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REVIEW

Genetic engineering of millets: current status and future prospects

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Abstract This review summarizes progress on the genetic transformation of millets and discusses the future prospects for the development of improved varieties. Only a limited number of studies have been carried out on genetic improvement of millets despite their nutritional importance in supplying minerals, calories and protein. Most genetic transformation studies of millets have been restricted to pearl millet and bahiagrass and most studies have been limited to the assessment of reporter and marker gene expression. Biolistic-mediated gene delivery has been frequently used for the transformation of millets but *Agrobacterium*-mediated transformation is still lagging. Improved transformation of millets, allied to relevant gene targets which may offer, for example, improved nutritional quality, resistance to abiotic and biotic stresses, and resistance to fungal infection will play important roles in millet improvement.

Keywords *Agrobacterium* · Biolistic · Millet · Transgenic cells

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Introduction

Plant biotechnology is founded on the demonstrated totipotency of plant cells, combined with the delivery, stable integration, and expression of transgene in plant cells, the regeneration of transformed plants, and the Mendelian transmission of transgenes to the progeny (Vasil 2008). Transgenic technology has been utilized in the past decade for the improvement of many crop plants including major cereals such as maize, wheat and rice. Many of these transgenic cereals have already reached the field for large scale cultivation. But a genetic improvement program for millets has been initiated only in recent years and has been paid less attention despite their nutritional importance.

Millet crops [pearl millet, *Pennisetum glaucm* (L.) R.Br; finger millet, *Eleusine coracana* (L) Gaertn; kodo millet, *Paspalum scorbiculatum* Linn; bahiagrass, *Paspalum notatum* Flugge; foxtail millet, *Setaria italica* (L.) P. Beauv; little millet, *Panicum miliare* Lam; proso millet, *Panicum miliaceum* (L.); barnyard millet, *Echinochola crusgalli* (Linn.) P. Beauv; guinea grass, *Panicum maximum* Jacq; elephant grass, *Pennisetum purpurium* Schum] are grasses that belong to the Family Poaceae of the monocotolydon group. Millets are staple foods that supply a major proportion of calories and protein to large segments of populations in the semi-arid tropical regions of Africa and Asia (O'Kennedy et al. 2006). The semi-arid tropics are characterized by unpredictable weather, limited and erratic rainfall,

nutrient-poor soils and suffer from a host of agricultural constraints (Sharma and Ortiz 2000). The projected food demand for 2025 will require the yield of cereals, including millets, to rise from 2.5 to 4.5 t ha^{-1} (Borlaug 2002). Production of millets, like finger millet, is also constrained by fungal diseases; so genetic engineering of millets is essential for conferring high level of resistance to improve the yield (Ceasar and Ignacimuthu 2008). Genetic engineering technology can also be harnessed for improving the nutritional quality of millets and conferring resistance to abiotic stresses (drought and saline).

Among the available reports of millet transformation, the biolistic method has been mostly used for gene delivery. *Agrobacterium*-mediated transformation of millets lags behind although it is a versatile method of gene delivery for many cereals which also belong to the Poaceae. In contrast, *Agrobacterium*-mediated transformation of other cereals is effective with rice (Ignacimuthu et al. 2000; Bajaj and Mohanty 2005; Shrawat and Lörz 2006), wheat (Jones et al. 2005), barley and sorghum (Shrawat and Lörz 2006; O'Kennedy et al. 2006). We have also reported the *Agrobacterium*-mediated transformation system for Indian rice cultivars (Ignacimuthu and Arockiasamy 2006; Arockiasamy and Ignacimuthu 2007). Efforts made for the *Agrobacterium*-mediated transformation of cereals need to be extended to millet crops in the near future for their genetic improvement to meet the growing demand for food.

Millets are capable of surviving in some of the most inhospitable ecosystems of the world providing food and fodder to millions where other quality cereals fail to grow. Hence with an ultimate aim of supplementing conventional breeding efforts the genetic transformation protocols for millets need to be developed so that important quality traits may be incorporated across the barriers of incompatibility (Gupta et al. 2001). Two reviews published on millets describe the widespread role of biotechnology for the improvement of millets in general (Kothari et al. 2005) and pearl millet in particular (O'Kennedy et al. 2006). The scope of the current review is narrow and specifically describes the recent advances in genetic engineering and transgenic aspects of millets; we review the plasmid, promoter, marker and reporter genes and different methods of gene delivery used for the transformation of millets; we also discuss the future prospects for the *Agrobacterium*-mediated transformation of millets.

In vitro culture of millets

Establishment of an efficient in vitro regeneration system is a vital prerequisite for the successful transformation and recovery of transgenic millet crops. Many reports are available for the in vitro culture of millets and different methods of in vitro culture have been reported in millets (reviewed by Kothari et al. 2005). Development of in vitro regeneration protocol through somatic embryogenesis seems to be more helpful for the efficient transformation and recovery of transgenic plant (Veluthambi et al. 2003). Somatic embryogenesis and plant regeneration protocols have been reported for pearl millet, finger millet, kodo millet and foxtail millet (Kothari et al. 2005). Recently we have also developed this protocol for finger millet (Ceasar and Ignacimuthu 2008). Similar protocols need to be developed in future for the rest of the millets to produce transgenic millets more efficiently, particularly for the *Agrobacterium*-mediated approaches.

Genetic engineering of millets

Pearl millet

Pearl millet (*Pennisetum glaucm*) has been given the highest priority among millets in transformation studies; in all the available reports, the pearl millet was transformed by biolistic method of gene delivery (Table 1). The first pearl millet transformation was made by Taylor et al. (1991) through microprojectile bombardment (biolistic); immature embryos were used as the target explants. Plasmid pMON 8678 was used for transformation; it contained β-glucuronidase (*GUS* or *uidA*) gene under the control of maize alcohol dehydrogenase gene (*adh1*) promoter. Transformation was confirmed by *GUS* histochemical assay. Later the pearl millet was transformed in the same laboratory using plasmids pBAR*GUS* and pAHC25 (Taylor et al. 1993); they observed that expression of the *uidA* gene in plasmid pAHC25 was superior to pBAR*GUS*. Following this, Lambe et al. (1995) transformed pearl millet by the biolistic method with two plasmids (p35SGUS and pROB5). The p35SGUS contained the *GUS* gene and pROB5 contained the hygromycin phosphotransferase gene (*hpt*) conferring resistance to hygromycin; expression

Table 1 Details of the genetic engineering of millets

Name of the millet	Method of transformation	Target tissue used for transformation	Promoter/ reporter gene used	Promoter/ selectable marker gene used	Reference
Pearl millet	Biolistic	Immature embryo	*Adh1* *uidA*	–	Taylor et al. (1991)
	Biolistic	Immature embryo	*Adh1* *uidA*	*CaMV35S* *bar*	Taylor et al. (1993)
	Biolistic	Embryonic callus and cell suspension culture	*CaMV35S* *uidA*	*CaMV35S* *hptII*	Lambe et al. (1995, 2000)
	Biolistic	Scutellar tissue	*CaMV35S* *uidA*	*CaMV35S* *bar*	Girgi et al. (2002)
	Biolistic	Apical meristem	*Act1* *uidA*	–	Devi and Sticklen (2002)
	Biolistic	Embryo, meristem and inflorescence	*uq1*; *CaMV35S* *uidA*; *gfp*	*uq1* *bar*	Goldman et al. (2003)
	Biolistic	Immature zygotic embryo	–	*uq1* *manA*	O'Kennedy et al. (2004)
	Biolistic	Somatic embryo	–	*CaMV35S* *pat*	Girgi et al. (2006)
	Biolistic	Embryonic callus	–	*CaMV35S* *bar*	Latha et al. (2006)
Bahiagrass	Biolistic	Embryonic callus	–	*CaMV35S* *bar*	Smith et al. (2002)
	Biolistic	Embryonic callus	*uq1* *uidA*	–	Grando et al. (2002)
	Biolistic	Callus	*Act1* *uidA*	*CaMV35S* *Bar*	Gondo et al. (2003, 2005)
	Biolistic	Embryonic callus	–	*uq1* *nptII*	Altpeter and James (2005)
	Biolistic	Embryonic callus	–	*uq1* *nptII*	Hangning et al. (2007)
	Biolistic	Callus	*CaMV35S* *uidA*	*Uq1* *nptII*	James et al. (2008)
Finger millet	Biolistic	Callus	*CaMV35S/ActI/UqI/ RbcS/Ft* *uidA*	–	Gupta et al. (2001)
	Biolistic	Embryonic callus	*CaMV35S* *uidA*	*CaMV35S* *bar*	Latha et al. (2005)
Foxtail millet	*Agrobacterium*-mediated	Callus	*CaMV35S* *uidA*	*CaMV35S* *nptII*	Liu et al. (2005)
	Agrobacterium-mediated	Callus	*Zm13*[a]	*Zm13*[a]	Fang et al. (2007)
Barnyard millet	Biolistic	Callus	*CaMV35S/ActI/UqI/ RbcS/Ft* *uidA*	–	Gupta et al. (2001)

Table 1 continued

Name of the millet	Method of transformation	Target tissue used for transformation	Promoter/ reporter gene used	Promoter/ selectable marker gene used	Reference
Elephant grass	Electroporation	Protoplast	–	*CaMV35S* *Cat;nptII;hph*	Hauptmann et al. (1987, 1988)
	Biolistic	Immature embryo	*Adh1* *uidA*	*CaMV35S* *bar*	Taylor et al. (1993)
Guinea grass	Electroporation	Protoplast	–	*CaMV35S* *Cat;nptII;hph*	Hauptmann et al. (1987, 1988)
	Biolistic	Immature embryo	*Adh1* *uidA*	*CaMV35S* *bar*	Taylor et al. (1993)

Adh1 alcohol dehydrogenase gene promoter, *CaMV35S* cauliflower mosaic virus 35S promoter, *Act1* rice actin gene promoter, *GUS or uidA* β-glucuronidase gene, *bar or pat* phospinothricin acetyl transferase gene, *hpt or hph* hygromycin phosphotransferase gene, *nptII* neomycin phosphotransferase gene, *Uq1* ubiquitin gene promoter from maize, *RbcS* rice ribulose 1,5 biphosphate carboxylase small subunit gene promoter, *Ft5* promoter of C4 isoform of phosphoenolpyruate carboxylase gene from *Flaveria trinervia*; *Zm13*, Zea mays 13 promoter; *Cat*, chlorophenicol acetyl transferase gene

[a] Promoter used for the expression of pollen specific protein

of both genes was under the control of separate CaMV35S promoters. 1–2 μm tungsten particles coated with plasmids were used for bombardment and a distance of 8 cm was maintained between the target material (embryonic calli or embryonic cell suspension) and the stopping plate of macroprojectile. They also confirmed the integration of transgene by Southern blot analysis (Lambe et al. 1995).

Another approach for pearl millet transformation was made by Girgi et al. (2002) who delivered genes to scutellar tissue by two alternative transformation protocols, particle gun 1,000/He (PDS) or particle inflow gun (PIG). Plasmid pAHC25 was used for PIG transformation; it harbored the *GUS* reporter gene and *bar* (phospinothricin acetyltransferase) marker gene; both the *GUS* and *bar* genes were put under the control of *uq1* (ubiquitin from maize) promoter. Plasmid p35SAcS and pubi.gus were used in co-transformation experiments with PDS; p35SAcS contained the *bar* selection marker gene under the control of CaMV35S promoter and pubi.gus had the *GUS* gene (*uidA*) driven by the *uq1* promoter. However, in this approach Girgi et al. (2002) obtained very low (0.18%) frequency of pearl millet transformation in spite of employing two different gene delivery methods (PIG and PDS).

Genotype-independent pearl millet transformation system was developed by Devi and Sticklen (2002) using shoot apical meristem as target tissue for gene delivery. Plasmid pAct1-F containing *uidA* gene driven by rice *Act1* (rice actin) promoter was used for transformation; they used tungsten particles of two different sizes (0.9 and 1.2 μm). The important parameters influencing the transformation like size of the tungsten particles, density of the particles, pressure of the helium gas, distance from the target and osmoticum of the culture medium were optimized (Devi and Sticklen 2002).

The transformation frequency of pearl millet was greatly increased by Goldman et al. (2003) using three different explants (embryogenic tissue, inflorescences and apical meristems); the transformation frequency ranged from 5 to 85%. They used plasmids pAHC25 and p524EGFP; plasmid pAHC25 contained the selectable *bar* gene and the reporter gene *uidA*, both under the control of separate *uq1* promoter. Plasmid p524EGFP.1 contained the double cauliflower mosaic virus *35S* promoter sequence followed by the alfalfa mosaic virus enhancer sequence controlling expression of an enhanced green fluorescent protein-encoding (*gfp*) gene**.** They coated the DNA on 0.6 or 0.75 μm gold particles; the distance of 6 cm was maintained between the target and stopping screen.

The transformation protocol for pearl millet was also optimized by O'Kennedy et al. (2004) using a phosphomannose isomerase (*manA*) transgene driven

under the control of the *uq1* promoter placed in the plasmid pNOV3604ubi. Transgenic *manA* expressing cells acquired the ability to convert mannose 6-phosphate to fructose 6-phosphate while the non-transgenic cells accumulated mannose 6-phosphate and could not survive (positive selection). The *manA* gene has been shown to be superior to antibiotic or herbicide (*pat* or *bar*) resistant selectable marker genes for transformation of many plants including maize and wheat (Reed et al. 2001). This gene could be utilized in future for the transformation of other millets.

The first transgenic pearl millet expressing functionally active foreign gene conferring resistance to fungal disease (downy mildew) has only recently been produced by Girgi et al. (2006). They have used the antifungal protein (*afp*) gene isolated from the ascomycete, *Aspergillus giganteus*; immature zygotic embryos have been used as target for biolistic transformation. Transformation of pearl millet was confirmed by molecular analysis; the disease resistance was also increased up to 90% when compared to non transformed control plants.

Latha et al. (2006) have also developed a transgenic pearl millet conferring resistance to downy mildew disease by inserting a chemically synthesized prawn antifungal protein encoding gene (*pin*); embryogenic calli were used as target explants for bombardment. The transformed plants were selected by incorporating phosphinothricin (PPT) in the selection medium and were subsequently confirmed by PCR, Southern blot and northern blot analysis. They have also confirmed the Mendelian inheritance pattern of *par* and *pin* genes in T1 progenies. The transformed plants showed high level of resistance to fungal pathogen when compared to untransformed control after challenging with fungal spores.

Even though the transformation system for pearl millet was developed earlier (Taylor et al. 1991) only two fertile transgenic pearl millets expressing functional foreign genes have been reported so far (Girgi et al. 2006; Latha et al. 2006). Since downy mildew is a major constraint in pearl millet production (Rachie and Majumdar 1980) genes like chitinase and glucanase could be transferred to pearl millet in the near future in order to offer the prospect of conferring high levels of resistance against fungal pathogens and thereby improving pearl millet production. These genes (chitinase and glucanase) seem to be promising in the fight against fungal pathogens (Kumar et al. 2003; Kalpana et al. 2006; Maruthasalam et al. 2007). In rice these genes were introduced by *Agrobacterium*-mediated transformation but there is no *Agrobacterium*-mediated transformation yet reported in pearl millet.

Bahiagrass

Bahiagrass (*Paspalum notatum*) is a major subtropical forage species that is extensively grown in the southeastern United States and from Central Mexico to Argentina. The transformation system for this important forage grass was first established by Smith et al. (2002) using the biolistic method. Transformed plants were selected on phosphinothricin and later confirmed by PCR and Southern blot analysis. Gondo et al. (2003) conducted preliminary experiments for particle inflow gun-mediated transformation of diploid bahiagrass. Later the same laboratory transformed diploid bahiagrass with efficient plant recovery system employing modified callus induction and plant regeneration protocols; 22 transgenic plants were recovered out of 360 explants bombarded (Gondo et al. 2005).

Altpeter and James (2005) have developed a transformation protocol for bahiagrass using the *nptll* (neomycin phosphotransferase) marker gene expressed under the control of *uql* promoter using biolistic method. Later the same laboratory also developed a transgenic bahiagrass with improved turf quality (Hangning et al. 2007). Over-expression of *Arabidopsis* ATHB16 transcription factor in bahiagrass produced significantly more vegetative and fewer reproductive tillers (Hangning et al. 2007). *ATH1* is an *Arabidopsis* homeobox transcription factor-encoding gene; in ryegrass the expression of this gene also produced late-heading or non-flowering plants with more vegetative parts (Van der Valk et al. 2004). They utilized the same protocol developed by Altpeter and James (2005) for the delivery of the *ATHB16* transcription factor-encoding gene. Transformed plants were selected on 50 mg paromomycin sulfate l^{-1} and later confirmed by molecular (PCR and Southern blot) analysis.

Recently James et al. (2008) developed a transgenic bahiagrass resistant to abiotic stress by expressing the stress-inducible gene *HsDREB1A*; transformed plants survived under severe salt and repeated cycles of dehydration stresses. *HsDREB1A*

encodes a transcription factor; it was isolated from barley and subcloned under the control of the stress-inducible barley *HVAls* promoter. Tolerance of transgenic plants to drought stress and salt stress was confirmed by exposing them to severe drought and salt stresses (James et al. 2008). This study can be of great use in the development of abiotic (drought and salt) stress resistant millets in the future. Since millets are the native of semi-arid tropics where unpredicted drought is most prevalent, transferring the genes like *HsDREB1A* to important millets like pearl millet and finger millet will help to improve the productivity of these millets and offer food security in this region.

Finger millet

Finger millet (*Eleusine coracana*) is the primary food source for millions of people in tropical dry land regions. Finger millet also has nutritional qualities superior to that of rice and is on par with that of wheat (Latha et al. 2005). One of the major biotic constraints in the finger millet crop is the high seed yield loss (>50%) due to leaf blast disease caused by the pathogenic fungus, *Pyricularia grisea*; this demands genetic improvement of finger millet by transferring fungal resistance genes (Ceasar and Ignacimuthu 2008). Only a little work has been done for the improvement of this millet in spite of its nutritional importance.

Preliminary work on finger millet transformation was performed by Gupta et al. (2001) who compared the efficiency of five gene promoters (*CaMV35*, *Actl*, *uql*, rice ribulose 1, 5-biphosphate carboxylase small subunit (*RbcS*) gene promoter and *Flaveria trinervia* (*Ft*) gene promoter) for the expression of the *GUS* reporter gene. They confirmed that promoters *Actl* and *uql* gave best response for the *GUS* gene expression; *RbcS* and *CaMV35S* promoters produced medium response and *Ft* promoter was found to be ineffective for *GUS* expression (Gupta et al. 2001). This study may be more helpful to choose the type of promoter for the high level expression of valuable genes in finger millet in future.

A transgenic finger millet resistant to fungal blast disease was developed by Latha et al. (2005). Antifungal protein (PIN) of prawn was chemically synthesized and cloned in the plasmid pPin35S and the *bar* reporter gene was cloned in the plasmid pBar35S; both the genes were put under the control of *CaMV35S* promoter. The transformed plants were selected on PPT-supplemented medium. Stable integration and expression of the PIN gene were confirmed by Southern and northern blot analyses. This is the first and only report available for transgenic finger millet exhibiting high-level of resistance to leaf blast disease. The genetic improvement of finger millet to improve the grain yield by conferring resistance to fungal disease and other stresses is a priority.

Foxtail millet

Foxtail millet (*Setaria italica*) is an important food crop grown in India, China and Japan on salinity-prone lands as well as during adverse conditions such as severe drought. It is also planted in Australia, North Africa and South America for hay and silage (Sreenivasulu et al. 2004). Only two reports are available so far for the transformation of foxtail millet; in both cases *Agrobacterium*-mediated transformation was used. *Agrobacterium*-mediated transformation system for foxtail millet was first developed by Liu et al. (2005). The transformed explants were selected on 50 mg kanamycin l^{-1} and assayed for *GUS* gene expression and later confirmed by Southern blot analysis; they obtained 6.6% transformation frequency through this approach. Afterwards the same protocol was utilized by Fang et al. (2007) for the transformation of foxtail millet with pollen specific gene *Si401* expressed under the control of pollen specific promoter of *Zea mays* (*Zm*13) cloned into the plasmid pBIZm13Si401; the vector also contained *nptII* gene for kanamycin selection. They reported that *Si401* expression in foxtail millet resulted in multiple abnormalities during the late stages of anther development including premature degeneration of the tapetum, pre-deposition of Wbrous bands in endothecium cells, and aborted pollen grains. These interesting findings may be harnessed in the near future for the development of agronomically important male sterile cereal crops.

Since these two studies on foxtail millet have confirmed the potential of *Agrobacterium*-mediated transformation of millet, efforts need to be taken to extend this protocol to many other important millet crops for the transfer of essential genes and production of improved traits using *Agrobacterium*-mediated approaches.

Other millets

A limited number of studies have been reported on other millets including barnyard millet, elephant grass and guinea grass for transformation studies; preliminary experiments alone were conducted but the transgenic crops expressing functionally active foreign genes are yet to be produced in these millets. Elephant grass and guinea grass were transformed by Hauptmann et al. (1987, 1988). Protoplasts were used as the target explant and DNA was delivered by electroporation. They used three plasmids, viz. pMON145, pMON273 and pMON41, containing marker genes chlorophenicol acetyl transferase (*cat*), *nptII* and *hpt*, respectively; all three genes were put under the control of separate *CaMV35S* promoters. Transformed protoplasts were selected on the antibiotics and later confirmed by Southern hybridization; they obtained very low (10^{-5} to 2×10^{-6}) frequency of transformation. Taylor et al. (1993) also conducted transformation studies using biolistic method in these two grasses (elephant grass and guinea grass) along with pearl millet; details of this transformation was discussed in the pearl millet section. Only one report is available for the transformation of barnyard millet; this millet was also used along with finger millet for assessment of the efficiency of five gene promoters as discussed in finger millet transformation part of this review (Gupta et al. 2001). In barnyard millet, only the *uq1* promoter was effective for the expression of *GUS* gene; other four promoters (*CaMV35S*, *Act1*, *RbcS* and *Ft*) were ineffective based on the histochemical analysis.

Future prospects for *Agrobacterium*-mediated transformation of millets

The biolistic method of gene delivery offered a genotype-independent transformation system for many cereals and made a great breakthrough in the transformation of monocots which were earlier thought to be inaccessible for *Agrobacterium*-mediated system. But a major drawback in the biolistic system is the considerable variation seen in stability, integration and expression of the introduced transgene (Kohli et al. 1999). The *Agrobacterium*-mediated system on the other hand offers the precise integration of a small number of gene copies into the plant genome and shows a greater degree of stability for the transgene (Dai et al. 2001). One of the major barriers in the use of *Agrobacterium* to transform cereals is the absence of wound responses and the associated activation of virulence genes. These problems were overcome with the use of actively dividing embryogenic cells such as immature embryos and calli induced from scutella which were co-cultivated with *Agrobacterium* in the presence of acetosyringone, a potent inducer of virulence genes (Vasil 2005). After a detailed study on the molecular events occurring during T-DNA transfer many laboratories have transformed cereals using *Agrobacterium* by manipulating transformation conditions and successfully obtained transgenic cereals. The natural ability of *Agrobacterium* to deliver a discrete segment of DNA into the recipient genome has been exploited in *Agrobacterium*-mediated transformation of cereals (Cheng et al. 2004; Shrawat and Lörz 2006). The possibility of utilizing *Agrobacterium*-mediated system for millet transformation in the future is presented in the following section of this review.

Several factors are shown to be involved in the *Agrobacterium*-mediated transformation of cereals; these include the screening of most responsive genotype and explant, *Agrobacterium* strain, binary vector, selectable marker gene and promoter, inoculation and co-culture conditions and tissue culture medium (Shrawat and Lörz 2006). Screening of genotypes for tissue culture response is an important aspect for subsequent transformation by *Agrobacterium*; genotypes responding well in tissue culture are readily amenable for transformation in wheat, maize, barley and rice (Cheng et al. 2004; Shrawat and Lörz 2006). Genotype response in tissue culture studies were reported in a few millets (Kothari et al. 2005); based on these reports, elite genotypes can be selected which may be readily amenable for *Agrobacterium*-mediated transformation. These model genotypes need to be isolated from the rest of the millets which give good response on tissue culture and can be utilized for the subsequent transgenic plant production. In wheat, maize, barley and sugarcane model genotypes have worked well for *Agrobacterium*-mediated transformation. These model genotypes include maize cv. A188 or its hybrids, wheat cvs. Bobwhite and Fielder, barley cvs. Golden Promise and Igri and sugarcane cv. Ja 60-5. (Cheng et al. 2004).

Somatic embryo is the more suitable explant for co-cultivation with *Agrobacterium* and efficient transformation in cereals, like rice, due to its active cell division (Kumar et al. 2003). This approach may be more helpful for the efficient *Agrobacterium*-mediated transformation of millets. Shoot apical meristems have also been successfully employed as starting material to recover stably transformed maize, wheat, rice, oat, barley, sorghum and millet (Sticklena and Orabya 2005).

The type of *Agrobacterium* strain also played a key role for the efficient cereal transformation. Strains LBA4404 and EHA101 and their derivatives (EHA105 from EHA101, AGL0 and AGL1 from EHA101) were most frequently used for transformation of cereals (Cheng et al. 2004). In millets the strain LBA4404 alone was used to deliver the gene into millet and only one millet (foxtail millet) has been transformed so far (Liu et al. 2005; Fang et al. 2007); other millets are still not tested for the transformation efficiency of any *Agrobacterium* strains. So screening of different *Agrobacterium* strains with various superbinary vectors can be of great use in the successful transformation of millets.

Recently Ding et al. (2007) have optimized the *Agrobacterium*-mediated transformation protocol for wheat using mature embryos as explants; mature embryos pre-cultured for 14 days before co-cultivation gave better response to transformation. This could also be attempted in millets in the future; since millet seeds can be stored for long periods without any insect damage, transformation experiments can be performed at any period of the year using mature embryos. Desiccation of explants prior to or post *Agrobacterium* infection has proved to be an important factor in increasing the efficiency of transformation in cereals (reviewed by Cheng et al. 2004). Though the molecular events involved in desiccation are still not clear it is believed that desiccation treatment had suppressed the over-growth of *Agrobacterium* and subsequently facilitated better cell recovery after co-culture when compared to the control without desiccation (Shrawat and Lörz 2006).

Other factors that influence the *Agrobacterium*-mediated transformation of cereals are addition of a *vir* gene-inducing compound (acetosyringone) in the co-culture medium, osmotic treatment, antinecrotic treatment, *Agrobacterium* density, addition of surfactants (e.g. silwet L77; pluronic acid F68) and temperature maintenance during co-culture (Cheng et al. 2004). Testing of these factors in millets could be helpful in *Agrobacterium*-mediated transformation. *Agrobacterium* infection using techniques such as desiccation, antinecrotic mixture for pre-induction as well as plant growth regulation treatment are emerging. Understanding the molecular basis of several factors such as desiccation and antinecrotic treatments affecting both T-DNA delivery and stable transformation may facilitate application of these treatments to other species including millets in the near future (Opabode 2006).

In conclusion, the millet transformation is dominated by physical methods of gene delivery (biolistic; electroporation). Many reports of millet transformation are also restricted to the analysis of marker or reporter gene expression. Production of transgenic millets expressing functional foreign genes has been initiated only in recent years. Efforts made in the *Agrobacterium*-mediated transformation of other cereals need to be extended to millets in the near future to produce transgenic millets expressing agronomically important foreign genes. This will greatly help to improve millet production by conferring resistance to biotic and abiotic stresses.

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