

Review

# Gene technology for grain legumes: can it contribute to the food challenge in developing countries?

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## Abstract

Grain legumes play a crucial role in the sustainability of agricultural systems and in food protein supply in developing countries. Several constraints that limit crop production or quality have been addressed by conventional breeding and enhanced management, but there are situations where the existing germplasm lacks the required traits. Genetic transformation could help provide solutions to certain constraints, thus improving food security in developing countries. The potential benefits of this form of genetic improvement have not yet been realised, mainly because efficient and reproducible gene transfer systems are not available. We review the state of the art of gene technology for genetic improvement of those grain legumes of major importance to developing countries. Protocols are evaluated for their reproducibility, efficiency and robustness.

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## 1. Introduction

Among the grain crops, grain legumes (also known as pulses or food legumes) rank third behind cereals and oilseeds in world production, but constitute an important dietary constituent for humans and animals. Production of cereals (2029 MMT) dominates world food production while pulses (55 MMT) play an often-underestimated role as a break crop that fixes nitrogen. Legumes associate with nitrogen fixing bacteria and play a central role in low input production systems, particularly on small-scale farms [1]. Grain legumes are mainly cultivated in developing countries where they accounted for 61.3 million hectares in 2002, compared to 8.5 million hectares in developed countries (Table 1).

Pulses are richer in protein than cereals and represent an important source of dietary protein, especially when intake

from animal or fish sources is low or not available. Proteins of grain legumes are generally high in lysine, but low in methionine and cysteine. However, combined with cereals they result in a balanced diet of energy and protein. Pulses provide a large variety of food alternatives and are a source of income and livestock feed, matching perfectly the requirements of small-scale, low-income farmers in developing countries.

Gene transfer is the introduction of defined genetic information from any living organism into a new host and can help provide a solution to certain constraints that limit crop production or quality. Such crops are genetically modified (GM) or transgenic. Transgenic crops that have been commercialized include maize, soya, cotton and canola. The largest areas of production have been in industrialized countries, but this technology may have far greater utility for farmers in developing countries [2]. For instance, 75% of the 5.5 million farmers who grew GM crops in 2002 were in developing countries [3]. It will be important to ensure that biosafety regulations and regulatory compliance systems are in place in each of the countries using the technology. This may not be the

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Table 1  
Important legumes for food security in the developing world

Legume species	Center of origin	Production (MMT <sup>a</sup> in 2002)		Major producers (% of world production)	
			World		DC <sup>b</sup>
Peanut ( <i>A. hypogaea</i> )	South America		34.07	32.32	China (44) India (16) Nigeria (7.9)
Common bean ( <i>P. vulgaris</i> )	Central America	Dry	18.33	15.75	Brazil (17) India (16) Mexico (9.0)
		Green	5.65	4.28	China (34) Indonesia (13) Turkey (8.2)
Chickpea ( <i>C. arietinum</i> )	Turkey		7.80	7.41	India (86) Turkey (7.6) Pakistan (4.6)
Cowpea ( <i>V. unguiculata</i> )	Ethiopia		3.68	3.64	Nigeria (65) Niger (9.5) Burkina (9.0)
Pigeon pea ( <i>C. cajan</i> )	India		2.99	2.99	India (81) Myanmar (10) Uganda (2.6)
Lentil ( <i>L. culinaris</i> )	Near East		2.94	2.24	India (33) Turkey (16) Canada (12)
Field pea ( <i>P. sativum</i> )	South-West Asia	Dry	9.87	2.50	France (17) Russian (16) Canada (14)
		Green	9.06	6.28	India (42) China (18)
Faba bean ( <i>V. faba</i> )	Near East	Dry	3.73	2.88	China (42) Egypt (12) Ethiopia (10)
		Green	1.02	0.80	Algeria (12) China (11) Morocco (10)
Grass pea ( <i>L. sativus</i> )	Balkan Peninsula			>1.00	India Bangladesh

Source: FAOSTAT 2002.

<sup>a</sup> MMT: million metric tones.

<sup>b</sup> DC: developing countries.

case at present. Thus, issues relating to food safety, labeling, traceability, trans-border movement and trade must receive attention prior to any potential release of a food legume.

Two popular strategies for gene transfer to plants [4–7] are the *Agrobacterium* method [8,9] and direct DNA introduction by micro-particle bombardment [10]. The efficient production of transgenic plants requires stringent selection procedures supported by a selectable marker gene that confers resistance to agents such as antibiotics or herbicides. Several such selection systems have recently been described for grain legumes, based on the marker genes neomycin phosphotransferase II (*nptII*), hygromycin phosphotrans-

ferase (*hph*, *aph IV* or *hyg*), phosphinotricin acetyltransferase (*bar* or *pat*), conferring resistance to kanamycin, hygromycin and the herbicide phosphinotricin (BASTA<sup>TM</sup>), respectively.

The prerequisites for a successful gene technology system for plants include:

- robust, reproducible and efficient plant-regeneration in tissue culture;
- a suitable selectable marker gene with corresponding selective agent;
- a gene (s) of interest, reconstructed for expression in the appropriate organ, tissue, or cell type;

- (d) recovery of many independent transgenic events;
- (e) stable transmission of the transgene(s) to the following generations;
- (f) stable expression of the transgene(s) proven over many generations;
- (g) proven efficacy of the transgenic phenotype;
- (h) freedom to operate, i.e. licenses to all the technologies being used;
- (i) regulatory approval for safe use with respect to health and environment;
- (j) access to improved germplasm for different agro-ecological zones.

Genes encoding insecticidal proteins have been extensively used in transgenic plants, although not in Legumes. These genes have been isolated from microorganisms, such as *Bacillus thuringiensis* [11], as well as from higher plants [12]. The latter includes genes for lectins [13] diverse proteases [14], protease inhibitors [15] and  $\alpha$ -amylase inhibitors [16].

Prospects for improvement of the nutritional quality of legume seeds have been reviewed recently [17]. Proof of concept has been obtained by transferring a gene encoding a methionine-rich seed 2S albumin from sunflower to lupins [18]. The albumin constituted 5% of the total protein in the seeds of the transgenic lupins and led to the doubling of the seed protein methionine. The potential of such approaches is still largely unrealized but should yield seeds with enhanced protein quality for the future [17,19].

While genetic transformation could provide major advances in developing insect and disease resistant cultivars or improve product quality (usually monogenic traits), the

contribution of these techniques to polygenic traits such as tolerance to drought, salt, heat or cold, will largely depend on better physiological understanding and molecular insights. For example, the complexities involved in conferring robust protection to abiotic stress may be a challenge for gene technology in the immediate future [20].

Here, we highlight the state of the art of gene technology for genetic improvement of grain legumes and focus on the development of transformation protocols for the production of transgenic plants (Table 2). The focus is on those grain legumes of major importance to developing countries (Fig. 1), namely peanuts (*Arachis hypogaea*), common beans (*Phaseolus vulgaris*), chickpeas (*Cicer arietinum*), cowpeas (*Vigna unguiculata*), pigeon peas (*Cajanus cajan*) and lentils (*Lens culinaris*). Other legumes such as field peas (*Pisum sativum*), faba bean (*Vicia faba*) grass pea (*Lathyrus sativus*) or mung bean (*Vigna radiata*) can however play an important role in local areas and achievements on these species are mentioned. Soybean (*Glycine max*) is playing an increasingly important role in developing countries, often replacing traditionally grown grain legumes. Because of its economic importance there have been numerous excellent reviews on gene technology applications in soybean [21–24].

## 2. Peanuts (*Arachis hypogaea*)

### 2.1. Importance

Peanut, also known as groundnut, is grown in all tropical and sub-tropical regions of the world (Fig. 1A). Two

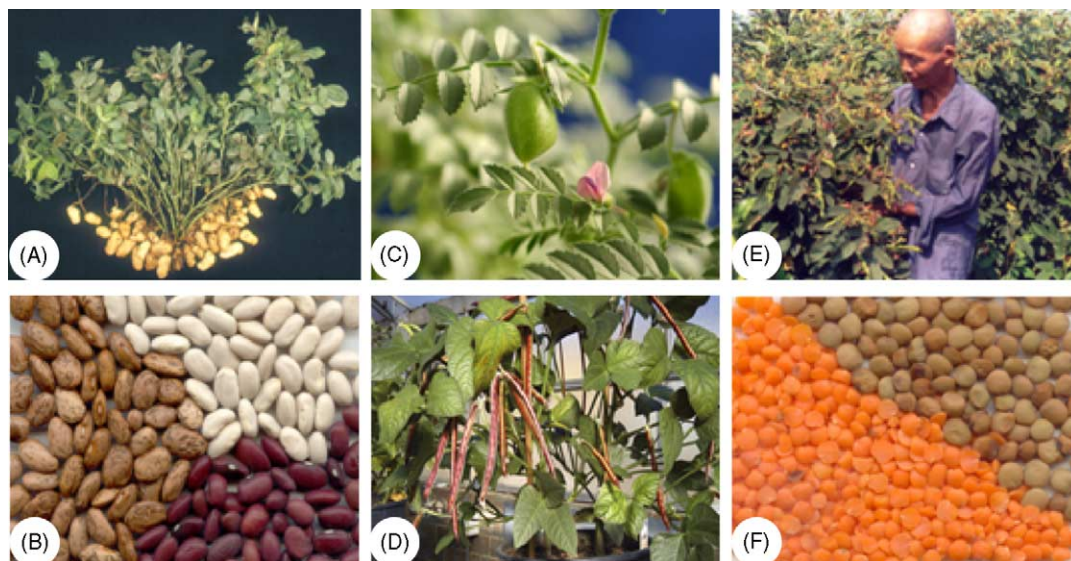


Fig. 1. Grain legumes of major importance to developing countries: (A) peanut (*Arachis hypogaea*) (image courtesy of H.C. Sharma); (B) common bean (*Phaseolus vulgaris*); (C) chickpea (*Cicer arietinum*); (D) cowpea (*Vigna unguiculata*); (E) pigeon pea (*Cajanus cajan*) (image courtesy of Shiyang Yang); (F) lentil (*Lens culinaris*).

Table 2  
Promising methods for gene transfer to selected grain legumes

Legume species, cultivar	Transformation method	Expressed gene (s)	Evidence	Reproducibility and robustness	Reference(s)
Peanut ( <i>A. hypogaea</i> ) NM Val A	At (EHA 105)	<i>NptII</i> , <i>uidA</i> , TSMV-L N	Transmission and expression in T <sub>1</sub> (Southern blot, northern blot), resistance to TSWV	Good	[29,122]
Florunner, Georgia Runner, MARC-1J	MPB	<i>hph</i> , <i>uidA</i> , TSWV N	Transmission and expression in T <sub>1</sub> (Southern blot, northern blot)	Good	[30,123]
JL-24	At (C58)	<i>nptII</i> , <i>uidA</i> , IPCVcp	Transmission to T <sub>1</sub> (PCR, Southern blot)	Low	[124]
Common bean ( <i>P. vulgaris</i> ) Olathe	MPB	<i>uidA</i> :: <i>neo</i> , 2S albumin	Transmission to T <sub>1</sub> (Southern blot)	Low	[47]
Tepary bean ( <i>P. acut. vulg.</i> ) NI 576 among others	At (C58)	<i>nptII</i> , <i>uidA</i> , <i>arc5-1</i>	Transmission and expression in T <sub>1</sub> (Southern blot and western blot)	Good	[42,43]
Chickpea ( <i>C. arietinum</i> ) Turkey and Chafa	At (EHA 101 and C58C1)	<i>pat</i> , <i>nptII</i>	Transmission to T <sub>1</sub> (PCR)	Low	[57]
Sensen	At	<i>nptII</i> , <i>αAI</i>	Transmission and expression in T <sub>1</sub> (Southern and western blot); functionality of <i>αAI</i>	Good	[59]
Cowpea ( <i>V. unguiculata</i> ) C-152	At	<i>Hpt</i>	Transgenic T <sub>0</sub> (Southern blot), no germination of T <sub>1</sub> seeds	Low	[78]
Pigeon pea ( <i>C. cajan</i> ) ICPL 87	At (LBA 4404), MPB	<i>nptII</i> , <i>uidA</i>	Transmission T <sub>1</sub> (PCR)	TETE	[90]
Hyderabad	At (EHA 105)	<i>nptII</i> , <i>H</i>	Transmission and expression in T <sub>1</sub> (Southern and western blot)	TETE	[91]
Lentil ( <i>L. culinaris</i> ) Laird	MPB	<i>als</i>	Transmission T <sub>1</sub> (PCR, Southern blot)	TETE	[105]
Field pea ( <i>P. sativum</i> ) Greenfeast, Rondo	At	<i>bar</i> , <i>nptII</i> , <i>αAI</i>	Transmission and expression in advanced generations	Good	[112,118]
Faba bean ( <i>V. faba</i> ) Mythos	At (EHA 101, EHA 105)	<i>uidA</i> , <i>nptII</i> , <i>sfa8</i> , <i>lysC</i>	Transmission and expression in advanced generations	TETE	[113]
Mung bean ( <i>V. radiata</i> ) K-851	At (LBA 4404)	<i>nptII</i> , <i>uidA</i>	Stable integration and expression in T <sub>0</sub> plants	TETE	[119]
Blackgram ( <i>V. mungo</i> ) K-851	At (EHA 105)	<i>nptII</i> , <i>uidA</i>	Stable integration and expression in T <sub>0</sub> plants, transmission to T <sub>1</sub> progeny	TETE	[120]

Abbreviations: At: *Agrobacterium tumefaciens*; MPB: micro-particle bombardment; TETE: to early to evaluate.

subspecies, *A. hypogaea hypogaea* and *A. hypogaea fastigiata* are cultivated. They have unique phenotypic and genetic characteristics [25] which determine crop management and final use. The early history of this crop has been reviewed by Hammons [26].

China accounts for more than 40% of world production (Table 1). Peanuts are mainly utilized for human consumption, as whole seed (roasted) or processed to a wide range of products. Due to its high protein content (up to 32%) it also provides excellent livestock feed. Peanut shells serve as roughage in fodder, as fuel, and even in particleboard manufacture. The groundnut is a valuable cash crop for small-scale farmers generating employment on the farm and during marketing and processing. In addition, due to its high oil content (up to 54%) it is, like soybean, a crop with commercial importance worldwide.

## 2.2. Crop constraints

This ancient crop has a narrow germplasm base, without satisfactory resistance to many major pathogenic fungi and viruses. Leaf spots (*Cercospora arachinidicola* and *Cercosporidium personatum*) as well as rust (*Puccinia arachidis*) are the most destructive peanut pathogens [27]. In the USA, tomato spotted wilt virus (TSWV) [28] has been responsible for many epidemics. Insects may have a direct impact on yield as well as serving as vectors for viruses. Beside aphids and thrips, termites and ants can also cause major problems.

Abiotic factors including drought as well as water logging are critical yield limiting factors. Water logging can have a negative impact on seed development and lead to aflatoxin contamination of the harvest.

In the USA, fungal diseases and insect pests are controlled by chemicals. Breeding programs have therefore neglected the development of disease resistant cultivars and have been focused primarily on raising yield potential. With increasing severity of difficult-to-control diseases and reduction in price support, this situation is changing and pathogen resistance is considered to be an important breeding goal [27].

## 2.3. Advances in peanut transformation

Transgenic peanut plants have been produced using both Agrobacterium and biolistic transformation approaches [29] (Table 2). A gene for a nucleocapsid protein (N) from TSWV has been successfully introduced [30,31] although large numbers of transformants with the appropriate level of N expression are yet to be produced [30]. Commercial peanut cultivars were transformed by micro particle bombardment of embryogenic callus cultures, using the *hpt* gene [31]. Rohini and Rao [32] described a non-tissue culture based method for the production of transgenic peanut plants. There is no published confirmation of this study.

## 3. Common bean

### 3.1. Importance

Common bean (*P. vulgaris*) is an important food legume and the *Phaseolus* genus contains four other domesticated species [33]: *P. acutifolius*, tepary bean, *P. coccineus*, the scarlet runner, *P. lunatus*, lima bean and *P. polyanthus*, yard-long bean. The common bean is the most widely cultivated and occupies more than 90% of the area of *Phaseolus* species ([34], <http://www.phaseolus.net>).

Common bean has been a dominant staple in Central and South America for thousands of years and shows an extremely high morphological variability (growth habit, seed colour and shape) (Fig. 1B). They have been adapted to a wide range of environments and cultivation methods and are mainly used as mature dry grain.

Except for Argentina, beans are usually cultivated on small land holdings. In Africa, intercropping of beans with cereals (maize, millet or sorghum), plantains or root crops, is a common practice. Genetic improvement of common bean by classical breeding has progressed enormously over the past decade [35].

### 3.2. Crop constraints

Common bean has several production constraints [36]. Six major diseases, anthracnose, rust, angular leaf spot, common bacterial blight, Bean Golden Mosaic Virus and Bean Common Mosaic Virus are widespread, and several others are regionally important. Leafhoppers, white flies, bean pod weevils and bean beetles cause severe damage in the field and bean weevils can be a problem during storage.

Drought stress, soil toxicities and nutritional deficiencies can also limit productivity. Beans are frequently produced on acid soils that are low in available phosphorus. Over 50% of bean-growing areas in Latin America and 65–80% in Africa are thought to be critically deficient in phosphorus. On acid soils beans are frequently affected by aluminium toxicity.

### 3.3. Advances in *Phaseolus* transformation

Common bean can be regenerated in vitro from different explants, including shoot tips, leaf petioles, seedlings, embryonic axes, cotyledons, seedling nodes or meristematic calli.

Susceptibility to Agrobacterium has been shown to be genotype dependent [37,38]. The first attempts to produce stable transgenic *Phaseolus* plants resulted in non-regenerable transgenic callus [39] or chimeric tissues [40,41]. There is no confirmed report of stably transgenic *P. vulgaris* plants using an *A. tumefaciens* based system.

On the other hand, *P. acutifolius* can be routinely transformed using Agrobacterium [42,43] (Table 2). The wild *P. acutifolius* line NI576, and the large seeded cultivar, TB1, have been used successfully [44]. Transformation is based

on callus explants, and between 5 and 10 independent, fertile transformants can be obtained in a routine experiment starting from 50 bean seeds. Since *P. acutifolius* can be crossed with *P. vulgaris* and fertile plants can be obtained by embryo rescue [45], this may be the most efficient way to improve the common bean with gene technology.

In contrast to the situation with *Agrobacterium*, a system to generate transgenic common beans has been developed using particle bombardment of apical meristems [46–49] (Table 2). Although this often results in chimeric plants, the progeny can be screened for stable integration of the transgene. Routine use of this protocol by many laboratories has not been demonstrated.

## 4. Chickpea

### 4.1. Importance

This ancient crop probably originated in Turkey over 7000 years ago, and spread to the Middle East, South Asia and North Africa where it became an important crop (Fig. 1C). The small seeded desi-type chickpea now accounts for about 85% of world production and is the principal type grown in India, Pakistan, Iran, Afghanistan and Ethiopia. The less common large seeded kabuli-type is grown in Middle East, India, Mexico as well as in North America, Australia and Spain. Chickpeas are mostly consumed as a mature pulse (cooked whole, dehulled or as flour), but are also served as a vegetable (immature shoots and seeds). Seeds average about 20% protein, 5% fat and 55% carbohydrate and represent a basic food crop in many developing countries, especially India, where they have a high economic value. Chickpea is a low input crop that often completes its lifecycle in drought and heat stress.

### 4.2. Crop constraints

The average yield of chickpea is about 0.8 t/ha [50], but it has an estimated yield potential of 5 t/ha. Drought stress, poor management practices and diseases are the main yield limiting factors in chickpeas. Fungal diseases, such as *Ascochyta* blight, *Rhizoctonia* root rot, *Pythium* rot, *Fusarium* wilt, white mold, as well as bacterial blight and certain viruses can cause great damage to the crop. The exudation of malic and oxalic acid from granular hairs covering leaves, stems and pods, make chickpea less susceptible to direct damage from aphids and other insects. Damage due to the pod borer (*Helicoverpa armigera*) is however a major threat. Stored chickpeas are highly susceptible to attack by bruchid beetles (*Callosobruchus maculatus* and *C. chinensis*). Germplasm with some degree of resistance to bruchids has been identified, but it appears to be correlated with undesired physical characteristics of the seed coat. Since dark colour, roughness, altered chemical composition and thickness of the seed coat makes bruchid resistant chickpeas less

desirable for human consumption, the introduction of unlinked resistance genes via gene transfer technology would be advantageous.

### 4.3. Advances in chickpea transformation

Plant regeneration via somatic embryogenesis after callus induction with 2,4-D or other synthetic auxins was an early success. For instance, “cotyledon like structures” can be induced by combining zeatin and low levels of indole-3-acetic acid (IAA) [51]. Transformation experiments relying on callus failed due to limited shoot regeneration [52] but demonstrated the potential of *A. tumefaciens* as a transformation vector for chickpea [53,54].

The first report of chickpea transformation [55] also provided molecular evidence for the transgenic nature of sexual progeny of chickpea after co-cultivation of embryonic axes with *A. tumefaciens*. Genes encoding  $\beta$ -D-glucuronidase (GUS) and neomycin phosphotransferase (NPTII) were expressed and the transgenes were transmitted up to the T<sub>2</sub> generation. Using similar experimental protocols the formation of multiple shoots from different genotypes and the production of primary transgenic plants has been reported [56,57]. Multiple shoot formation was achieved on MS medium supplemented with 6-benzylaminopurine (BAP) [57], BAP and  $\alpha$ -naphthalene acetic acid (NAA) [58] or BAP, NAA and kinetin [55]. Transgenic plants were selected via multiple culture cycles on media containing kanamycin [55,56] or phosphinotricin [57] (Table 2). Transformation frequencies were low and reproducibility was a limiting factor.

There are two reports of transgenic plants expressing potentially useful transgenes in chickpeas. Transgenic chickpea plants produced via biolistic transformation expressed the bacterial *cryIAc* gene from *B. thuringiensis* together with *nptII* as the selectable marker [56]. Insect feeding trials with one primary transgenic plant demonstrated an inhibitory effect on growth of larvae of the chickpea pod-borer *H. armigera*. Transmission to T<sub>1</sub> progeny was demonstrated although further analysis has not been reported.

*Agrobacterium* was used to transform desi type chickpeas with a seed specific  $\alpha$ -amylase inhibitor ( *$\alpha$ A11*) gene from *P. vulgaris* and the *nptII* gene as selectable marker [59]. Stable transmission and expression of the transgenes in subsequent generations was demonstrated. The high level of expression of the  *$\alpha$ A11* gene protected chickpea seeds from insect damage by severely inhibiting the development of cowpea weevils (*C. maculatus*) and adzuki bean weevils (*C. chinensis*) [59] (Table 2).

## 5. Cowpea

### 5.1. Importance

Cowpea (Fig. 1D) is a widely adapted and nutritious grain legume. The majority (92%) of world production occurs in

Africa (Table 1) where its importance derives from its relative tolerance to poor, dry soil conditions and many uses. Besides providing a variety of foods (the leaves, stems, shelled green seed and dried seeds are eaten), cowpeas are a source of income and livestock feed (fodder, silage or hay). It is estimated that in Africa more than 200 million people consume cowpea on a daily basis and rely on it as a protein source.

### 5.2. Crop constraints

Yield is limited by abiotic and biotic stresses [60,61]. Natural variation in tolerance to abiotic stress and resistance to most fungal and viral diseases as well as to nematodes and parasitic weeds justify the current vigorous classical breeding approach [61]. However, a major constraint to cowpea production is the insects, which attack virtually every developmental stage of the crop. The cowpea leaf beetle (*Oothea mutabilis*), the cowpea bud or flower thrips (*Megalurothrips sjostedti*), the cowpea aphid (*Aphis craccivora*), the cowpea pod borer (*Maruca vitrata*), the pod sucking bug (*Clavigralla tomentosicollis*) and the cowpea weevil (*C. maculatus*), are the most damaging insects on cowpea. Acceptable levels of resistance to *Maruca* pod borer and bruchids were found in the wild relative *Vigna vexillata*, but transfer of resistance genes into cowpea cultivars and elimination of undesired characters by backcrossing have not been successful [61] making conventional breeding approaches unlikely to provide a feasible solution to the problem.

The introduction of genes for insect resistance into cowpea by genetic transformation has the potential to address the problem and could have an important impact on food security especially for sub-Saharan Africa [62].

### 5.3. Advances in cowpea transformation

Regeneration of cowpeas via somatic embryogenesis has been attempted using different tissues including cell suspensions derived from callus [63,64] and callus derived from young leaves [65], mature embryonic axes [66] and immature cotyledons [67]. Cowpea callus failed to regenerate plants at an acceptable frequency. Therefore, organogenic regeneration is the current focus for cowpea transformation.

Direct organogenesis from tissue derived from axenic hypocotyls [68], epicotyl, cotyledon, hypocotyls [69] and mature cotyledons [70,71] has been achieved with moderate levels of cytokinins. The easy-to-culture cotyledonary node-explants seem the most promising with reproducible responses reported by several laboratories [72,73]. Recently, fertile cowpea plants were regenerated from cotyledonary node thin cell layer explants by application of the cytokinin analogue, thidiazuron [74]. If a high proportion of the shoot buds give rise to independent, phenotypically normal plants, this system could be an attractive route to transformation.

Several *Agrobacterium* strains were shown to infect cowpeas [75–77] although no regenerated cowpea plants were

Table 3  
Are the prerequisites for successful gene technology in grain legumes being met?

Criterion	<i>A. hypogaea</i>	<i>P. vulgaris</i>	<i>P. sativum</i>	<i>C. arietinum</i>	<i>V. unguiculata</i>	<i>C. cajanus</i>	<i>L. culinaris</i>	<i>P. sativum</i>	<i>V. faba</i>	<i>V. radiata</i>	<i>V. mungo</i>
Robust, reproducible and efficient tissue culture, suitable for routine transformation experiments	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Suitable selectable marker gene and selective agent	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Recovery of multiple transgenic events in independent experiments	Yes	No	Yes	Yes	No	Yes	No	Yes	Yes	No	No
Stable transmission of transgene(s) to the following generations	Yes	Yes	Yes	Yes	–	–	–	Yes	Yes	–	Yes
Stable expression of transgene(s) proven over many generations	Yes	Yes	Yes	Yes	–	–	–	Yes	–	–	–
Proven efficacy of the transgenic phenotype	Yes	Yes	Yes	Yes	–	–	–	Yes	–	–	–

In some cases the prerequisites have not been met because the research is still in progress, as shown (–).

obtained. By avoiding the callus regeneration step and using mature de-embryonated cotyledons on hygromycin selection, four plants were obtained that produced seeds [78]. Southern blot analysis of one transgenic plant was positive but the T<sub>1</sub> seeds failed to germinate (Table 2). The particle gun has been used for cowpea transformation and although transformation may have occurred, Mendelian transmission of the transgene could not be confirmed [79]. In summary, to date there is no evidence for a robust transformation system in cowpeas despite the great need for such a development (Table 3).

## 6. Pigeon pea

### 6.1. Importance

Pigeon pea (Fig. 1E) is an important crop for subsistence farmers in the warm semi-arid and sub-humid tropics, particularly in India where more than 80% of world production occurs (Table 1). The crop is often grown on marginal soils without inputs other than seed. Traditional pigeon pea farming systems are intercropped with cereals. The plant is well adapted and remarkably hardy to both low and high temperatures. People eat the mature seeds cooked whole, dehulled or as flour. The immature green seeds are also eaten, mainly in the Caribbean. The plant's woody stems are used as firewood, thatch and fencing.

### 6.2. Crop constraints

Among many insect pests, the pod sucking bug (*Clavigralla* spp.), the African bollworm (*H. armigera*), the pod fly (*Megalurthrips* spp.) and bruchids (*Callosobruchus* spp.) are of major importance. *Helicoverpa* can completely devastate a crop if left untreated. This is further complicated by the prolonged and uneven pattern of flowering and pod-fill in the crop. Pigeon pea is susceptible to a form of fusarium wilt caused by *Fusarium udum*, particularly in humid regions.

Pigeon pea seed protein (21%) is low in methionine and cysteine, but unlike other pulses, it is also low in lysine [80].

### 6.3. Advances in pigeon pea transformation

In vitro cultures have been initiated from different tissue sources [81] and organogenesis as well as somatic embryogenesis is possible [82,83]. Regenerated plants were also obtained via callus [84] and by direct differentiation from leaves [85]. Multiple shoots were induced on explants of cotyledonary nodes [86] or epicotyls [87]. Organogenesis from the distal half of cotyledon explants without pre-existing meristems was genotype dependent [88].

Transgenic pigeon peas expressing a cowpea protease inhibitor gene in leaves of the primary transformants have been obtained [89] and stable transmission and expression of

transgenes in pigeon peas have been demonstrated [90,91] (Table 2) Seeds germinated on a callus-inducing medium were subjected to Agrobacterium mediated gene transfer or bombarded with micro-particles. Both methods resulted in transgenic plants which were confirmed by Southern analysis. In several lines, the *np1II* gene was transmitted to the segregating progeny [90].

A protective antigen of the Rinderpest virus has been expressed in pigeon peas and stable transmission to T<sub>1</sub> progeny shown by integration and expression analysis [91]. Transformation relies on BAP-induced shoot formation on callus induced from germinating seedlings [90], cotyledonary nodes or embryonic axes [91]. A transformation system based on micro-particle bombardment of leaf explants was described [92]. Transgenic plants were obtained after inducing adventitious shoot formation from the petiolar cut end with equimolar concentrations of BAP and kinetin and by promoting shoot elongation with gibberellic acid [92].

## 7. Lentils

### 7.1. Importance

The lentil is thought to be one of the earliest domesticated crops, and has been traced back to 8000 B.C. in the Near East. It is important in West- and Central Asia, as well as in North Africa (Table 1). Traditionally lentils have been important for the largely vegetarian population of South Asia. Lentils are used in many ways ranging from soups and stews to salad dishes. In addition to human consumption, the high-quality straw is highly valued as fodder for small ruminants in the Middle East and North Africa.

Lentil seed size can vary from 2 to 7 mm and the color from light tan to brown, purple and black (Fig. 1F). They are traditionally grown in semi-arid conditions, are well adapted to all types of soil and have a relatively good tolerance to cool weather conditions. Some types can survive winter in cold highland areas.

### 7.2. Crop constraints

Lentils are characterized by poor yields and susceptibility to insect and fungal diseases [93]. Lentil breeding has led to high-yielding varieties with greater yield of biomass, increased tolerance to drought, insects and diseases. Breeding for resistance to lodging, pod shattering and a different plant growth habit has increased the yield potential considerably and facilitated mechanized harvesting. Genetic engineering may also have a role in eliminating anti-nutritional factors and improving the nutritional quality of lentil proteins [21].

### 7.3. Advances in lentil transformation

Tissue culture of lentils has been achieved [94] with shoot apical meristem tips [95,96], nodal segments [97] and intact

seedlings [98]. Cotyledonary nodes showed the highest frequency and genotype independence for shoot regeneration [99,100].

T-DNA was integrated into the lentil genome via axenic tumors on in vivo and in vitro inoculated stems [101]. Transient expression of the reporter gene GUS was achieved by electroporation, particle bombardment and by Agrobacterium transfer methods [102–107].

Fertile transgenic lentil plants were produced after bombarding cotyledonary nodes with a mutant of the tobacco acetolactate synthase gene, which confers resistance to sulfonyleurea herbicides [105]. Shoots were micro-grafted on non-transgenic plantlets. Seven transgenic plants survived leaf-painting tests and were phenotypically normal. Southern hybridisation of the T<sub>1</sub> progeny provided evidence for the transmission of the transgenes [105] (Table 2).

## 8. Minor pulses

### 8.1. Importance

Legumes with minor importance in developing countries can play an important role in local areas. Field peas are an important crop of the temperate regions, with several thousand varieties existing throughout the world [108]. In the genus *V. faba* bean is the most important for human consumption. However, it has poor yield stability, which is the main breeding goal [109]. Mung bean is mainly grown in India where it is also known as green or golden gram and is consumed as dry beans or fresh sprouts. It is closely related to blackgram (*Vigna mungo*). A grain legume which is drought tolerant and therefore important in certain countries such as Ethiopia, is grass pea (*L. sativus*). However, the grains contain variable amounts of a neurotoxic amino acid beta-oxalyl-L-alpha,beta-diaminopropionic acid, that in periods of high Lathyrus consumption can lead to the crippling disease called neurolathyrism [110]. A gene technology approach to reduce the levels of this toxin could be an option here. Although transformation has not been reported for this crop, a regeneration protocol is available [111]. Transformation of other grain legumes, such as adzuki bean (*Vigna angularis*), asparagus bean (*Vigna sesquipedalis*) or lupins (*Lupinus* ssp.) was reviewed elsewhere [121,24]; these are less important for human consumption in developing countries.

### 8.2. Advances in transformation of other pulses

Reliable transformation protocols for field peas and faba beans have been reported [112,113]. The pea system has proven to be applicable to a wide range of genotypes [114–117]. The introduction of the  $\alpha$ AI gene from common bean, expressed in peas, led to protection against the cowpea, adzuki bean and pea weevils [16,118].

## 9. Conclusion

Despite its potential to complement current breeding programs, genetic transformation technology is not yet routinely available for most legumes of importance in developing countries (Table 3). However, while the availability of robust and efficient protocols for gene transfer could have a major impact on food security for such growing populations, long-term progress will depend upon consumer acceptance of the use of gene technology in food production. Thus, in addition to the biological prerequisites that must be met, this is a political challenge that must also be addressed.

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