelements, which are effective in somatic embryogenesis [16]. In bahiagrass, CuSO_4 is effective for somatic embryogenesis, and changing it to 50 μM from 0.1 μM in normal MS medium enhanced somatic embryogenesis frequency and produced high quality embryogenic callus, compact and dense with pro-embryos (Figure 1d). This modified culture minimized the problems associated with the loss of regeneration and increase in albinism which frequently occur in long term cultures of warm-season grasses [60,68,89].

Plant growth regulators are needed to control callus formation, proliferation, somatic embryo formation, plant regeneration, and rooting. Auxins [2,4-dichlorophenoxyacetic acid (2,4-D), dicamba, 1-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), picloram] and cytokinins [6-benzylaminopurine (BAP), kinetin (KN), zeatin] were used for in vitro culture in a wide range of plant species. 2,4-D is used for callus induction, somatic embryogenesis, and proliferation in most warm-season grasses. It is often applied at 2–10 mg L^{-1} , and is combined with low concentrations of cytokinins to control somatic embryogenesis. For plant regeneration, BAP is often used at concentrations of 1–3 mg L^{-1} , and low concentrations of NAA added, or sometimes hormone-free media.

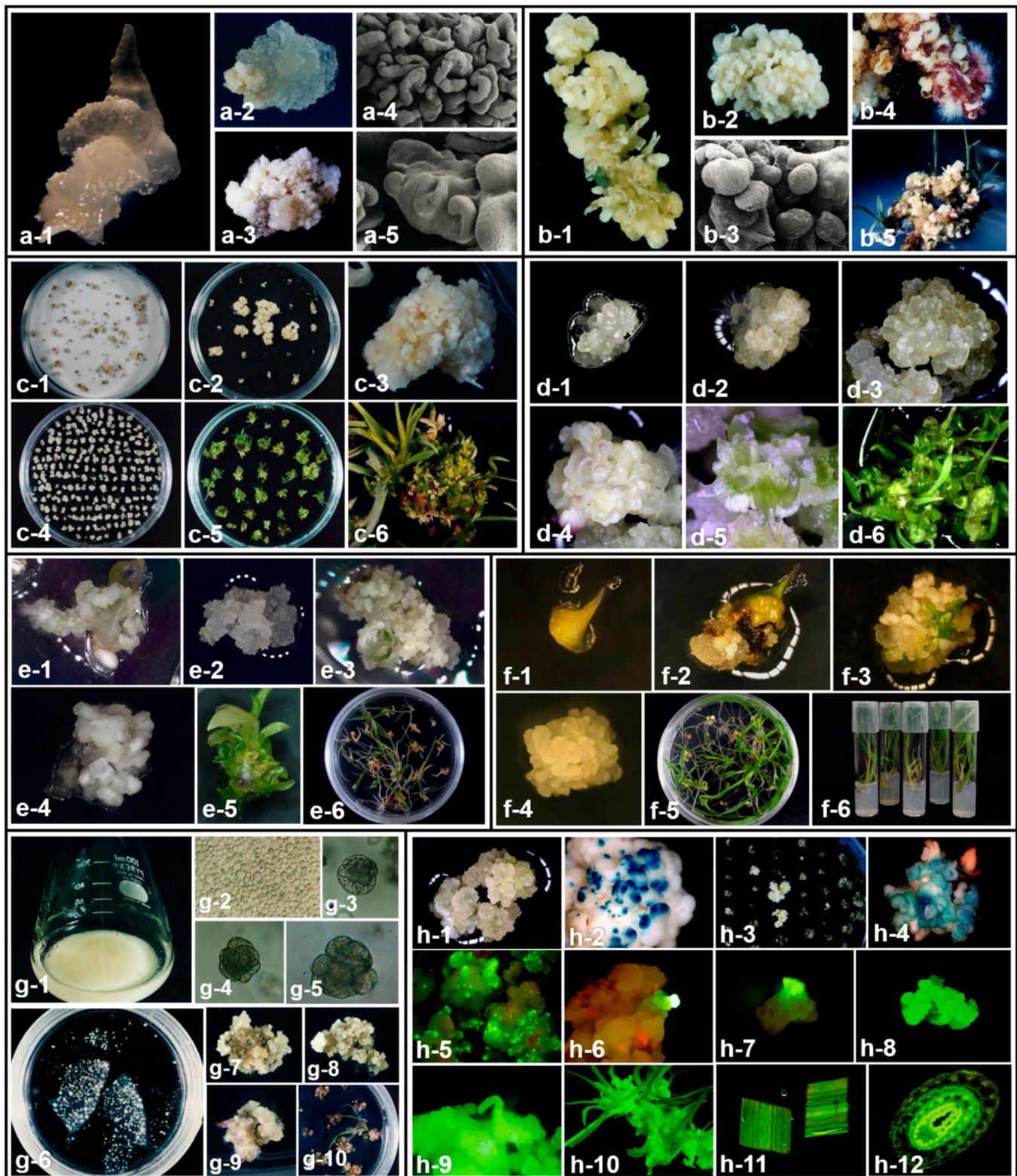


Figure 1. Somatic embryogenesis and its application to protoplast culture and genetic transformation in some warm-season grasses. (a) Somatic embryogenesis from immature zygotic embryos in Guinea grass (*Panicum maximum*). (1) Immature zygotic embryo, (2) non-embryogenic callus, (3) embryogenic callus, (4,5) SEM of somatic embryos at different stages of development. (b) Somatic embryogenesis from immature inflorescences and plant regeneration in dallisgrass (*Paspalum dilatatum*). (1) Primary callus after 14 d of culture, (2) embryogenic callus, (3) SEM of pro-embryogenic structures, (4,5) plant

regeneration from somatic embryos. (c) Somatic embryogenesis from mature seeds and plant regeneration in bahiagrass (*Paspalum notatum*). (1) Primary callus after 14 d of culture, (2,3) embryogenic callus after 28 d of culture, (4) A sub-cultured micro-callus after 60 d culture, (5,6) Plant regeneration from micro-callus. (d) Developmental stages and plant regeneration of highly regenerative embryogenic callus cultured in CuSO₄-supplemented medium. (1–3) Embryogenic callus cultured after 0 (1), 3 (2), and 14 (3) d on CuSO₄ additional medium, (4,5) Shoot germination with scutellum formation, (6) elongation of germinated shoot. (e) Somatic embryogenesis from mature seeds and plant regeneration in ruzigrass (*Urochloa ruziziensis*). (1) Primary callus after 14 d of culture, (2–4) three types callus after 30 d of culture, non-embryogenic callus (2), friable embryogenic callus (3), compact embryogenic callus (4), (5). Plant regeneration from embryogenic callus, (6) rooting. (f) Somatic embryogenesis from apical meristem and plant regeneration in Napier grass (*Pennisetum purpureum*). (1) Apical meristem, (2,3) primary callus after 10 (2) and 45 (3) d of culture, (4) compact and proliferating uniform embryogenic callus. (5) Plant regeneration from embryogenic callus, (6) rooting. (g) Cell colony formation and plant regeneration from suspension protoplasts of dallisgrass. (1) Typical suspension cells, (2) isolated protoplasts from suspension cells, (3–5) cell division and cell colony formation from protoplasts after 5 (3), 7 (4) and 10 (5) d of culture, (6) colonies formed from protoplasts after 20 d of culture, (7,8) somatic embryos formation from protoplast-derived colonies, (9,10) plant regeneration from somatic embryos. (h) Stable transformation of bahiagrass mediated by particle inflow gun with bialaphos screening (1–4) and GFP (green fluorescent protein) visual screening (5–12). (1) Highly regenerative embryogenic callus for target tissue, (2) transient GUS (β -glucuronidase) expression 16 h after bombardment, (3) Bialaphos resistant callus under selection. (4) Stable GUS expression on bialaphos resistant callus. (5) Transient GFP expression 16 h after bombardment, (6,7) GFP expressing callus 14 d after bombardment, (8–10) GFP expression from transformed callus to plant regeneration, (11,12) GFP expression in leaf (11) and stem (12) of transgenic plants.

The authors have established tissue culture systems for several warm-season grasses, from callus induction to plant regeneration. Embryogenic callus was initiated from immature embryos on MS medium supplemented with 10 mg L⁻¹ 2,4-D, 10% coconut water and solidified with 0.8% agar in Guinea grass (*Panicum maximum*) (Figure 1a). Initially various types of calli were obtained and embryogenic responses were found to be correlated with the genotypes investigated. For somatic embryo germination and plant formation, MS medium supplemented with gibberellic acid and kinetin were used. The twelve genotypes analyzed can be classified into three groups by the frequency of somatic embryo formation and degree of apomixis. One of the groups consisted of highly apomictic genotypes with a high embryogenic capacity [40]. Plant regeneration from cultured immature inflorescences of dallisgrass (*Paspalum dilatatum*) was obtained by somatic embryogenesis (Figure 1b). Embryogenic callus was initiated from immature inflorescences on MS medium supplemented with 2, 5, and 10 mg L⁻¹ 2,4-D and solidified with 0.2% Gellan Gum. Somatic embryos developed and germinated precociously when embryogenic calli were transferred to a medium containing kinetin and gibberellic acid. All regenerants were successfully grown to maturity [47]. In bahiagrass, embryogenic callus was initiated from mature seeds on MS liquid and solid medium supplemented with 2 mg L⁻¹ 2,4-D with a two-step callus induction method (Figure 1c). Selection of high-quality callus from a large number of mature seeds could be obtained by this modified culture. In addition, the selected good quality callus was cultured in MS medium with 2 mg L⁻¹ 2,4-D, 0.1 mg L⁻¹ BAP and 50 μ M CuSO₄, which resulted in dense pro-embryos on the surface of the callus and maintained high potential regeneration capacity for long-time culture (Figure 1d) [89].

Urochloa species are widely cultivated in tropical and subtropical regions, and are utilized as a main forage grass in South America. Ruzigrass (*Urochloa ruziziensis*) is one of several diploids with a sexual reproduction mode in the *Urochloa* genus, and we have established tissue culture system for this species. Embryogenic callus was induced from mature seeds on MS medium containing 4 mg L⁻¹ 2,4-D and 0.2 mg L⁻¹ BAP. Plant regeneration was achieved by culturing on MS medium with 2.0 mg L⁻¹ BAP and 0.1 mg L⁻¹ NAA (Figure 1e) [73]. However, in long-term tissue culture periods, spontaneous appear-

ances of polyploids (tetraploid and octoploid) in plants regenerated from embryogenic calli were reported. At present, it is recommended that two-months-old or younger embryogenic calli are best suited for ruzigrass transformation since these calli generate fertile diploid plants [91]. As application for the breeding program, a tetraploid ruzigrass was produced by colchicine treatment of in vitro multiple-shoot clumps or in vitro germinated seedlings [92]. Subsequently, a new cultivar 'Isan' produced from the hybrids between the tetraploid ruzigrass and Mulato was selected for variety registration and investigation for initial growth (degree of plant growth between two to four weeks after sowing) and vigor in the tropical islands of Okinawa, Japan [93]. Likewise, the tetraploid ruzigrass have expanded the breeding material of the genus *Urochloa* by crossing it with tetraploid apomixis cultivars [94,95]. Napier grass (*Pennisetum purpureum* Schumach.) is a highly productive C₄ tropical forage grass that has been targeted as a high potential bioenergy crop. Apical meristems were used as explants and cultured on MS medium with 2 mg L⁻¹ 2,4-D, 0.5 mg L⁻¹ BAP, and 50 μM CuSO₄ in four accessions. A dwarf type with late-heading (DL line) had the best response for embryogenic callus formation. Highly regenerative calli that formed dense polyembryogenic clusters were selected through 14 d interval of subculture and maintained regeneration for six months (Figure 1f) [68]. These culture systems based on somatic embryogenesis are fundamental techniques for protoplast culture, genetic transformation and genome editing in warm-season grasses.

3. Somatic Embryogenesis Related Genes and Relationship to Apomixis in Warm-Season Grasses

Various genes that are involved in somatic embryogenesis include (a) housekeeping genes [96], (b) auxin-inducible genes, (c) ABA inducible genes [97], (d) transcription factors [98], (e) homeobox genes [99–101], and (f) maturation genes [102]. Apomixis, an asexual mode of reproduction of avoiding meiosis, is abundant in warm-season grasses and can produce seeds of the same genotype. Apomixis has the potential to maintain hybrid vigor for many generations in economically important plant genotypes. The evolution and genetics of asexual seed production are unclear, and much more effort will be required to determine the genetic architecture of this phenomenon. Somatic embryonic receptor-like kinases (SERKs) consist of plasma membrane receptor genes that have been characterized in a variety of species and have been found to be associated with several aspects of plant development, including reproduction. The expression of SERK is observed from competent cell stage up to the globular and heart stage of somatic embryos [103]. In Guinea grass, highly apomictic genotypes indicated a high embryogenic capacity [40], which suggested the involvement of a similar gene in somatic embryogenesis and apomictic seed formation. *SERK* genes are involved in another development and in competent cell stage up to the globular stage of somatic embryos and early embryo development in sexual and asexual seed formation in *Paspalum notatum* [104] and *Urochloa* genus [105]. Stronger expression of *PnSERK2* in embryogenic calli of apomicts compared to those of sexual plants suggested an association with apomixis [104]. However, *SERK3* was differentially expressed from other *SERKs* and was possibly down regulated in associated with apomictic development [105]. Somatic embryogenesis and apomixis embryo development have many similarities, and in addition to the *SERK* gene, *BBM*, *LEC1*, *LEC2*, *LEC3*, *FUS3*, etc. have common functions [106,107]. Warm-season grasses are suitable materials for studying apomixis, and it is essential to accumulate genetic information on somatic embryogenesis and use them as candidate genes for isolating apomixis-related genes.

4. Suspension Cell and Protoplast Culture

Suspension cell culture uses single cells or small aggregates of cells that multiply while suspended in agitated liquid medium. The establishment of single cell cultures in warm-season grasses has given excellent opportunity to develop somatic embryos and support protoplast systems. Moreover, suspension cell culture allows accelerated culture of embryogenic cells, including transgenic cells, which could lead to somatic embryos and their

regeneration [108]. Such systems and protocols have been fundamental towards advancing breeding objectives especially in tropical grasses with economic importance to bioenergy, forage feed, cereals, and turfgrass. Suspension culture protocols have been established in many warm-season grasses including pearl millet [59], Guinea grass [109], dallisgrass [47], bahiagrass [13], zoysiagrass [87], Bermuda grass [110], and switchgrass [111] etc. With the current trends in “omics” and genome editing technologies, there have been a high preference to use single cells such as protoplast for DNA delivery and regeneration; and in that suspension culture is an indispensable tool to achieve such platforms [112–115].

Suspension cell-derived protoplasts that regenerate via somatic embryogenesis have been reported in few warm-season grasses such as switchgrass (*P. virgatum* L.) [111] and Guinea grass (*Panicum maximum*) [80]. However, some reported the failure of protoplast-derived microcalli to regenerate despite high protoplast yield from Finger millet (*Eleusine coracana*) [116]. Also, high exposure to enzyme treatment could cause cell toxicity and mitotic disorder in pearl millet (*P. glaucum*) [117]. Prior to protoplast isolation from dallisgrass, suspension cells previously derived from immature embryo-derived calli were conditioned with MS liquid medium without sucrose and growth regulators [83]. This treatment resulted to an increase in protoplast yield and colony formation. Embryogenic structures could be maintained proliferating in suspension culture only between one to two months to avoid the loss of regeneration. On the other hand, recalcitrance to plant regeneration has been said to be a major bottleneck in the application of protoplast in many warm-season grasses [111]. Factors such as genotype, source of explant, isolation method, culture medium, and the physical environment affect regeneration [118].

Protoplast fusion and somatic hybridization offers the potential to produce novel crops and overcome breeding obstacles in polyploid and apomictic warm-season grasses [7,119–121]. It could provide alternative ways to produce hybrids from sexually incompatible species and offers opportunity for intergeneric hybridization [16]. Although protoplast fusion was more successful in Solanaceae family, such approach has produced somatic hybrids in Gramineae species including wheat (*Triticum aestivum*) and maize (*Zea mays*) [122,123], pearl millet (*Pennisetum americanum*) and sugarcane (*Saccharum officinarum*) [124,125], Guinea grass (*Panicum maximum*) and dallisgrass (*Paspalum dilatatum*) [119], and Guinea grass (*Panicum maximum*) and pearl millet (*Pennisetum americanum*) [126]. On the other hand, C₄ photosynthesis genes could be introduced in C₃ crops by somatic hybridization as demonstrated by the protoplast fusion of C₄ *Z. mays* and C₃ *Triticum* sect. *tritirigia* MacKey [127] which formed somatic hybrids. Also, protoplast fusion between C₃ rice and C₄ Guinea grass produced somatic hybrids that exhibited abnormal floral structure and low fertility [128]. On the other hand, fusion of pearl millet and oat (*Avena sativa*) produced haploid embryos and karyoptically stable hybrids [129,130]. Although somatic hybridizations between C₃ and C₄ grasses have been moderately successful [131], these reports show the capacity for gene transfer between C₄ warm-season and C₃ cool-season grasses by protoplast fusion.

Gene-editing technology has great potential for efficient and accelerated improvement in warm-season grasses. Recently, there has been a renewed interest in using protoplasts in gene silencing and genome editing using CRISPR technologies [132–135]. Due to the naked nature of protoplasts, it makes it an ideal material for direct gene transfer to individual plant cells. Cereal crops and few forage grasses utilized protoplasts to evaluate CRISPR systems including maize [112], millet [112], sorghum [114], Zoysia [136], and switchgrass [132,137]. Transgene-free edited plants have used CRISPR–Cas RNP delivery using protoplasts [138], and recently, Banakar et al. [139] developed the protoplast-based RNP delivery approach and successfully demonstrated it in dicots and monocots, including *Setaria viridis*. The use of protoplast with new plant breeding technologies could offer more precise results and unique advantages for bioenergy and forage crops [118,140].

5. Somaclonal Variation

The application and advancements in somatic embryogenesis in tissue culture has made regeneration possible for various recalcitrant warm-season grass species in vitro for

mass production, embryo rescue, and breeding. Culture and preservation of elite genotypes, which are selected for their superior traits, need a high degree of genetic uniformity amongst the regenerated plants. However, cells and tissues that have been subjected to long-term culture and repetitive stresses may lead to the production of somaclonal variation (SV), a genetic variability caused by gene mutation or changes in epigenetic marks. SV is a genetically stable variation produced in plant tissue culture and has been useful in creating novel variants [16,141,142], and as strategy in overcoming strict transgenic regulations [143]. It has also been an alternative tool to increase genetic variation specially when there is a narrow genetic base such as apomictic species.

Propagation, breeding and genetic improvement in warm-season grasses require the selection of important genotypes from diverse genetic resources. With an effort to isolate somaclonal variants, Li et al. [144] reported a large-scale tissue culture that regenerated approximately 7900 St. Augustine grass 'Raleigh' (*Stenotaphrum secundatum*) plants in vitro and characterized 119 morphological variants which focused on plant variants that had semi-dwarf growth habit and still maintained growth vigour. Somaclonal variants with improved agronomic traits include high seed sets in *Paspalum dilatatum* [145], fall-army worm resistance in *Cynodon dactylon* 'Brazos R3' [146,147], herbicide resistance in seashore paspalum (*Paspalum vaginatum*) [143,148] and freezing tolerance in St. Augustine grass (*S. secundatum*) [149], seashore paspalum (*P. vaginatum*) [150], and centipedegrass (*Eremochloa ophiuroides*) [151,152]. Likewise, a somaclonal triploid Bermuda grass (*Cynodon transvaalensis* × *C. dactylon*) with increased drought tolerance was obtained following 2-year cell suspension culture and subsequent regeneration of somatic embryos [110,153]. Grasses have tremendous potential for phytoremediation of trace element-polluted soils [154], and breeding by in vitro culture can be a feasible approach to enhancing heavy metal accumulation properties such as lead uptake as reported in *Cynodon dactylon* [155]. Considered an important breeding approach that could increase genetic diversity and expand germplasm pool, there is yet more to explore on somaclonal variation for the development of new and improved cultivars in warm season grasses.

6. Genetic Transformation

To develop molecular breeding of plants, the establishment of tissue culture systems, genetic transformation technology, and the isolation of useful genes are three critical points. In the case of warm-season grasses, only few species have sufficiently established these systems. One of the main reasons for this is the need to establish a stable and efficient tissue culture system and the choice of target tissues with competence for transformation. Target tissues for genetic transformation in warm-season grasses used embryogenic callus, suspension cell, protoplast, and stolon nodes [16]. Among them, embryogenic calli are frequently used for transformation, and their transformation efficiency greatly depends on their characteristics. Gondo et al. [89] found that callus culture with 50 µM CuSO₄ resulted in the formation of compact callus with dense polyembryogenic clusters on the surface, and the modified callus shape produced a 3-fold increase in transient GUS expression. In addition, transformed callus could be recovered frequently and transgenic plants have been produced stably without loss of regenerative ability and increase in albinism.

Transgenic warm-season grass by direct gene transfer to protoplasts was first obtained in *Zoysia japonicus* [156]. Although transformation by protoplasts has been reported in temperate grasses; creeping bentgrass (*Agrostis stolonifera*), orchardgrass (*Dactylis glomerata*), tall fescue (*Festuca arundinacea*), Italian ryegrass (*Lolium multiflorum*) etc. [157], it is limited to a few warm-season grass species due to the difficulty in culturing protoplasts. As an alternative technology, microprojectile bombardment and *Agrobacterium*-based transformation have been developed and became the major methods for producing transgenic warm-season grasses (Table 2). Microprojectile particle gun is an effective transformation method for warm-season grass since it has let to introduce foreign genes into any cell or tissues without protoplast culture and *Agrobacterium* host specificity. *Agrobacterium*-mediated method is also applicable to several species due to the improvement of vectors [158–160]

and has become the major genetic transformation method for some species due to its stability of gene insertion, low cost and simplicity (Table 2).

Table 2. Summary of genetic transformation in warm-season grasses.

Plant Species	Transformation Method ¹	Transgenes ²	Outcome ³	References
<i>Bouteloua gracilis</i>	PB	<i>npt, gusA</i>	PL	[161]
<i>Chloris gayana</i>	PB	<i>bar, gusA</i>	PL	[162]
<i>Cynodon dactylon</i>	PB	<i>hph, gusA</i>	PL	[163]
	AG	<i>hph</i>	PL	[164]
<i>Cynodon dactylon</i> × <i>C. transvaalensis</i>	PB	<i>hph</i>	PL	[165]
<i>Digitaria sanguinalis</i>	PB	<i>bar, gusA</i>	PL	[166]
<i>Eragrostis tef</i>	AG	<i>npt, gusA, PcGA2ox</i>	PL	[167]
<i>Miscanthus sinensis</i>	PB	<i>hph, gfp</i>	PL	[168]
	AG	<i>bar, gfp</i>	PL	[169]
	AG	<i>npt, MsCOMT</i>	PL	[170]
	AG	<i>npt, gusA</i>	PL	[171]
<i>Panicum meyerianum</i>	AG	<i>hpt, gusA, ddsA</i>	PL	[172]
<i>Panicum virgatum</i>	PB	<i>bar, gfp</i>	PL	[173]
	AG	<i>hph, PvCOMT</i>	PL	[174]
	AG	<i>hph, PvCAD</i>	PL	[175]
	AG	<i>bar, hph, gusA</i>	PL	[176]
	AG	<i>hph, Pv4CL</i>	PL	[177]
	AG	<i>hph, gusA, pporRFP</i>	PL	[158]
	AG	<i>hph, gfp</i>	PL	[178]
	AG	<i>hph, PvMYB4</i>	PL	[179]
	AG	<i>hph, PvBMY1, PvBMY3</i>	PL	[180]
	AG	<i>hph, gusA, PuP5CS</i>	PL	[181]
	AG	<i>hph, OsAT10</i>	PL	[182]
	AG	<i>hph, LpP5CS</i>	PL	[183]
	AG	<i>hph, pporRFP</i>	PL	[108]
AG	<i>hph, vPIP2;9</i>	PL	[184]	
<i>Paspalum dilatatum</i>	PB	<i>bar</i>	CL	[185]
	PB	<i>npt, PdCCR</i>	PL	[186]
<i>Paspalum notatum</i>	PB	<i>bar,</i>	PL	[187]
	PB	<i>bar, gusA</i>	PL	[89]
	PB	<i>npt, AtGA2ox1</i>	PL	[188]
	PB	<i>npt, AtHB16</i>	PL	[189]
	PB	<i>npt, HsDREB1A</i>	PL	[190]
	PB	<i>bar, npt</i>	PL	[191]
	PB	<i>gfp</i>	PL	[192]
	PB	<i>npt, HvWRKY38</i>	PL	[193]
	PB	<i>bar, 1-SST, 6-SFT</i>	PL	[194]
	PB	<i>bar, gfp</i>	PL	[195]
	PB	<i>bar, CAD</i>	PL	[196]
<i>Paspalum vaginatum</i>	AG	<i>hph, gusA</i>	PL	[197]
	AG	<i>hph, CdtNF-YC1</i>	PL	[198]
<i>Pennisetum glaucum</i>	PB	<i>hph, gusA</i>	CL	[199]
	PB	<i>bar, gusA</i>	PL	[200]
	PB	<i>bar, gusA, egfp</i>	PL	[201]
	PB	<i>pat, affp</i>	PL	[202]
	PB	<i>bar, gusA, pin</i>	PL	[203]
	AG	<i>hph, gusA</i>	PL	[204]
	AG	<i>bar, mag</i>	PL	[205]

Table 2. Cont.

Plant Species	Transformation Method ¹	Transgenes ²	Outcome ³	References
<i>Pennisetum purpureum</i>	PB	<i>bar, gusA</i>	PL	[68]
<i>Setaria italica</i>	AG	<i>hph, gfp</i>	PL	[206]
	AG	<i>hpt, ntp, gusA</i>	PL	[207]
<i>Setaria viridis</i>	AG	<i>hph, pporRFP</i>	PL	[208]
<i>Urochloa ruziziensis</i>	PB	<i>bar, gusA</i>	PL	[91]
<i>Zoysia japonica</i>	PP	<i>hph, gusA</i>	PL	[156]
	AG	<i>hph, gusA</i>	PL	[209]
	AG	<i>cryIA(b), hph, gusA</i>	PL	[210]
	AG	<i>bar, ICE1</i>	PL	[211]
	AG	<i>bar, gusA, AHLs</i>	PL	[212]
<i>Zoysia tenuifolia</i>	AG	<i>hph, gusA</i>	PL	[213]
<i>Zoysia sinica</i>	AG	<i>bar, CBF1</i>	PL	[214]

¹ PB, particle bombardment; AG, *Agrobacterium*-mediated; PP, protoplast transformation. ² *afp*, the antifungal protein from *Aspergillus giganteus*; AHLs, AT-hook motif nuclear-localized genes from *Arabidopsis thaliana*; *AtGA2ox1*, gibberellin 2- β -dioxxygenase gene from *Arabidopsis thaliana*; *AtHB16*, homeobox gene from *Arabidopsis thaliana*; *bar*, phosphinothricin N-acetyltransferase gene from *Streptomyces hygrosopicus*; *CBF1*, a cold inducible transcription factor from *Arabidopsis thaliana*; *CdtNF-YC1*; a nuclear factor Y transcription factor from hybrid Bermuda grass (*Cynodon dactylon* \times *Cynodon transvaalensis*); *CryIA(b)*, synthetic insecticidal protein genes from *Bacillus thuringiensis*; *ddsA*, decaprenyl diphosphate synthase gene from *Gluconobacter suboxydans*; *gfp*, green fluorescent protein gene from *Aequorea victoria*; *gusA*, β -glucuronidase gene from *Escherichia coli*; *hph*, hygromycin phosphotransferase gene from *Escherichia coli*; *HsDREB1A*, The dehydration-responsive element binding proteins gene from *Hordeum spontaneum*; *HvWRKY38*, WRKY transcription factor from *Hordeum vulgare*; *ICE1*, a regulator of cold-induced transcriptome from *Arabidopsis thaliana*; *LpP5CS*, proline biosynthesis gene from *Lolium perenne*; *npt*, neomycin phosphotransferase II gene from *Escherichia coli*; *mag*, a synthetic magainin gene from *Xenopus laevis*; *MsCOMT*, caffeic acid O-methyltransferase gene from *Miscanthus sinensis*; *OsAT10*, BAHD acyltransferase gene from *Oryza sativa*; *pat*, phosphinothricin N-acetyltransferase from *Streptomyces viridochromogenes*; *PcGA2ox*, GA inactivating gene from *Phaseolus coccineus*; *pin*, a synthetic prawn antifungal protein gene; *PdCCR*, cinnamoyl-CoA reductase gene from *Paspalum dilatatum*; *pporRFP*, red fluorescence protein gene from *Porites porites*; *PuP5CS*, proline biosynthesis gene from *Puccinellia chinampoensis*; *PvCAD*, cinnamyl alcohol dehydrogenase gene from *Panicum virgatum*; *PvCOMT*, caffeic acid O-methyltransferase gene from *Panicum virgatum*; *PvMYB1*, 3, 4, transcriptional repressors of monolignol biosynthetic genes from *Panicum virgatum*; *PvPIP2;9*, aquaporin gene from *Panicum virgatum*; *Pv4CL*, 4-coumarate:CoA ligases gene from *Panicum virgatum*; *1-SST*, sucrose:sucrose 1-fructosyltransferase from *Triticum aestivum*; *6-SFT*, sucrose:fructan 6-fructosyltransferase from *Triticum aestivum*. ³ PL, Plantlet; CL, Callus.

In the last two decades, genetic transformation has been successfully carried out in many species and produced several transgenic plants with agronomically useful genes in warm-season grasses (Table 2). The target genes are also highly dependent on the utilization of the grass species, which in the case of warm-season grasses is characterized by a wide range of uses, including forage grass, turfgrass, and bioenergy. Many warm-season grasses have high biomass but have low forage quality, and requires a continuous selection and breeding for improved forage quality. In bahiagrass, transgenic plants carrying *CAD* (cinnamyl alcohol dehydrogenase) gene for lignin biosynthesis with a RNAi vector were developed. The *CAD* down-regulated transgenic lines had significantly reduced lignin content and improved digestibility within 5–10% [196]. Transgenic plants overexpressing the wheat-derived fructan synthesis genes, sucrose: sucrose 1-fructosyltransferase (1-SST) gene and sucrose: fructan 6- fructosyltransferase (6-SFT) gene, have also been produced. Fructan biosynthesis, a metabolic property found in cool-season grasses, was established for the first time in warm-season bahiagrass as transgenic plants generated fructan and consequentially increased sugar content [194].

Bioenergy refers to renewable energy from biological sources. Lignocellulosic ethanol, a second-generation biofuel, has the potential to fill most global transportation fuel needs and does not present a conflict between energy demands and the food supply [215]. More importantly, grass biomass is one of the world's most productive and sustainable lignocellulosic bioenergy sources [216,217]. Decreasing lignin content and increasing sugar content will lead to efficient bioethanol production, which is the same breeding strategy for

improving forage grass quality. An efficient transformation system has been established in switchgrass (*Panicum virgatum*), a model bioenergy crop, and is reported to down-regulate many lignin synthesis genes and its transcription factors genes [218]. Transformation systems in other grass species with higher biomass production, such as *Miscanthus sinensis* [169,170] and Napier grass (*Pennisetum purpureum*) [68], have also been developed. Warm-season grasses with fine texture and dense growth are used as turfgrasses in parks, gardens, golf courses, and sports ground. They are also industrially important and are used as greening materials in various situations. In particular, *Zoysia*, *Cynodon*, and *Paspalum* are the most common turfgrasses, and transgenic plants have been successfully produced (Table 2). Environmental stress resistance is an important trait for turfgrasses, and drought-resistant transgenic plants over-expressing *DREB* and *WRKY* transcription factors and *GA2ox1* gibberellin synthesis gene for drought resistance, and those with *ICE1* and *CBF1* transcription factors for cold tolerance were developed (Table 2).

The authors have established genetic transformation systems through somatic embryogenic cultures in the following warm-season grasses. Bahiagrass (*Paspalum notatum*) is a typical warm-season pasture grass, this transformation system employs a marker selection with *bar* genes [89] and a visual screening with *GFP* gene [192] (Figure 1h). Both methods produce stable transgenic plants with around 3.0% transformation efficiency, which is similar to other apomictic warm-season grasses [7]. Other transformation systems have been established in ruzigrass [91], which is widely cultivated in South America, and Napier grass [68] which is utilized as forage and bioenergy crops. In Rhodes grass, which is used for hay production and silage, transgenic plants have been successfully produced through organogenesis by using multiple shoot clumps as the target tissue [162]. Thus, we have successfully developed genetic transformation systems for many warm-season grasses by applying the appropriate culture system for each species.

7. Genome Editing

The genome editing is a breakthrough technology that can cut a targeted sequence at a pinpoint with artificial nucleases (ZFN, TALEN etc.) and RNA-inducible nucleases (CRISPR/Cas9), and can perform gene knockout and knock-in of target genes. The technology is being used in various research fields such as medicine, industry, science, and agronomics. In the field of plants, it is focused as a new breeding technology different from genetic transformation. On the other hand, the way of regulation of genome editing is discussed in the world.

Recently, highly functional soybeans with high oleic acid, developed by genome editing, have actually been produced without under the GM regulation and sold commercialization as soybean oil in the US [219]. Most of countries, except the EU and New Zealand, have accepted the technology and adapted legislations to these technologies or released guidelines supporting the use of genome editing [140]. This technology of plants is applied not only to model plants but also to crops such as corn, wheat, and sorghum [220,221]. Although the research has been advanced for practical use, genome editing of forage and turf grass has been successful in a few species such as *Lolium perenne* [185] and switchgrass [137,222].

In common, plant genome editing technology was performed to insert the CRISPR/Cas9 vector into the genome. The genome-edited mutant has the inserted vector in its genome, so the vector must be removed at the next generation by self-pollination. Therefore, this genome editing system is difficult to apply to forage grass or turfgrass due to their varying reproductive modes which include vegetative propagation, apomixis, and cross fertilization. In recent years, new genome editing systems have been developed which introduce Cas9 protein-gRNA ribonucleoproteins (RNPs) into plant cells and make genome editing [223]. This technique is completely free of DNA so the risk of transgene integration into the genome can be excluded. It is expected that this technology will be applied to many plant species. Although this system has been used mainly with protoplasts in plants, it has not been possible to apply it to a wide range of plant species due to the difficulty for

plant regeneration. On the other hand, genome editing using immature zygotic embryos has been successfully achieved by introducing RNPs with a particle bombardment in wheat [224–226], and maize [227,228], but this method cannot be applied to some warm-season grasses with a vegetative propagation and a hard to produce seeds.

The authors are working on a new genome editing system for warm-season grass and turf, focusing on somatic embryos as an alternative target tissue, which have high transformation efficiency and vigorous cell division with high potential of regeneration. In bahiagrass somatic embryo culture system, high dense pro-embryos on the surface of the callus can be continuously renewed by forming and proliferating secondary somatic embryos. Similar to genome editing of immature zygotic embryos, RNPs are introduced into somatic embryo cells by particle bombardment. Immediately after the RNPs are introduced into the cells, the genome-edited events occur at the cellular level, but through the culture system above, the cells can be developed into somatic embryos and regenerated into new plants. In our current research, we have already confirmed mutation at the target site at the cellular level after the delivery of RNPs into the somatic embryos of bahiagrass (unpublished). This genome editing method is completely free of DNA introduction and can be applied to vegetative propagated plants and apomictic plants, especially included warm-season grasses, and could be a novel method to produce genome-edited plants in its generation.

8. Conclusions

In the recent two decades, tissue culture systems have been established in many warm-season grasses. Also, there have been many reports of genetic transformation, which hardly succeeded before. Most of the plant regenerations are based on somatic embryogenesis, and a stable and efficient culture system ensures the applications to micropropagation, protoplast culture, genetic transformation and genome editing. In recent years, whole genome sequences have been determined in foxtail millet (*Setaria italica*) [229], switchgrass [230], *Miscanthus sinensis* [231], and *Zoysia* spp. [232], and progress in research using genomic information is expected in warm-season grasses, where genetic information has been limited until now. In particular, the genes for somatic embryogenesis are deeply involved in apomixis seed formation, thus exploration and identification of these candidate genes are expected to be applied to future breeding technology. In addition, genome editing technology is being applied to warm-season grasses, and the practical genomic breeding, unlike genetic transformation, is becoming possible. Our research team has been developing molecular breeding of some warm-season grasses, and has established a step-by-step process starting from somatic embryo formation, plant regeneration, suspension culture, protoplast culture, and genetic transformation. At this stage, we have just begun to focus on practical and applied research. We are now working on genome editing using genetic information and will develop new breeding materials in the next stage.

Author Contributions: Conceptualization, M.B.M. and T.G.; Original draft preparation, M.B.M. and T.G.; Review and editing, M.B.M., T.G., G.I., Y.S., N.U., P.N., M.M.R. and R.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by JSPS KAKENHI grant number 17K07608 (2017–2019) and the Ito Foundation grant number 52 (2020), 151 (2021).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chaudhury, A.; Qu, R. Somatic embryogenesis and plant regeneration of turf-type bermudagrass: Effect of 6-benzyladenine in callus induction medium. *Plant Cell Tissue Organ Cult.* **2000**, *60*, 113–120. [CrossRef]
2. Wilkins, P.W.; Humphreys, M.O. Progress in breeding perennial forage grasses for temperate agriculture. *J. Agric. Sci.* **2003**, *140*, 129–150. [CrossRef]
3. Food and Agriculture Organization (FAO). *The Future of Food and Agriculture—Trends and Challenges*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2017; Volume 296, pp. 1–180. Available online: <http://www.fao.org/3/a-i6583e.pdf> (accessed on 23 January 2021).
4. Alexandros, N.; Bruinsma, J. *World Agriculture: Towards 2030/2050: The 2012 Revision*; Agricultural Development Economics Division, Food and Agriculture Organization of the United Nations: Rome, Italy, 2012. [CrossRef]
5. Soreng, R.J.; Peterson, P.M.; Romaschenko, K.; Davidse, G.; Teisher, J.K.; Clark, L.G.; Barberá, P.; Gillespie, L.J.; Zuloaga, F.O. A worldwide phylogenetic classification of the Poaceae (Gramineae) II: An update and a comparison of two 2015 classifications. *J. Syst. Evol.* **2017**, *55*, 259–290. [CrossRef]
6. Sunny, A.; Razaqat, A.G.; Ki-Hong, J.; Aroosha, F.; Muhammad, U.Q.; Mustansar, M.; Weijun, Z. Conventional and molecular techniques from simple breeding to speed breeding in crop plants: Recent advances and future outlook. *Int. J. Mol. Sci.* **2020**, *21*, 2590. [CrossRef]
7. Bellido, A.M.; Souza Canad a, E.D.; Permingeat, H.R.; Echenique, V. Genetic transformation of apomictic grasses: Progress and constraints. *Front. Plant Sci.* **2021**, *12*, 768393. [CrossRef] [PubMed]
8. Mordhorst, A.P.; Toonen, M.A.; de Vries, S.C.; Meinke, D. Plant embryogenesis. *Crit. Rev. Plant Sci.* **1997**, *16*, 535–576. [CrossRef]
9. Steward, F.C.; Mapes, M.O.; Mears, K. Growth and organized development of cultured cells. II. Organization in cultures grown from freely suspended cells. *Am. J. Bot.* **1958**, *45*, 705–708. [CrossRef]
10. Gleddie, S.; Keller, W.; Setterfield, G. Somatic embryogenesis and plant regeneration from leaf explants and cell suspensions of *Solanum melongena* (eggplant). *Can. J. Bot.* **1983**, *61*, 656–666. [CrossRef]
11. Duncan, D.R.; Williams, M.E.; Zehr, B.E.; Widholm, J.M. The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* genotypes. *Planta* **1985**, *165*, 322–332. [CrossRef]
12. Tautorus, T.E.; Fowke, L.C.; Dunstan, D.I. Somatic embryogenesis in conifers. *Can. J. Bot.* **1991**, *69*, 1873–1899. [CrossRef]
13. Akashi, R.; Hashimoto, A.; Adachi, T. Plant regeneration from seed-derived embryogenic callus and cell suspension cultures of bahiagrass (*Paspalum notatum*). *Plant Sci.* **1993**, *90*, 73–80. [CrossRef]
14. Jain, S.M.; Gupta, P.K.; Newton, R.J. *Somatic Embryogenesis in Woody Plants Volume 2—Angiosperms*; Springer Science & Business Media, B.V.: Dordrecht, The Netherlands, 1995.
15. Faure, O.; Dewitte, W.; Nougarede, A.; Van Onckelen, H. Precociously germinating somatic embryos of *Vitis vinifera* have lower ABA and IAA levels than their germinating zygotic counterparts. *Physiol. Plant* **1998**, *102*, 591–595. [CrossRef]
16. Giri, C.C.; Praveena, M. In vitro regeneration, somatic hybridization and genetic transformation studies: An appraisal on biotechnological interventions in grasses. *Plant Cell Tissue Organ Cult.* **2015**, *120*, 843–860. [CrossRef]
17. Norstog, K. Induction of embryolike structures by kinetin in cultured barley embryos. *Dev. Biol.* **1970**, *23*, 665–670. [CrossRef]
18. Lu, C.Y.; Vasil, I.K. Somatic embryogenesis and plant regeneration from leaf tissues of *Panicum maximum* Jacq. *Theor. Appl. Genet.* **1981**, *59*, 275–280. [CrossRef] [PubMed]
19. Vasil, V.; Vasil, I.K. Somatic embryogenesis and plant regeneration from tissue cultures of *Pennisetum americanum*, and *P. americanum* × *P. purpureum* hybrid. *Amer. J. Bot.* **1981**, *68*, 864–872. [CrossRef]
20. Aguado-Santacruz, G.A.; Cabrera-Ponce, J.L.; Ram rez-Ch avez, E.; Le n-Ram rez, C.G.; Rasc n-Cruz, Q.; Herrera-Estrella, L.; Olalde-Portugal, V. Establishment, characterization and plant regeneration from highly chlorophyllous embryogenic cell cultures of blue grama grass, *Bouteloua gracilis* (H.B.K.) Lag. ex Steud. *Plant Cell Rep.* **2001**, *20*, 131–136. [CrossRef]
21. Kumar, S.; Sahu, N.; Singh, A. High-frequency in vitro plant regeneration via callus induction in a rare sexual plant of *Cenchrus ciliaris* L. *Vitr. Cell. Dev. Biol. Plant* **2015**, *51*, 28–34. [CrossRef]
22. Shashi; Bhat, V. Enhanced somatic embryogenesis and plantlet regeneration in *Cenchrus ciliaris* L. *Vitr. Cell. Dev. Biol. Plant* **2021**, *57*, 499–509. [CrossRef]
23. Gondo, T.; Matsumoto, J.; Yamakawa, K.; Tsuruta, S.; Ebina, M.; Akashi, R. Somatic embryogenesis and multiple-shoot formation from seed-derived shoot apical meristems of rhodesgrass (*Chloris gayana* Kunth). *Grassl. Sci.* **2007**, *53*, 138–142. [CrossRef]
24. Ahn, B.J.; Huang, F.H.; King, J.W. Plant regeneration through somatic embryogenesis in common bermudagrass tissue culture. *Crop. Sci.* **1985**, *25*, 1107–1109. [CrossRef]
25. Ahn, B.J.; Huang, F.H.; King, J.W. Regeneration of bermudagrass cultivars and evidence of somatic embryogenesis. *Crop. Sci.* **1987**, *27*, 594–597. [CrossRef]
26. Artunduaga, I.R.; Taliaferro, C.M.; Johnson, B.L. Effects of auxin concentration on induction and growth of embryogenic callus from young inflorescence explants of Old World bluestem (*Bothriochloa* spp.) and bermuda (*Cynodon* spp.) grasses. *Plant Cell Tissue Organ Cult.* **1988**, *12*, 13–19. [CrossRef]
27. Le, B.V.; Jeanneau, M.; Do My, N.T.; Vidal, J.; Thanh V n, K.T. Rapid regeneration of whole plants in large crabgrass (*Digitaria sanguinalis* L.) using thin-cell-layer culture. *Plant Cell Rep.* **1998**, *18*, 166–172. [CrossRef]
28. Mekbib, F.; Mantell, S.H.; Buchanan-Wollaston, V. Callus induction and in vitro regeneration of tef [*Eragrostis tef* (Zucc.) Trotter] from leaf. *J. Plant Physiol.* **1997**, *151*, 368–372. [CrossRef]

29. Kebebew, A.; Gaj, M.D.; Maluszynski, M. Somatic embryogenesis and plant regeneration in callus culture of tef, *Eragrostis tef* (Zucc.) Trotter. *Plant Cell Rep.* **1998**, *18*, 154–158. [[CrossRef](#)]
30. Akashi, R.; Ikeda, H. Callus formation and plant regeneration from immature inflorescences and apical meristem of cogongrass (*Imperata cylindrica* L.). *Glassl. Sci.* **1989**, *34*, 333–335. [[CrossRef](#)]
31. Umami, N.; Gondo, T.; Tanaka, H.; Rahman, M.M.; Akashi, R. Efficient nursery plant production of dwarf cogongrass (*Imperata cylindrica* L.) through mass propagation in liquid culture. *Grassl. Sci.* **2012**, *58*, 201–207. [[CrossRef](#)]
32. Nielsen, J.M.; Brandt, K.; Hansen, J. Long-term effects of thidiazuron are intermediate between benzyladenine, kinetin or isopentenyladenine in *Miscanthus sinensis*. *Plant Cell Tissue Organ Cult.* **1993**, *35*, 173–179. [[CrossRef](#)]
33. Zhang, Q.X.; Sun, Y.; Hu, H.K.; Chen, B.; Hong, C.T.; Guo, H.P.; Pan, Y.H.; Zheng, B.S. Micropropagation and plant regeneration from embryogenic callus of *Miscanthus sinensis*. *Vitr. Cell. Dev. Biol. Plant* **2012**, *48*, 50–57. [[CrossRef](#)]
34. Bajaj, Y.P.S.; Sidhu, B.S.; Dubey, V.K. Regeneration of genetically diverse plants from tissue cultures of forage grass—*Panicum* spp. *Euphytica* **1981**, *30*, 135–140. [[CrossRef](#)]
35. Seo, M.S.; Takahara, M.; Ebina, M.; Takamizo, T. Evaluation of tissue culture response from mature seeds of *Panicum* spp. *Grassl. Sci.* **2008**, *54*, 125–130. [[CrossRef](#)]
36. Seo, M.S.; Takahara, M.; Takamizo, T. Optimization of culture conditions for plant regeneration of *Panicum* spp. through somatic embryogenesis. *Grassl. Sci.* **2010**, *56*, 6–12. [[CrossRef](#)]
37. Fladung, M.; Hesselbach, J. Callus induction and plant regeneration in *Panicum bisulcatum* and *P. milioides*. *Plant Cell Rep.* **1986**, *5*, 169–173. [[CrossRef](#)]
38. Lu, C.Y.; Vasil, I.K. Somatic embryogenesis and plant regeneration from freely suspended cells and cell groups of *Panicum maximum* in vitro. *Ann. Bot.* **1981**, *48*, 543–548. [[CrossRef](#)]
39. Lu, C.Y.; Vasil, I.K. Somatic embryogenesis and plant regeneration in tissue cultures of *Panicum maximum* Jacq. *Amer. J. Bot.* **1982**, *69*, 77–81. [[CrossRef](#)]
40. Akashi, R.; Adachi, T. High frequency somatic embryo formation in culture of immature embryos of guineagrass, *Panicum maximum*. *Jpn. J. Breed.* **1991**, *41*, 85–93. [[CrossRef](#)]
41. Rangan, T.S. Morphogenic investigations on tissue cultures of *Panicum miliaceum*. *Z. Pflanzenphysiol.* **1974**, *72*, 456–459. [[CrossRef](#)]
42. Rangan, T.S.; Vasil, I.K. Somatic embryogenesis and plant regeneration in tissue cultures of *Panicum miliaceum* L. and *Panicum miliare* Lamk. *Z. Pflanzenphysiol.* **1983**, *109*, 49–53. [[CrossRef](#)]
43. Rajasekaran, K.; Vasil, I.K. Somatic embryogenesis and plant regeneration from cultured segments of young leaves and inflorescences of *Panicum virgatum* L. (Switch grass). *J. Plant Physiol.* **1986**, *126*, 41–48.
44. Dutta, S.; Conger, B.V. Somatic embryogenesis and plant regeneration from suspension cultures of switchgrass. *Crop. Sci.* **1999**, *39*, 223–227. [[CrossRef](#)]
45. Burris, J.N.; Mann, D.G.J.; Joyce, B.L.; Stewart, C.N., Jr. An improved tissue culture system for embryogenic callus production and plant regeneration in switchgrass (*Panicum virgatum* L.). *Bioenerg. Res.* **2009**, *2*, 267–274. [[CrossRef](#)]
46. Bovo, O.A.; Mroginski, L.A. Tissue culture in *Paspalum* (Gramineae): Plant regeneration from cultured inflorescences. *J. Plant Physiol.* **1986**, *124*, 481–492. [[CrossRef](#)]
47. Akashi, R.; Adachi, T. Somatic embryogenesis and plant regeneration from cultured immature inflorescences of apomictic dallisgrass (*Paspalum dilatatum* Poir.). *Plant Sci.* **1992**, *82*, 213–218. [[CrossRef](#)]
48. Bovo, O.A.; Mroginski, L.A. Somatic embryogenesis and plant regeneration from cultured mature and immature embryos of *Paspalum notatum* (Gramineae). *Plant Sci.* **1989**, *65*, 217–223. [[CrossRef](#)]
49. Marousky, F.J.; West, S.H. Somatic embryogenesis and plant regeneration from cultured mature caryopses of bahiagrass (*Paspalum notatum* Flugge). *Plant Cell Tissue Organ Cult.* **1990**, *20*, 125–129. [[CrossRef](#)]
50. Grando, M.F.; Franklin, C.I.; Shatters, R.G. Optimizing embryogenic callus production and plant regeneration from “Tifton 9” bahiagrass (*Paspalum notatum* Flugge) seed explants for genetic manipulation. *Plant Cell Tissue Organ Cult.* **2002**, *71*, 213–222. [[CrossRef](#)]
51. Chen, L.; Anami, E.; Guan, L.; Adachi, T. Somatic embryogenesis and plant regeneration from leaflets of “Nanou” bahiagrass. *Plant Biotechnol.* **2001**, *18*, 119–123. [[CrossRef](#)]
52. Rangan, T.S. Growth and plantlet regeneration in tissue cultures of some Indian millets; *Paspalum scrobiculatum* L., *Eleusine coracana* Gaertn. and *Pennisetum typhoideum* Pers. *Z. Pflanzenphysiol.* **1976**, *78*, 208–216. [[CrossRef](#)]
53. Nayak, P.; Sen, S.K. Plant regeneration through somatic embryogenesis from suspension cultures of a minor millet, *Paspalum scrobiculatum*. *Plant Cell Rep.* **1989**, *8*, 296–299. [[CrossRef](#)]
54. Rashid, A. Somatic embryogenesis from immature and mature embryos of a minor millet *Paspalum scrobiculatum* L. *Plant Cell Tissue Organ Cult.* **2002**, *69*, 71–77. [[CrossRef](#)]
55. Vikrant; Rashid, A. Induction of multiple shoots by thidiazuron from caryopsis cultures of minor millet (*Paspalum scrobiculatum* L.) and its effect on the regeneration of embryogenic callus cultures. *Plant Cell Rep.* **2002**, *21*, 9–13. [[CrossRef](#)]
56. Ceasar, S.A.; Ignacimuthu, S. Effects of cytokinins, carbohydrates and amino acids on induction and maturation of somatic embryos in kodo millet (*Paspalum scrobiculatum* Linn.). *Plant Cell Tissue Organ Cult.* **2010**, *102*, 153–162. [[CrossRef](#)]
57. Neibaur, I.; Gallo, M.; Altpeter, F. The effect of auxin type and cytokinin concentration on callus induction and plant regeneration frequency from immature inflorescence segments of seashore paspalum (*Paspalum vaginatum* Swartz). *Vitr. Cell. Dev. Biol. Plant* **2008**, *44*, 480–486. [[CrossRef](#)]

58. Vasil, V.; Vasil, I.K. Somatic embryogenesis and plant regeneration from suspension cultures of pearl millet (*Pennisetum americanum*). *Ann. Bot.* **1981**, *47*, 669–678. [[CrossRef](#)]
59. Vasil, V.; Vasil, I.K. Characterization of an embryogenic cell suspension culture derived from inflorescences of *Pennisetum americanum* (pearl millet; Gramineae). *Am. J. Bot.* **1982**, *69*, 1441–1449. [[CrossRef](#)]
60. Lambé, P.; Mutambel, H.S.N.; Deltour, R.; Dinant, M. Somatic embryogenesis in pearl millet (*Pennisetum glaucum*): Strategies to reduce genotype limitation and to maintain long-term totipotency. *Plant Cell Tissue Organ Cult.* **1998**, *55*, 23–29. [[CrossRef](#)]
61. Devi, P.; Zhong, H.; Sticklen, M.B. In vitro morphogenesis of pearl millet [*Pennisetum glaucum* (L.) R.Br.]: Efficient production of multiple shoots and inflorescences from shoot apices. *Plant Cell Rep.* **2000**, *19*, 546–550. [[CrossRef](#)]
62. Oldach, K.; Moregenstern, A.; Rother, S.; Girgi, M.; O’Kennedy, M.; Lörz, H. Efficient in vitro plant regeneration from immature zygotic embryos of pearl millet [*Pennisetum glaucum* (L.) R. Br.] and *Sorghum bicolor* (L.) Moench. *Plant Cell Rep.* **2001**, *20*, 416–421. [[CrossRef](#)]
63. Jha, P.; Yadav, C.B.; Anjaiah, V.; Bhat, V. In vitro plant regeneration through somatic embryogenesis and direct shoot organogenesis in *Pennisetum glaucum* (L.). *R. Br. Vitro. Cell Dev. Biol. Plant* **2009**, *45*, 145–154. [[CrossRef](#)]
64. Haydu, Z.; Vasil, I.K. Somatic embryogenesis and plant regeneration from leaf tissues and anthers of *Pennisetum purpureum* Schum. *Theor. Appl. Genet.* **1981**, *59*, 269–273. [[CrossRef](#)] [[PubMed](#)]
65. Wang, D.; Vasil, I.K. Somatic embryogenesis and plant regeneration from inflorescence segments of *Pennisetum purpureum* Schum, (Napier or Elephant grass). *Plant Sci. Lett.* **1982**, *25*, 147–154. [[CrossRef](#)]
66. Chandler, S.F.; Vasil, I.K. Optimization of plant regeneration from long term embryogenic callus cultures of *Pennisetum purpureum* Schum. (Napier grass). *J. Plant Physiol.* **1984**, *117*, 147–156. [[CrossRef](#)]
67. Umami, N.; Gondo, T.; Ishigaki, G.; Rahman, M.M.; Akashi, R. Efficient nursery production and multiple-shoot clumps formation from shoot tiller-derived shoot apices of dwarf napiergrass (*Pennisetum purpureum* Schumach.). *JWARAS* **2012**, *55*, 121–127. [[CrossRef](#)]
68. Gondo, T.; Umami, N.; Muguerza, M.; Akashi, R. Plant regeneration from embryogenic callus derived from shoot apices and production of transgenic plants by particle inflow gun in dwarf napiergrass (*Pennisetum purpureum* Schumach.). *Plant Biotech.* **2017**, *34*, 143–150. [[CrossRef](#)]
69. Xu, Z.H.; Wang, D.Y.; Yang, L.J.; Wei, Z.M. Somatic embryogenesis and plant regeneration in cultured immature inflorescences of *Setaria italia*. *Plant Cell Rep.* **1984**, *3*, 149–150. [[CrossRef](#)]
70. Rao, A.M.; Kishor, P.B.; Reddy, L.A.; Vaidyanath, K. Callus induction and high frequency plant regeneration in Italian millet (*Setaria italica*). *Plant Cell Rep.* **1988**, *7*, 557–559. [[CrossRef](#)]
71. Cabral, G.B.; Carneiro, V.T.C.; Lacerda, A.L.; do Valle, C.B.; Martinelli, A.P.; de Alencar Dusi, D.M. Somatic embryogenesis and organogenesis in apomictic and sexual *Brachiaria brizantha*. *Plant Cell Tissue Organ Cult.* **2011**, *107*, 271–282. [[CrossRef](#)]
72. Cabral, G.B.; Carneiro, V.T.C.; Rossi, M.L.; Silva, J.P.D.; Martinelli, A.P.; Dusi, D.M.A. Plant regeneration from embryogenic callus and cell suspensions of *Brachiaria brizantha*. *Vitr. Cell Dev. Biol. Plant* **2015**, *51*, 369–377. [[CrossRef](#)]
73. Ishigaki, G.; Gondo, T.; Suenaga, K.; Akashi, R. Multiple shoot formation, somatic embryogenesis and plant regeneration from seed-derived shoot apical meristems in ruzigrass (*Brachiaria ruziziensis*). *Grassl. Sci.* **2009**, *55*, 46–51. [[CrossRef](#)]
74. Asano, Y. Somatic embryogenesis and protoplast culture in Japanese lawngrass (*Zoysia japonica*). *Plant Cell Rep.* **1989**, *8*, 141–143. [[CrossRef](#)] [[PubMed](#)]
75. Asano, Y.; Katsumoto, H.; Inokuma, C.; Kaneko, S.; Ito, Y.; Fujiie, A. Cytokinin and thiamine requirements and stimulative effects of riboflavin and α -ketoglutaric acid on embryogenic callus induction from the seeds of *Zoysia japonica* steud. *J. Plant Physiol.* **1996**, *149*, 413–417. [[CrossRef](#)]
76. Liu, L.; Fan, X.; Zhang, J.; Yan, M.; Bao, M. Long-term cultured callus and the effect factor of high-frequency plantlet regeneration and somatic embryogenesis maintenance in *Zoysia japonica*. *Vitr. Cell. Dev. Biol. Plant.* **2009**, *45*, 673–680. [[CrossRef](#)]
77. Dhandapani, M.; Hong, S.B.; Aswath, C.R.; Kim, D.H. Regeneration of zoysia grass (*Zoysia matrella* L. Merr.) cv. Konhee from young inflorescences and stem nodes. *Vitr. Cell. Dev. Biol. Plant.* **2008**, *44*, 8–13. [[CrossRef](#)]
78. Chai, M.; Jia, Y.; Chen, S.; Gao, Z.; Wang, H.; Liu, L.; Wang, P.; Hou, D. Callus induction, plant regeneration, and long-term maintenance of embryogenic cultures in *Zoysia matrella* [L.] Merr. *Plant Cell Tissue Organ Cult.* **2011**, *104*, 187–192. [[CrossRef](#)]
79. Lu, C.Y.; Vasil, V.; Vasil, I.K. Isolation and culture of protoplasts of *Panicum maximum* Jacq. (Guineagrass): Somatic embryogenesis and plantlet formation. *Z. Pflanzenphysiol.* **1981**, *104*, 311–318. [[CrossRef](#)]
80. Akashi, R.; Lachmann, S.; Hoffmann, F.; Adachi, T. Embryogenic callus formation from protoplasts derived from suspension cells of apomictic guineagrass (*Panicum maximum*). *Breed. Sci.* **1995**, *45*, 445–448. [[CrossRef](#)]
81. Heyser, J.W. Callus and shoot regeneration from protoplasts of Proso millet (*Panicum miliaceum* L.). *Z. Pflanzenphysiol.* **1984**, *113*, 292–299. [[CrossRef](#)]
82. Nayak, P.; Sen, S.K. Plant regeneration through somatic embryogenesis from suspension culture-derived protoplasts of *Paspalum scrobiculatum* L. *Plant Cell Rep.* **1991**, *10*, 362–365. [[CrossRef](#)]
83. Akashi, R.; Adachi, T. Plant regeneration from suspension cultured-derived protoplasts of apomictic dallisgrass (*Paspalum dilatatum* Poir.). *Plant Sci.* **1992**, *82*, 219–225. [[CrossRef](#)]
84. Vasil, V.; Vasil, I.K. Isolation and culture of cereal protoplasts I. Callus formation from pearl millet (*Pennisetum americanum*) protoplasts. *Z. Pflanzenphysiol.* **1979**, *92*, 379–383. [[CrossRef](#)]

85. Vasil, V.; Vasil, I.K. Isolation and culture of cereal protoplasts. Part 2: Embryogenesis and plantlet formation from protoplast of *Pennisetum americanum* L. *Theor. Appl. Genet.* **1980**, *56*, 97–99. [[CrossRef](#)] [[PubMed](#)]
86. Vasil, V.; Wang, D.Y.; Vasil, I.K. Plant regeneration from protoplasts of Napier grass (*Pennisetum purpureum* Schum.). *Z. Pflanzenphysiol.* **1983**, *111*, 233–239. [[CrossRef](#)]
87. Inokuma, C.; Sugiura, K.; Cho, C.; Okawara, R.; Kaneko, S. Plant regeneration from protoplasts of Japanese lawngrass. *Plant Cell Rep.* **1996**, *15*, 737–741. [[CrossRef](#)] [[PubMed](#)]
88. Yang, X.; Zhang, X. Regulation of somatic embryogenesis in higher plants. *Crit. Rev. Plant Sci.* **2010**, *29*, 36–57. [[CrossRef](#)]
89. Gondo, T.; Tsuruta, S.; Akashi, R.; Kawamura, O.; Hoffmann, F. Green, herbicide-resistant plants by particle inflow gun-mediated gene transfer to diploid bahiagrass (*Paspalum notatum*). *J. Plant Physiol.* **2005**, *162*, 1367–1375. [[CrossRef](#)]
90. Murashige, S.; Skoog, F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant* **1962**, *15*, 473–497. [[CrossRef](#)]
91. Ishigaki, G.; Gondo, T.; Suenaga, K.; Akashi, R. Fertile transgenic *Brachiaria ruziziensis* (ruzigrass) plants by particle bombardment of tetraploidized callus. *J. Plant Physiol.* **2012**, *169*, 546–549. [[CrossRef](#)]
92. Ishigaki, G.; Gondo, T.; Suenaga, K.; Akashi, R. Induction of tetraploid ruzigrass (*Brachiaria ruziziensis*) plants by colchicine treatment of in vitro multiple-shoot clumps and seedlings. *Grassl. Sci.* **2009**, *55*, 164–170. [[CrossRef](#)]
93. JIRCAS, Japan International Research Center for Agricultural Sciences. *Annual Reports 2018 (Apr. 2018–Mar. 2019)*; JIRCAS: Ibaraki, Japan, 2019; No. 25; p. 11.
94. Nitthaisong, P.; Ishigaki, G.; Tanaka, H.; Akashi, R. Chromosome number, genomic variation, and molecular markers to assess genetic diversity of *Brachiaria* species. *Crop. Sci.* **2016**, *56*, 312–321. [[CrossRef](#)]
95. Nitthaisong, P.; Ishigaki, G.; Suenaga, K.; Muguerza, M.; Tanaka, H.; Akashi, R. Pentaploid apomicts by interspecific hybridization between diploid *Urochloa ruziziensis* and tetraploid apomictic *U. decumbens*. *Crop. Sci.* **2019**, *59*, 1648–1656. [[CrossRef](#)]
96. Aleith, F.; Richter, G. Gene expression during induction of somatic embryogenesis in carrot cell suspensions. *Planta* **1990**, *183*, 17–24. [[CrossRef](#)] [[PubMed](#)]
97. Busk, P.K.; Pages, M. Regulation of abscisic acid-induced transcription. *Plant Mol. Biol.* **1998**, *37*, 425–435. [[CrossRef](#)] [[PubMed](#)]
98. Guo, F.; Liu, C.; Xia, H.; Bi, Y.; Zhao, C.; Zhao, S.; Hou, L.; Li, F.; Wang, X. Induced expression of AtLEC1 and AtLEC2 differentially promotes somatic embryogenesis in transgenic tobacco plants. *PLoS ONE* **2013**, *8*, e71714. [[CrossRef](#)]
99. Sinha, N.R.; Williams, R.E.; Hake, S. Overexpression of the maize homeo box gene, KNOTTED-1, causes a switch from determinate to indeterminate cell fates. *Gene Dev.* **1993**, *7*, 787–795. [[CrossRef](#)]
100. Kawahara, R.; Komamine, A.; Fukuda, H. Isolation and characterization of homeobox-containing genes of carrot. *Plant Mol. Biol.* **1995**, *27*, 155–164. [[CrossRef](#)]
101. Hiwatashi, Y.; Fukuda, H. Tissue-specific localization of mRNA for carrot homeobox genes, CHBs, in carrot somatic embryos. *Plant Cell Physiol.* **2000**, *41*, 639–643. [[CrossRef](#)]
102. Holk, A.; Kaldenhoff, R.; Richter, G. Regulation of an embryogenic carrot gene (*DC 2.15*) and identification of its active promoter sites. *Plant Mol. Biol.* **1996**, *31*, 1153–1161. [[CrossRef](#)]
103. Pilarska, M.; Malec, P.; Salaj, J.; Bartnicki, F.; Konieczny, R. High expression of SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE coincides with initiation of various developmental pathways in in vitro culture of *Trifolium nigrescens*. *Protoplasma* **2016**, *253*, 345–355. [[CrossRef](#)]
104. Podio, M.; Felitti, S.A.; Siena, L.A.; Delgado, L.; Mancini, M.; Seijo, J.G.; González, A.M.; Pessino, S.C.; Ortiz, J.P.A. Characterization and expression analysis of SOMATIC EMBRYOGENESIS RECEPTOR KINASE (*SERK*) genes in sexual and apomictic *Paspalum notatum*. *Plant Mol. Biol.* **2014**, *84*, 479–495. [[CrossRef](#)]
105. Koehler, A.D.; Irsigler, A.S.T.; Carneiro, V.T.C.; Cabral, G.B.; Rodrigues, J.C.M.; Gomes, A.C.M.M.; Togawa, R.C.; Costa, M.M.C.; Martinelli, A.P.; de Alencar Dusi, D.M. *SERK* genes identification and expression analysis during somatic embryogenesis and sporogenesis of sexual and apomictic *Brachiaria brizantha* (Syn. *Urochloa brizantha*). *Planta* **2020**, *252*, 39. [[CrossRef](#)] [[PubMed](#)]
106. Brukhin, V. Is sex irreplaceable? Towards the molecular regulation of apomixis. *Int. J. Plant Reprod. Biol.* **2017**, *9*, 153–169.
107. Brukhin, V. Molecular and genetic regulation of apomixis. *Russ. J. Genet.* **2017**, *53*, 943–964. [[CrossRef](#)]
108. Ondzighi-Assoume, C.A.; Willis, J.D.; Ouma, W.K.; Allen, S.M.; King, Z.; Parrott, W.A.; Liu, W.; Burris, J.N.; Lenaghan, S.C.; Stewart, C.N., Jr. Embryogenic cell suspensions for high-capacity genetic transformation and regeneration of switchgrass (*Panicum virgatum* L.). *Biotechnol. Biofuels* **2019**, *12*, 290. [[CrossRef](#)] [[PubMed](#)]
109. Karlsson, S.B.; Vasil, I.K. Morphology and ultrastructure of embryogenic cell suspension cultures of *Panicum maximum* (Guinea grass) and *Pennisetum purpureum* (Napier grass). *Am. J. Bot.* **1986**, *73*, 894–901. [[CrossRef](#)]
110. Lu, S.; Wang, Z.; Peng, X.; Guo, Z.; Zhang, G.; Han, L. An efficient callus suspension culture system for triploid bermudagrass (*Cynodon transvaalensis* × *C. dactylon*) and somaclonal variations. *Plant Cell Tissue Organ Cult.* **2006**, *87*, 77–84. [[CrossRef](#)]
111. Mazarei, M.; Al-Ahmad, H.; Rudis, M.R.; Joyce, B.L.; Stewart, C.N., Jr. Switchgrass (*Panicum virgatum* L.) cell suspension cultures: Establishment, characterization, and application. *Plant Sci.* **2011**, *181*, 712–715. [[CrossRef](#)]
112. Lin, C.S.; Hsu, C.T.; Yang, L.H.; Lee, L.Y.; Fu, J.Y.; Cheng, Q.W.; Wu, F.H.; Hsiao, H.C.W.; Zhang, Y.; Zhang, R.; et al. Application of protoplast technology to CRISPR/Cas9 mutagenesis: From single-cell mutation detection to mutant plant regeneration. *Plant Biotechnol. J.* **2018**, *16*, 1295–1310. [[CrossRef](#)]
113. Chen, K.; Wang, Y.; Zhang, R.; Zhang, H.; Gao, C. CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annu. Rev. Plant Biol.* **2019**, *70*, 667–697. [[CrossRef](#)]

114. Meng, R.; Wang, C.; Wang, L.; Liu, Y.; Zhan, Q.; Zheng, J.; Li, J. An efficient sorghum protoplast assay for transient gene expression and gene editing by CRISPR/Cas9. *PeerJ* **2020**, *8*, e10077. [[CrossRef](#)]
115. Gao, C. Genome engineering for crop improvement and future agriculture. *Cell* **2021**, *184*, 1621–1635. [[CrossRef](#)]
116. Mariani, T.S.; Miyake, H.; Taniguchi, T. Isolation and culture of protoplast from Finger Millet (*Eleusine coracana*) Callus. *Jpn. J. Crop. Sci.* **1992**, *61*, 668–675. [[CrossRef](#)]
117. Tiécoura, K.; Kouassi, A.B.; N’nan-Alla, O.; Bi, S.G.; Dinant, M.; Ledou, L. Isolation and culture of protoplasts of Côte d’Ivoire’s pearl millet (*Pennisetum glaucum* (L.) R.) varieties. *J. Appl. Biosci.* **2015**, *92*, 8620–8929. [[CrossRef](#)]
118. Reed, K.M.; Bargmann, B.O.R. Protoplast regeneration and its use in new plant breeding technologies. *Front. Genome Ed.* **2021**, *3*, 734951. [[CrossRef](#)] [[PubMed](#)]
119. Akashi, R. Some trials of genetic manipulation of apomictic species in *Gramineae*: Protoplast culture and fusion of guineagrass (*Panicum maximum*) and dallisgrass (*Paspalum dilatatum*). *Proc. ICOBB Miyazaki* **1991**, *1991*, 25–40.
120. Spangenberg, G.; Wang, Z.Y.; Potrykus, I. Biotechnology in forage and turf grass improvement. In *Monographs on Theoretical and Applied Genetics*; Frankel, R., Grossman, M., Linskens, H.F., Maliga, P., Riley, R., Eds.; Springer: Heidelberg, Germany, 1998; Volume 23, p. 192.
121. Davey, M.R.; Anthony, P.; Power, J.B.; Lowe, K.C. Plant protoplasts: Status and biotechnological perspectives. *Biotechnol. Adv.* **2005**, *23*, 131–171. [[CrossRef](#)]
122. Zhi, D.; Xiang, F.; Chen, X.; Xia, G.; Chen, H. Production of plants from somatic hybridization between common wheat and maize (*Zea mays* L.). *Sci. China Life Sci.* **2002**, *37*, 80–83.
123. Xu, C.H.; Xia, G.M.; Zhi, D.Y.; Xiang, F.N.; Chen, H.M. Integration of maize nuclear and mitochondrial DNA into the wheat genome through somatic hybridization. *Plant Sci.* **2003**, *165*, 1001–1008. [[CrossRef](#)]
124. Tabaeizadeh, Z.; Ferl, R.J.; Vasil, I.K. Somatic hybridization in the Gramineae: *Saccharum officinarum* L. (sugarcane) and *Pennisetum americanum* (L.) K. Schum. (pearl millet). *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 5616–5619. [[CrossRef](#)]
125. Tabaeizadeh, Z.; Pring, D.R.; Vasil, I.K. Analysis of mitochondrial DNA from somatic hybrid cell lines of *Saccharum officinarum* (sugarcane) and *Pennisetum americanum* (pearl millet). *Plant Mol. Biol.* **1987**, *8*, 509–513. [[CrossRef](#)]
126. Ozias-Akins, P.; Ferl, R.J.; Vasil, I.K. Somatic hybridization in the gramineae: *Pennisetum americanum* (L.) K. Schum. (pearl millet) + *Panicum maximum* Jacq. (Guinea grass). *Mol. Gen. Genet.* **1986**, *203*, 365–370. [[CrossRef](#)]
127. Wang, T.B.; Niizeki, M.; Harada, T.; Ishikawa, R.; Qian, Y.; Saito, K. Establishment of somatic hybrid cell lines between *Zea mays* L. (maize) and *Triticum* sect. *trititrigia* MacKey (*trititrigia*). *Theor. Appl. Genet.* **1993**, *86*, 371–376. [[CrossRef](#)] [[PubMed](#)]
128. Xin, H.W.; Sun, J.S.; Yan, Q.S.; Zhang, X.Q. Plant regeneration from asymmetric somatic hybrids of *Oryza sativa* and *Panicum maximum*. *J. Integr. Plant Biol.* **1997**, *39*, 717–724.
129. Laurie, D.A.; Bennett, M.D. Wheat × maize hybridization. *Can. J. Genet. Cytol.* **1986**, *28*, 313–316. [[CrossRef](#)]
130. Riera-Lizarazu, O.; Rines, H.W.; Phillips, R.L. Cytological and molecular characterization of oat × maize partial hybrids. *Theoret. Appl. Genet.* **1996**, *93*, 123–135. [[CrossRef](#)] [[PubMed](#)]
131. Simpson, C.J.; Reeves, G.; Tripathi, A.; Singh, P.; Hibberd, J.M. Using breeding and quantitative genetics to understand the C₄ pathway. *J. Expt. Bot.* **2021**, erab486. [[CrossRef](#)]
132. Burris, K.P.; Dlugosz, E.M.; Collins, A.G.; Stewart, C.N.; Lenaghan, S.C. Development of a rapid, low-cost protoplast transfection system for switchgrass (*Panicum virgatum* L.). *Plant Cell Rep.* **2016**, *35*, 693–704. [[CrossRef](#)]
133. Gong, Z.; Cheng, M.; Botella, J.R. Non-GM genome editing approaches in crops. *Front. Genome Ed.* **2021**, *3*, 817279. [[CrossRef](#)]
134. Yue, J.-J.; Yuan, J.-L.; Wu, F.-H.; Yuan, Y.-H.; Cheng, Q.-W.; Hsu, C.-T.; Lin, C.-S. Protoplasts: From isolation to CRISPR/Cas genome editing application. *Front. Genome Ed.* **2021**, *3*, 717017. [[CrossRef](#)]
135. Zhang, Y.; Iaffaldano, B.; Qi, Y. CRISPR ribonucleoprotein-mediated genetic engineering in plants. *Plant Commun.* **2021**, *2*, 100168. [[CrossRef](#)]
136. Kim, J.H.; Doan, P.P.T.; Lee, H.Y.; Kim, J. Transient gene expression system in zoysiagrass leaf mesophyll protoplasts. *Plant Biotechnol. Rep.* **2022**, *16*, 113–121. [[CrossRef](#)]
137. Liu, Y.; Merrick, P.; Zhang, Z.; Ji, C.; Yang, B.; Fei, S.Z. Targeted mutagenesis in tetraploid switchgrass (*Panicum virgatum* L.) using CRISPR/Cas9. *Plant Biotechnol. J.* **2018**, *16*, 381–393. [[CrossRef](#)] [[PubMed](#)]
138. Zhang, Z.; Hua, L.; Gupta, A.; Tricoli, D.; Edwards, K.J.; Yang, B.; Li, W. Development of an Agrobacterium-delivered CRISPR/Cas9 system for wheat genome editing. *Plant Biotechnol. J.* **2019**, *17*, 1623–1635. [[CrossRef](#)] [[PubMed](#)]
139. Banakar, R.; Schubert, M.; Kurgan, G.; Rai, K.M.; Beaudoin, S.F.; Collingwood, M.A.; Vakulskas, C.A.; Wang, K.; Zhang, F. Efficiency, specificity and temperature sensitivity of Cas9 and Cas12a RNPs for DNA- free genome editing in plants. *Front. Genome Ed.* **2022**, *3*, 760820. [[CrossRef](#)] [[PubMed](#)]
140. Menz, J.; Modrzejewski, D.; Hartung, F.; Wilhelm, R.; Sprink, T. Genome edited crops touch the market: A view on the global development and regulatory environment. *Front. Plant Sci.* **2020**, *11*, 586027. [[CrossRef](#)] [[PubMed](#)]
141. Larkin, P.J.; Scowcroft, W.R. Somaclonal variation: A novel source of variability from cell cultures for plant improvement. *Theor. Appl. Genet.* **1981**, *60*, 197–214. [[CrossRef](#)]
142. Bajaj, Y.P.S. Somatic Hybridization—A Rich Source of Genetic Variability. In *Somatic Hybridization in Crop Improvement I. Biotechnology in Agriculture and Forestry*; Bajaj, Y.P.S., Ed.; Springer: Berlin/Heidelberg, Germany, 1994; Volume 27, pp. 3–32. [[CrossRef](#)]

143. Acuna, C.A.; Martínez, E.J.; Zilli, A.L.; Brugnoli, E.A.; Espinoza, F.; Marcon, F.; Urbani, M.H.; Quarin, C.L. Reproductive systems in *Paspalum*: Relevance for germplasm collection and conservation, breeding techniques, and adoption of released cultivars. *Front. Plant Sci.* **2019**, *10*, 1377. [CrossRef]
144. Li, R.; Bruneau, A.H.; Qu, R. Tissue culture-induced morphological somaclonal variation in St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze]. *Plant Breed.* **2010**, *129*, 96–99. [CrossRef]
145. Burson, B.L.; Tischler, C.R. Regeneration and somaclonal variation in apomictic *Paspalum dilatatum* Poir. *Euphytica* **1993**, *67*, 71–78. [CrossRef]
146. Croughan, S.S.; Quisenberry, S.S. Enhancement of fall army-worm (Lepidoptera: Noctuidae) resistance in bermudagrass through cell culture. *J. Econ. Entomol.* **1989**, *82*, 236–238. [CrossRef]
147. Pitman, W.D.; Croughan, S.S.; Stout, M.J. Field performance of bermudagrass germplasm expressing somaclonal variation selected for divergent responses to fall armyworm. *Euphytica* **2002**, *125*, 103–111. [CrossRef]
148. Heckart, D.L.; Parrott, W.A.; Raymer, P.L. Obtaining sethoxydim resistance in seashore paspalum. *Crop. Sci.* **2010**, *50*, 2632–2640. [CrossRef]
149. Li, R.; Qu, R.; Bruneau, A.H.; Livingston, D.P. Selection for freezing tolerance in St. Augustine grass through somaclonal variation and germplasm evaluation. *Plant Breed.* **2010**, *129*, 417–421. [CrossRef]
150. Liu, J.; Yang, Z.; Li, W.; Yu, J. Improving cold tolerance through in vitro selection for somaclonal variations in Seashore Paspalum. *J. Am. Soc. Hort. Sci.* **2013**, *138*, 452–460. [CrossRef]
151. Liu, M.X.; Lu, S.Y.; Guo, Z.F. Chilling-tolerant variants screening and physiological identification of centipedegrass. *Acta Agrestia Sin.* **2011**, *19*, 652–656. (In Chinese) [CrossRef]
152. Yuan, X.J.; Wang, Z.Y.; Zheng, Y.Q.; Liu, J.X.; She, J.M. Acquisition and Identification of Cold-Resistant Somatic Mutants of Centipedegrass. *Acta Pratacul. Sin.* **2011**, *20*, 237–244. Available online: <http://cyxb.magtech.com.cn/EN/Y2011/V20/I6/237> (accessed on 15 February 2021). (In Chinese)
153. Lu, S.; Chen, C.; Wang, Z.; Guo, Z.; Li, H. Physiological responses of somaclonal variants of triploid bermudagrass (*Cynodon transvaalensis* × *Cynodon dactylon*) to drought stress. *Plant Cell Rep.* **2009**, *28*, 517–526. [CrossRef]
154. Rabêlo, F.H.S.; Vangronsveld, J.; Baker, A.J.M.; van der Ent, A.; Alleoni, L.R.F. Are grasses really useful for the phytoremediation of potentially toxic trace elements? A review. *Front. Plant Sci.* **2021**, *12*, 77827. [CrossRef]
155. Taghizadeh, M.; Kafi, M.; Fattahi Moghadam, M.R. Breeding by In Vitro Culture to Improve Tolerance and Accumulation of Lead in *Cynodon dactylon* L. *J. Agric. Sci. Technol.* **2015**, *17*, 1851–1860. Available online: <http://jast.modares.ac.ir/article-23-813-en.html> (accessed on 16 February 2021).
156. Inokuma, C.; Sugiura, K.; Imaizumi, N.; Cho, C. Transgenic Japanese lawngrass (*Zoysia japonica* Steud.) plants regenerated from protoplasts. *Plant Cell Rep.* **1998**, *17*, 334–338. [CrossRef]
157. Wang, Z.Y.; Ge, Y. Recent advances in genetic transformation of forage and turf grasses. *Vitr. Cell. Dev. Biol. Plant.* **2006**, *42*, 1–18. [CrossRef]
158. Mann, D.G.J.; LaFayette, P.R.; Abercrombie, L.L.; King, Z.R.; Mazarei, M.; Halter, M.C.; Poovaiah, C.R.; Baxter, H.; Shen, H.; Dixon, R.A.; et al. Gateway-compatible vectors for high throughput gene functional analysis in switchgrass (*Panicum virgatum* L.) and other monocot species. *Plant Biotechnol. J.* **2012**, *10*, 226–236. [CrossRef] [PubMed]
159. Hiei, Y.; Ishida, Y.; Komari, T. Progress of cereal transformation technology mediated by *Agrobacterium tumefaciens*. *Front. Plant Sci.* **2014**, *5*, 628. [CrossRef] [PubMed]
160. Peterson, D.; Barone, P.; Lenderts, B.; Schwartz, C.; Feigenbutz, L.; St. Clair, G.; Jones, S.; Svitashv, S. Advances in *Agrobacterium* transformation and vector design result in high-frequency targeted gene insertion in maize. *Plant Biotechnol. J.* **2021**, *19*, 2000–2010. [CrossRef]
161. Aguado-Santacruz, G.A.; Rascón-Cruz, Q.; Cabrera-Ponce, J.L.; Martínez-Hernández, A.; Olalde-Portugal, V.; Herrera-Estrella, L. Transgenic plants of blue grama grass, *Bouteloua gracilis* (H.B.K.) Lag. ex Steud., from microprojectile bombardment of highly chlorophyllous embryogenic cells. *Theor. Appl. Genet.* **2002**, *104*, 763–771. [CrossRef]
162. Gondo, T.; Matsumoto, J.; Tsuruta, S.; Yoshida, M.; Kawakami, A.; Terami, F.; Ebina, M.; Yamada, T.; Akashi, R. Particle inflow gun-mediated transformation of multiple-shoot clumps in rhodes grass (*Chloris gayana*). *J. Plant Physiol.* **2009**, *166*, 435–441. [CrossRef]
163. Li, L.; Qu, R. Development of highly regenerable callus lines and biolistic transformation of turf-type common bermudagrass [*Cynodon dactylon* (L.) Pers.]. *Plant Cell Rep.* **2004**, *22*, 403–407. [CrossRef]
164. Li, L.; Li, R.; Fei, S.; Qu, R. *Agrobacterium*-mediated transformation of common bermudagrass (*Cynodon dactylon*). *Plant Cell Tissue Organ Cult.* **2005**, *83*, 223–229. [CrossRef]
165. Zhang, G.; Lu, S.; Chen, T.A.; Funk, C.R.; Meyer, W.A. Transformation of triploid bermudagrass (*Cynodon dactylon* × *C. transvaalensis* cv. TifEagle) by means of biolistic bombardment. *Plant Cell Rep.* **2003**, *21*, 860–864. [CrossRef]
166. Chen, W.; Lennox, S.J.; Palmer, K.E.; Thomson, J.A. Transformation of *Digitaria sanguinalis*: A model system for testing maize streak virus resistance in Poaceae. *Euphytica* **1998**, *104*, 25–31. [CrossRef]
167. Gebre, E.; Gugsu, L.; Schlüter, U.; Kunert, K. Transformation of tef (*Eragrostis tef*) by *Agrobacterium* through immature embryo regeneration system for inducing semi-dwarfism. *S. Afr. J. Bot.* **2013**, *87*, 9–17. [CrossRef]

168. Wang, X.; Yamada, T.; Kong, F.-J.; Abe, Y.; Hoshino, Y.; Sato, H.; Takamizo, T.; Kanazawa, A.; Yamada, T. Establishment of an efficient in vitro culture and particle bombardment-mediated transformation systems in *Miscanthus sinensis* Anders., a potential bioenergy crop. *Glob. Chang. Biol. Bioenergy* **2011**, *3*, 322–332. [[CrossRef](#)]
169. Hwang, O.-J.; Cho, M.-A.; Han, Y.-J.; Kim, Y.-M.; Lim, S.-H.; Kim, D.-S.; Hwang, I.; Kim, J.-I. Agrobacterium-mediated genetic transformation of *Miscanthus sinensis*. *Plant Cell Tissue Organ Cult.* **2014**, *117*, 51–63. [[CrossRef](#)]
170. Yoo, J.H.; Seong, E.S.; Ghimire, B.K.; Heo, K.; Jin, X.; Yamada, T.; Clark, L.V.; Sacks, E.J.; Yu, C.Y. Establishment of *Miscanthus sinensis* with decreased lignin biosynthesis by Agrobacterium-mediated transformation using antisense COMT gene. *Plant Cell Tissue Organ Cult.* **2018**, *133*, 359–369. [[CrossRef](#)]
171. Wu, Y.; Zhou, N.; Ni, X.; Okoye, C.O.; Wang, Y.; Li, X.; Gao, L.; Zhou, G.; Jiang, J. Developing a long-term and powerful in vitro culture and Agrobacterium-mediated transformation system for *Miscanthus sinensis* (Poaceae). *Ind. Crops Prod.* **2021**, *161*, 113190. [[CrossRef](#)]
172. Seo, M.S.; Takahashi, S.; Kadowaki, K.I.; Kawamukai, M.; Takahara, M.; Takamizo, T. Expression of CoQ10-producing ddsA transgene by efficient Agrobacterium-mediated transformation in *Panicum meyerianum*. *Plant Cell Tissue Organ Cult.* **2011**, *107*, 325–332. [[CrossRef](#)]
173. Richards, H.A.; Rudas, V.A.; Sun, H.; McDaniel, J.K.; Tomaszewski, Z.; Conger, B.V. Construction of a GFP-BAR plasmid and its use for switchgrass transformation. *Plant Cell Rep.* **2001**, *20*, 48–54. [[CrossRef](#)]
174. Fu, C.; Mielenz, J.R.; Xiao, X.; Ge, Y.; Hamilton, C.Y.; Rodriguez, M., Jr.; Chen, F.; Foston, M.; Ragauskas, A.; Bouton, J.; et al. Genetic manipulation of lignin reduces recalcitrance and improves ethanol production from switchgrass. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 3803–3808. [[CrossRef](#)]
175. Saathoff, A.J.; Sarath, G.; Chow, E.K.; Dien, B.S.; Tobias, C.M. Downregulation of cinnamyl-alcohol dehydrogenase in switchgrass by RNA silencing results in enhanced glucose release after cellulase treatment. *PLoS ONE* **2011**, *6*, e16416. [[CrossRef](#)]
176. Xu, B.; Huang, L.; Shen, Z.; Welbaum, G.E.; Zhang, X.; Zhao, B. Selection and characterization of a new switchgrass (*Panicum virgatum* L.) line with high somatic embryogenic capacity for genetic transformation. *Sci. Hort.* **2011**, *129*, 854–861. [[CrossRef](#)]
177. Xu, B.; Escamilla-Treviño, L.L.; Sathitsuksanoh, N.; Shen, Z.; Shen, H.; Percival Zhang, Y.H.; Dixon, R.A.; Zhao, B. Silencing of 4-coumarate: Coenzyme a ligase in switchgrass leads to reduced lignin content and improved fermentable sugar yields for biofuel production. *New Phytol.* **2011**, *192*, 611–625. [[CrossRef](#)]
178. Ramamoorthy, R.; Kumar, P.P. A simplified protocol for genetic transformation of switchgrass (*Panicum virgatum* L.). *Plant Cell Rep.* **2012**, *31*, 1923–1931. [[CrossRef](#)]
179. Shen, H.; He, X.; Poovaiyah, C.R.; Wuddineh, W.A.; Ma, J.; Mann, D.G.J.; Wang, H.; Jackson, L.; Tang, Y.; Stewart, C.N., Jr.; et al. Functional characterization of the switchgrass (*Panicum virgatum*) R2R3-MYB transcription factor *PvMYB4* for improvement of lignocellulosic feedstocks. *New Phytol.* **2012**, *193*, 121–136. [[CrossRef](#)] [[PubMed](#)]
180. Ambavaram, M.M.R.; Ali, A.; Ryan, K.P.; Peoples, O.; Snell, K.D.; Somleva, M.N. Novel transcription factors *PvBMY1* and *PvBMY3* increase biomass yield in greenhouse-grown switchgrass (*Panicum virgatum* L.). *Plant Sci.* **2018**, *273*, 100–109. [[CrossRef](#)] [[PubMed](#)]
181. Guan, C.; Huang, Y.H.; Cui, X.; Liu, S.J.; Zhou, Y.Z.; Zhang, Y.W. Overexpression of gene encoding the key enzyme involved in proline-biosynthesis (*PuP5CS*) to improve salt tolerance in switchgrass (*Panicum virgatum* L.). *Plant Cell Rep.* **2018**, *37*, 1187–1199. [[CrossRef](#)] [[PubMed](#)]
182. Li, G.; Jones, K.C.; Eudes, A.; Pidatala, V.R.; Sun, J.; Xu, F.; Zhang, C.; Wei, T.; Jain, R.; Birdseye, D.; et al. Overexpression of a rice BAHD acyltransferase gene in switchgrass (*Panicum virgatum* L.) enhances saccharification. *BMC Biotech.* **2018**, *18*, 54. [[CrossRef](#)] [[PubMed](#)]
183. Guan, C.; Huang, Y.H.; Cen, H.F.; Cui, X.; Tian, D.Y.; Zhang, Y.W. Overexpression of the *Lolium perenne* L. delta1-pyrroline 5-carboxylate synthase (*LpP5CS*) gene results in morphological alterations and salinity tolerance in switchgrass (*Panicum virgatum* L.). *PLoS ONE* **2019**, *14*, e0219669. [[CrossRef](#)]
184. Zhang, J.; Wen, W.; Li, H.; Lu, Q.; Xu, B.; Huang, B. Overexpression of an aquaporin gene *PvPIP2;9* improved biomass yield, protein content, drought tolerance and water use efficiency in switchgrass (*Panicum virgatum* L.). *Glob. Chang. Biol. Bioenergy* **2020**, *12*, 979–991. [[CrossRef](#)]
185. Akashi, R.; Yuge, C.; Gondo, T.; Kawamura, O.; Hoffmann, F. Bialaphos-resistant cells of dallisgrass (*Paspalum dilatatum* Poir.) through particle bombardment with a simple self-built inflow gun. *Grassl. Sci.* **2002**, *47*, 588–593. [[CrossRef](#)]
186. Giordano, A.; Liu, Z.; Panter, S.N.; Dimech, A.M.; Shang, Y.; Wijesinghe, H.; Fulgueras, K.; Ran, Y.; Mouradov, A.; Rochfort, S.; et al. Reduced lignin content and altered lignin composition in the warm season forage grass *Paspalum dilatatum* by down-regulation of a Cinnamoyl CoA reductase gene. *Transgenic Res.* **2014**, *23*, 503–517. [[CrossRef](#)]
187. Smith, R.L.; Grando, M.F.; Li, Y.Y.; Seib, J.C.; Shatters, R.G. Transformation of bahiagrass (*Paspalum notatum* Flugge). *Plant Cell Rep.* **2002**, *20*, 1017–1021. [[CrossRef](#)]
188. Agharkar, M.; Lomba, P.; Altpeter, F.; Zhang, H.; Kenworthy, K.; Lange, T. Stable expression of *AtGA2ox1* in a low-input turfgrass (*Paspalum notatum* Flugge) reduces bioactive gibberellin levels and improves turf quality under field conditions. *Plant Biotechnol. J.* **2007**, *5*, 791–801. [[CrossRef](#)] [[PubMed](#)]
189. Zhang, H.; Lomba, P.; Altpeter, F. Improved turf quality of transgenic bahiagrass (*Paspalum notatum* Flugge) constitutively expressing the *ATHB16* gene, a repressor of cell expansion. *Mol. Breed.* **2007**, *20*, 415–423. [[CrossRef](#)]

190. James, V.A.; Neibaur, I.; Altpeter, F. Stress inducible expression of the *DREB1A* transcription factor from xeric, *Hordeum spontaneum* L. in turf and forage grass (*Paspalum notatum* Flugge) enhances abiotic stress tolerance. *Transgenic Res.* **2008**, *17*, 93–104. [[CrossRef](#)] [[PubMed](#)]
191. Sandhu, S.; Altpeter, F. Co-integration, co-expression and inheritance of unlinked minimal transgene expression cassettes in an apomictic turf and forage grass (*Paspalum notatum* Flugge). *Plant Cell Rep.* **2008**, *27*, 1755–1765. [[CrossRef](#)] [[PubMed](#)]
192. Himuro, Y.; Gondo, T.; Yamakawa, K.; Akashi, R. Genetic transformation of bahiagrass (*Paspalum notatum* Flugge) by visually screening cells expressing green fluorescent protein. *Grassl. Sci.* **2009**, *55*, 216–220. [[CrossRef](#)]
193. Xiong, X.; James, V.A.; Zhang, H.; Altpeter, F. Constitutive expression of the barley *HvWRKY38* transcription factor enhances drought tolerance in turf and forage grass (*Paspalum notatum* Flugge). *Mol. Breed.* **2010**, *25*, 419–432. [[CrossRef](#)]
194. Muguerza, M.; Gondo, T.; Yoshida, M.; Kawakami, A.; Terami, F.; Yamada, T.; Akashi, R. Modification of the total soluble sugar content of the C4 grass *Paspalum notatum* expressing the wheat-derived sucrose: Sucrose 1-fructosyltransferase and sucrose: Fructan 6-fructosyltransferase genes. *Grassl. Sci.* **2013**, *59*, 196–204. [[CrossRef](#)]
195. Mancini, M.; Woitovich, N.; Permingeat, H.R.; Podio, M.; Siena, L.A.; Ortiz, J.P.A.; Pessino, S.C.; Felitti, S.A. Development of a modified transformation platform for apomixis candidate genes research in *Paspalum notatum* (bahiagrass). *Vitr. Cell. Dev. Biol. Plant.* **2014**, *50*, 412–424. [[CrossRef](#)]
196. Muguerza, M.; Gondo, T.; Ishigaki, G.; Akashi, R. Lignin content and digestibility in transgenic bahiagrass (*Paspalum notatum* Flugge) obtained by genetic manipulation of cinnamyl alcohol dehydrogenase gene. *Asian J. Plant Sci.* **2014**, *13*, 8–17. [[CrossRef](#)]
197. Wu, X.; Shi, H.; Chen, X.; Liu, Y.; Guo, Z. Establishment of *Agrobacterium*-mediated transformation of seashore paspalum (*Paspalum vaginatum* O. Swartz). *Vitr. Cell. Dev. Biol. Plant.* **2018**, *54*, 545–552. [[CrossRef](#)]
198. Wu, X.; Shi, H.; Guo, Z. Overexpression of a *NF-YC* gene results in enhanced drought and salt tolerance in transgenic seashore paspalum. *Front. Plant Sci.* **2018**, *9*, 1355. [[CrossRef](#)] [[PubMed](#)]
199. Lambé, P.; Dinant, M.; Matagne, R.F. Differential long-term expression and methylation of the hygromycin phosphotransferase (*hph*) and β -glucuronidase (*GUS*) genes in transgenic pearl millet (*Pennisetum glaucum*) callus. *Plant Sci.* **1995**, *108*, 51–62. [[CrossRef](#)]
200. Girgi, M.; O’Kennedy, M.M.; Morgenstern, A.; Mayer, G.; Lörz, H.; Oldach, K.H. Transgenic and herbicide resistant pearl millet (*Pennisetum glaucum* L.) R.Br. via microprojectile bombardment of scutellar tissue. *Mol. Breed.* **2002**, *10*, 243–252. [[CrossRef](#)]
201. Goldman, J.J.; Hanna, W.W.; Fleming, G.; Ozias-Akins, P. Fertile transgenic pearl millet [*Pennisetum glaucum* (L.) R. Br.] plants recovered through microprojectile bombardment and phosphinothricin selection of apical meristem-, inflorescence-, and immature embryo-derived embryogenic tissues. *Plant Cell Rep.* **2003**, *21*, 999–1009. [[CrossRef](#)]
202. Girgi, M.; Breese, W.A.; Lörz, H.; Oldach, K.H. Rust and downy mildew resistance in pearl millet (*Pennisetum glaucum*) mediated by heterologous expression of the *afp* Gene from *Aspergillus giganteus*. *Transgenic Res.* **2006**, *15*, 313–324. [[CrossRef](#)]
203. Latha, A.M.; Rao, K.V.; Reddy, T.P.; Reddy, V.D. Development of transgenic pearl millet (*Pennisetum glaucum* (L.) R. Br.) plants resistant to downy mildew. *Plant Cell Rep.* **2006**, *25*, 927–935. [[CrossRef](#)]
204. Jha, P.; Shashi; Rustagi, A.; Agnihotri, P.K.; Kulkarni, V.M.; Bhat, V. Efficient *Agrobacterium*-mediated transformation of *Pennisetum glaucum* (L.) R. Br. using shoot apices as explant source. *Plant Cell Tissue Organ Cult.* **2011**, *107*, 501–512. [[CrossRef](#)]
205. Ramadevi, R.; Rao, K.V.; Reddy, V.D. *Agrobacterium tumefaciens*-mediated genetic transformation and production of stable transgenic pearl millet (*Pennisetum glaucum* [L.] R. Br.). *Vitr. Cell Dev. Biol. Plant.* **2014**, *50*, 392–400. [[CrossRef](#)]
206. Santos, C.M.; Romeiro, D.; Silva, J.P.; Basso, M.F.; Molinari, H.B.C.; Centeno, D.C. An improved protocol for efficient transformation and regeneration of *Setaria italica*. *Plant Cell Rep.* **2020**, *39*, 501–510. [[CrossRef](#)]
207. Sood, P.; Singh, R.K.; Prasad, M. An efficient *Agrobacterium*-mediated genetic transformation method for foxtail millet (*Setaria italica* L.). *Plant Cell Rep.* **2020**, *39*, 511–525. [[CrossRef](#)]
208. Nguyen, D.Q.; Eck, J.V.; Eamens, A.L.; Grof, C.P.L. Robust and reproducible *Agrobacterium*-mediated transformation system of the C4 genetic model species *Setaria viridis*. *Front. Plant Sci.* **2020**, *11*, 281. [[CrossRef](#)] [[PubMed](#)]
209. Ge, Y.; Norton, T.; Wang, Z.Y. Transgenic zoysiagrass (*Zoysia japonica*) plants obtained by *Agrobacterium*-mediated transformation. *Plant Cell Rep.* **2006**, *25*, 792–798. [[CrossRef](#)] [[PubMed](#)]
210. Zhang, L.; Wu, D.; Zhang, L.; Yang, C. *Agrobacterium*-mediated transformation of Japanese lawngress (*Zoysia japonica* Steud.) containing a synthetic *cryIA(b)* gene from *Bacillus thuringiensis*. *Plant Breed.* **2007**, *126*, 428–432. [[CrossRef](#)]
211. Zuo, Z.F.; Kang, H.G.; Park, M.Y.; Jeong, H.; Sun, H.J.; Yang, D.H.; Lee, Y.E.; Song, P.S.; Lee, H.Y. Overexpression of *ICE1*, a regulator of cold-induced transcriptome, confers cold tolerance to transgenic *Zoysia japonica*. *J. Plant Biol.* **2019**, *62*, 137–146. [[CrossRef](#)]
212. Jeong, H.N.; Sun, H.J.; Zuo, Z.F.; Lee, D.H.; Song, P.S.; Kang, H.G.; Lee, H.Y. Overexpression of *ATHG1/AHL23* and *ATPG3/AHL20*, *Arabidopsis* AT-hook motif nuclear-localized genes, confers salt tolerance in transgenic *Zoysia japonica*. *Plant Biotechnol. Rep.* **2020**, *14*, 351–361. [[CrossRef](#)]
213. Li, M.; Li, H.; Hu, X.; Pan, X.; Wu, G. An *Agrobacterium tumefaciens*-mediated transformation system using callus of *Zoysia tenuifolia* Willd. ex Trin. *Plant Cell Tissue Organ Cult.* **2010**, *102*, 321–327. [[CrossRef](#)]
214. Li, R.F.; Wei, J.H.; Wang, H.Z.; He, J.; Sun, Z.Y. Development of highly regenerable callus lines and *Agrobacterium*-mediated transformation of Chinese lawngress (*Zoysia sinica* Hance) with a cold inducible transcription factor, *CBF1*. *Plant Cell Tissue Organ Cult.* **2006**, *85*, 297–305. [[CrossRef](#)]

215. Sims, R.E.; Mabee, W.; Saddler, J.N.; Taylor, M. An overview of second generation biofuel technologies. *Bioresour. Technol.* **2010**, *101*, 1570–1580. [[CrossRef](#)]
216. Byrt, C.S.; Grof, C.P.; Furbank, R.T. C4 plants as biofuel feedstocks: Optimising biomass production and feedstock quality from a lignocellulosic perspective. *J. Integr. Plant Biol.* **2011**, *53*, 120–135. [[CrossRef](#)]
217. Mohapatra, S.; Mishra, S.S.; Bhalla, P.; Thatoi, H. Engineering grass biomass for sustainable and enhanced bioethanol production. *Planta* **2019**, *250*, 395–412. [[CrossRef](#)]
218. Nelson, R.S.; Stewart, C.N., Jr.; Gou, J.; Holladay, S.; Gallego-Giraldo, L.; Flanagan, A.; Mann, D.G.J.; Hisano, H.; Wuddineh, W.A.; Poovaiah, C.R.; et al. Development and use of a switchgrass (*Panicum virgatum* L.) transformation pipeline by the BioEnergy Science Center to evaluate plants for reduced cell wall recalcitrance. *Biotechnol. Biofuels* **2017**, *10*, 309. [[CrossRef](#)] [[PubMed](#)]
219. Calyxt Inc. *First Commercial Sale of Calyxt High Oleic Soybean Oil on the US Market*; Calyxt Inc.: Roseville, MN, USA, 2019.
220. Bortesi, L.; Fischer, R. The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnol. Adv.* **2015**, *33*, 41–52. [[CrossRef](#)] [[PubMed](#)]
221. Manghwar, H.; Lindsey, K.; Zhang, X.; Jin, S. CRISPR/Cas system: Recent advances and future prospects for genome editing. *Trends Plant Sci.* **2019**, *24*, 1102–1125. [[CrossRef](#)] [[PubMed](#)]
222. Liu, Y.; Wang, W.; Yang, B.; Currey, C.; Fei, S.Z. Functional analysis of the teosinte branched 1 gene in the tetraploid switchgrass (*Panicum virgatum* L.) by CRISPR/Cas9-directed mutagenesis. *Front. Plant Sci.* **2020**, *11*, 572193. [[CrossRef](#)]
223. Metje-Sprink, J.; Menz, J.; Modrzejewski, D.; Sprink, T. DNA-free genome editing: Past, present and future. *Front. Plant Sci.* **2019**, *9*, 1957. [[CrossRef](#)]
224. Liang, Z.; Chen, K.; Li, T.; Zhang, Y.; Wang, Y.; Zhao, Q.; Liu, J.; Zhang, H.; Liu, C.; Ran, Y.; et al. Efficient DNA-free genome editing of bread wheat using CRISPR/Cas9 ribonucleoprotein complexes. *Nat. Commun.* **2017**, *8*, 14261. [[CrossRef](#)]
225. Liang, Z.; Chen, K.; Zhang, Y.; Liu, J.; Yin, K.; Qiu, J.L.; Gao, C. Genome editing of bread wheat using biolistic delivery of CRISPR/Cas9 in vitro transcripts or ribonucleoproteins. *Nat. Protoc.* **2018**, *13*, 413–430. [[CrossRef](#)]
226. Liang, Z.; Chen, K.; Gao, C. Biolistic delivery of CRISPR/Cas9 with ribonucleoprotein complex in wheat. In *Plant Genome Editing with CRISPR Systems*; Humana Press: New York, NY, USA, 2019; pp. 327–335. [[CrossRef](#)]
227. Svitashv, S.; Schwartz, C.; Lenderts, B.; Young, J.K.; Mark Cigan, A. Genome editing in maize directed by CRISPR–Cas9 ribonucleoprotein complexes. *Nat. Commun.* **2016**, *7*, 13274. [[CrossRef](#)]
228. Dong, S.; Qin, Y.L.; Vakulskas, C.A.; Collingwood, M.A.; Marand, M.; Rigoulot, S.; Zhu, L.; Jiang, Y.; Gu, W.; Fan, C.; et al. Efficient targeted mutagenesis mediated by CRISPR-Cas12a ribonucleoprotein complexes in maize. *Front. Genome Ed.* **2021**, *3*, 670529. [[CrossRef](#)]
229. Bennetzen, J.L.; Schmutz, J.; Wang, H.; Percifield, R.; Hawkins, J.; Pontaroli, A.C.; Estep, M.; Feng, L.; Vaughn, J.N.; Grimwood, J.; et al. Reference genome sequence of the model plant *Setaria*. *Nat. Biotechnol.* **2012**, *30*, 555–561. [[CrossRef](#)]
230. Lovell, J.T.; MacQueen, A.H.; Mamidi, S.; Bonnette, J.; Jenkins, J.; Napier, J.D.; Sreedasyam, A.; Healey, A.; Session, A.; Shu, S.; et al. Genomic mechanisms of climate adaptation in polyploid bioenergy switchgrass. *Nature* **2021**, *590*, 438–444. [[CrossRef](#)] [[PubMed](#)]
231. Mitros, T.; Session, A.M.; James, B.T.; Wu, G.A.; Belaffif, M.B.; Clark, L.V.; Shu, S.; Holmes, J.R.; Mattick, J.E.; Bredeson, J.V.; et al. Genome biology of the paleotetraploid perennial biomass crop *Miscanthus*. *Nat. Commun.* **2020**, *11*, 5442. [[CrossRef](#)] [[PubMed](#)]
232. Tanaka, H.; Hirakawa, H.; Kosugi, S.; Nakayama, S.; Ono, A.; Watanabe, A.; Hashiguchi, M.; Gondo, T.; Ishigaki, G.; Mugerza, M.; et al. Sequencing and comparative analyses of the genomes of zoysiagrasses. *DNA Res.* **2016**, *23*, 171–180. [[CrossRef](#)] [[PubMed](#)]