

Biotechnology in Genus *Lotus*

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Introduction

Lotus corniculatus L. (birdsfoot trefoil) is presently used for pasture, hay and silage. It is fine-stemmed, leafy, slightly decumbent and does not cause bloat when grazed. It grows on a wide range of soil types and conditions, including moderately alkaline soil, shallow soil and moderately acid or infertile soil (RACHIE and SCHMID 1955). The feeding value of *L. corniculatus* is almost equal to that of *Medicago sativa* L. (alfalfa) (Seaney and Henson 1970). However, *L. corniculatus* has a number of undesirable characteristics which need to be improved. These are lack of seedling vigor, pod shattering (dehiscence) caused by indeterminate flowering, and the presence of hydrocyanic acid (HCN) in the leaves and stems (MACDONALD 1946 ; O DONOUGHUE and GRANT 1988). Modification of undesirable traits, however, has been hampered by breeding behavior and genetic nature of the species. *L. corniculatus* is largely an outcrossing species and the characters are mainly inherited tetrasomically (DAWSON 1941). In general, there has been increasing interest in the potential use of tissue and cell culture in generating new genetic variability (LARKIN and SCOWCROFT 1981 ; BAJAJ 1990).

Plant regeneration from *L. corniculatus* calli was established in an anther culture study by NIIZEKI and GRANT (1971). The plant is easy to regenerate from callus culture through both organogenesis (SWANSON and TOMES 1980) and somatic embryogenesis (MARIOTTI *et al.* 1984). Plant regeneration from the protoplast-derived calli of *L. corniculatus* has also been reported (AHUJA *et al.* 1983 ; WEBB *et al.* 1987). The cultivar Viking produces calli from protoplasts with a high potential for regeneration through adventitious buds (NIIZEKI and SAITO 1986) (Figure 1).

Somaclonal Variation

Plants regenerated from tissue or cell culture are considered as the clones of the tissue or cell donor. Generally, in one or more of the traits among the clones, there was variation which was termed somaclonal variation by LARKIN and SCOWCROFT (1981). Somaclonal variation has been found in forage legumes regenerated from cell culture (GROOSE and BINGHAM 1984 ; JOHNSON *et al.* 1984 ; PEZZOTTI *et al.* 1985 ; BINGHAM and MCCOY 1986 ; VESSABUTR and GRANT 1995). This variation may be used in breeding programs because the variants often occur at higher frequencies than from chemically induced mutagenesis (GAVAJI *et al.* 1987). Regenerated plants from *L. corniculatus*, an outbreeding leguminous forage crop, have proved to be suitable for the evaluation of somaclonal variation in morphological and agronomic traits and for comparison with those of a seed-produced population (see Table 1). Extensive work on the cytogenetic, molecular genetic, and morphological variations in regenerated plants of *L. corniculatus* from single protoplasts has been carried by NIIZEKI and coworkers (NIIZEKI *et al.* 1990b, 1991, 1994a, NIIZEKI 1993, 1996 ; NIIZEKI and KODAIRA 1994). The results indicated that Southern blots of the mitochondrial DNA (mtDNA) using mitochondrial genes showed some novel fragments found among the protoclonal. However, the novel fragments were the same as those fragments found in the polymorphism of the seed-derived population. On the other hand, Southern blots of the chloroplast DNA (cpDNA)

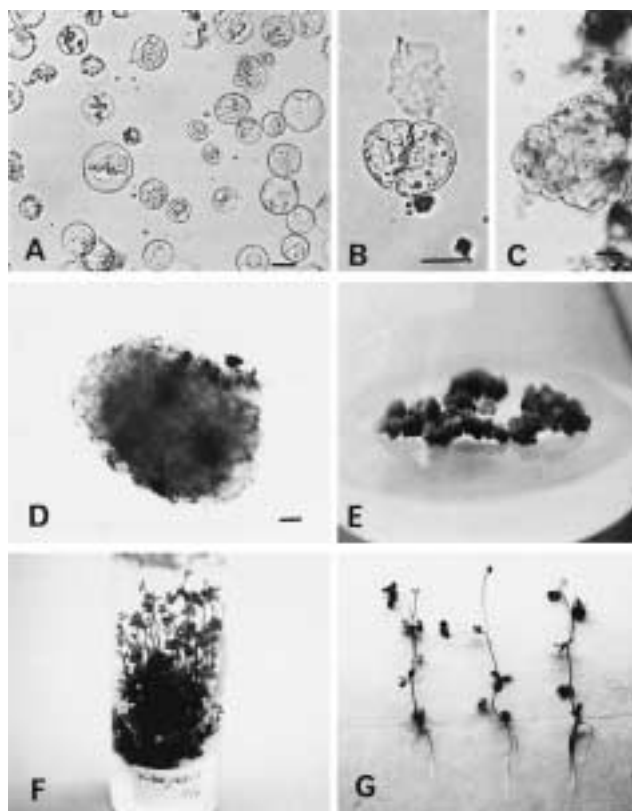


Fig. 1. Isolation and culture of protoplasts of birdsfoot trefoil, cv. Viking. a. Isolated protoplasts. b. Initiated cell division which was observed within 7 days of culture. c. A formed cell cluster which was observed 10 days after addition of fresh medium. d. A colony composed of numerous cells after 1 month of culture. e. Calli formed for a period of 1 month after transplantation of colonies to the callus culture medium. f. Shoot formation at 1.5 months after transplantation of calli to the regeneration medium. g. Complete plantlets which were obtained about 6 months after the initiation of protoplast culture. Bars represent 20 μ m.

Table 1. Summary of the work on somaclonal variation in *Lotus corniculatus* L.

Cultiver	Explant or tissue used	Somaclonal variation	Reference
Leo	Internode-derived calli	2, 4-D (2, 4-dichlorophenoxyacetic acid)-tolerant calli and regenerated plants	SWANSON and TOMES (1980)
Leo	Internode-derived calli	2, 4-D-tolerant callus, suspension culture lines and regenerated plants	SWANSON and TOMES (1983)
Leo	Internode-derived calli	Chlorophyllous callus	SWANSON <i>et al.</i> (1983)
Franco	Calli	Agronomical traits such as plant height, dry matter yield, etc.	DAMIANI <i>et al.</i> (1985)
Leo	Hypocotyl-derived calli	Tolerant suspension calli and regenerated plants for 2, 4-D and chlorosulfuron (2-chloro-N-[(4-methoxy-6-methyl-1, 3, 5-triazine-2-yl) amino] carbonyl]-benzenesulfonamide	MACLEAN and GRANT (1987)
Leo	Protoplasts of leaves	Morphological characters	WEBB <i>et al.</i> (1987)
Franco	Leaf-derived calli	Morphological and agronomical traits such as leaflet width and seed yield	DAMIANI <i>et al.</i> (1990)
Viking	Protoplasts of calli	Chromosome structure, agronomical traits and HCN content	NIIZEKI <i>et al.</i> (1990b)
Leo	Protoplasts of root hairs	Plant height and stem number	RASHEED <i>et al.</i> (1990)
Leo	Hypocotyl-derived calli	Tolerant plants for sulfonylurea herbicide Harmony {DPX-M6316 ; 3-[(4-methoxy-6-methyl-1, 3, 5-triazine-2-yl) amino] carbonyl] amino] sulfonyl-2-thiophenecarboxylate}	POFELIS <i>et al.</i> (1992)
Viking	protoplasts of calli	Mitochondrial and chloroplast genome banding pattern of Southern blot analysis	NIIZEKI (1996)

by using chloroplast genomic DNAs showed that there were few variations among protoclonal lines. In regard to the nuclear genes, there were no variations in Southern blots when using a small subunit of ribulose biphosphate carboxylase (RuBisCO), phenylalanine ammonia-lyase, and ribosomal DNA as probes. However, there was a considerable variation in traits such as plant height and stem diameter, which may be regulated by polygenes. Abnormal meiotic configuration such as univalents, lagging chromosomes, fragments, and bridges were frequently observed in the pollen mother cells and these tended to be related to low pollen fertility. Because very few physiologically and morphologically abnormal plants were found in the protoclonal population, the mutation of major genes is probably very rare and chromosomal aberrations may occur in that part of the heterochromatin which lacks genetic activity. However, the possibility still exists that plants containing mutations in major genes or with chromosomal aberrations in part of the euchromatin are eliminated during acclimatization. Variation in quantitative characters was inherited by the progeny and the elimination of abnormal chromosome configurations resulted in the recovery of plants with high pollen fertility. Therefore, the practicality of a breeding program for quantitative characters including seedling vigor and low HCN by using protoclonal lines in *L. corniculatus* is affirmed and recommended.

One obvious strategy for the use of somaclonal variation is to introduce the best available varieties into cell culture and to select among regenerated plants or their progeny for incremental improvements over existing varieties. Hence, the technique could be used to uncover new variants that retain all the favorable qualities of an existing variety, while adding one additional trait, such as disease resistance or herbicide resistance. For example, in *L. corniculatus* SWANSON and TOMES (1980, 1983) showed the resistance to a herbicide of 2, 4-dichlorophenoxyacetic acid (2, 4-D) (Table 1). While this technique can be used to select and propagate mutant cells and even give rise to tolerant plants, these are often epigenetic and are sometimes unstable. In species that are normally vegetatively propagated, however, this strategy has much to recommend it.

Somatic Cell Hybrids

The hybridization of distantly related species by protoplast fusion has been a practical tool for removing the barriers of incompatibility in sexual crossing of agriculturally important plant species. To date, there have been a limited number of reports on successful hybridization between leguminous species (SANO *et al.* 1988; NIIZEKI and SAITO 1989; KIHARA *et al.* 1992; KAIMORI *et al.* 1998). In addition, agriculturally useful hybrid production has been very difficult, since there is an imbalance in the genomes of the parents in most cases and results in the rearrangement or partial elimination of the chromosomes of one parent and an incapability to achieve morphogenesis (KAO 1977; CHIEN *et al.* 1982; SALA *et al.* 1985). However, some successes in asymmetric protoplast fusion based on the complementation of X-ray- or γ -ray-irradiated and iodoacetamide (IOA)-treated protoplast have been reported of leguminous species (SIDOROV *et al.* 1981; TANNO-SUENAGA *et al.* 1988; SAKAI *et al.* 1996; HAUSEN and EARLE 1997; LIU *et al.* 1999).

Wright *et al.* (1987) were the first to produce somatic hybrid plants in the genus *Lotus* in an attempt to transfer the seed pod indehiscence of *L. conimbricensis* Willd. into *L. corniculatus*, as the species are sexually incompatible. *L. corniculatus* hypocotyl protoplasts were inactivated with IOA to inhibit cell division prior to fusion with *L. conimbricensis* suspension culture protoplasts. *L. conimbricensis* protoplasts divided to form callus which did not regenerate plants. Thus, plant regeneration from protoplast-derived callus was used to tentatively identify somatic hybrid cell lines. Plants regenerated from three cell lines exhibited additive combinations of parental isozymes of phosphoglucosyltransferase, and *L. conimbricensis* specific esterases indicating that they were somatic hybrids, but chromosome numbers were variable and the hybrids were both male and female sterile. AZIZ *et al.* (1990) attempted to combine *L. corniculatus* and *L. tenuis* Waldst et Kit. A cell suspension of *L. tenuis* was established, as a source of protoplasts, from kanamycin resistant callus derived from roots transformed by *Agrobacterium rhizogenes*. Such protoplasts were treated with a sublethal dose of sodium iodoacetate prior to

their electrofusion with green cotyledon protoplasts of *L. corniculatus*. Putative somatic hybrid colonies were selected on medium containing kanamycin sulphate. The hybridity of plants regenerated from these selected colonies was confirmed by their morphology, esterase banding patterns, the presence of condensed tannins in leaves and stems, and chromosome complements, while plant fertility has not been reported. NIIZEKI and coworkers have also produced three asymmetric somatic hybrid calli and plants of *L. corniculatus* by protoplast fusion with *Oryza sativa* L. (rice, strain A58), *Glycine max* (L.) Merr. (soybean, cv. Harosoy) and *Medicago sativa* L. (alfalfa, cv. Rangelander) as follows. Asymmetric hybrid calli, which have only the nuclei of *L. corniculatus*, were produced by protoplast fusion between rice and *L. corniculatus*, and analyzed for their mtDNA and cpDNA (NIIZEKI *et al.* 1992a; NAKAJO and NIIZEKI 1995; NAKAJO *et al.* 1994). In the hybrid calli, novel mtDNA fragments were detected in Southern blot analysis. This result shows that some kind of alteration such as intergenomic and/or intragenomic recombinations of mtDNA occurred in the hybrid calli. On the other hand, the cpDNA fragment patterns of all hybrid callus lines observed by Southern blot analysis were found to be identical with those of *L. corniculatus*. Thus, it is suggested that the cpDNAs of these hybrid calli sorted out unidirectionally. Some regenerated plants from the hybrid calli were tolerant of low temperatures and low sunlight intensity. Also, in order to produce asymmetric hybrids containing a complete *L. corniculatus* nuclear genome and a small part of a soybean nuclear genome, or cybrid containing only a *L. corniculatus* nuclear genome, IOA-treated protoplasts of *L. corniculatus* were fused with X-ray-irradiated soybean protoplasts (KIYAHARA *et al.* 1992; NIIZEKI *et al.* 1990a, 1992b, 1994b). Peroxidase isozyme and karyotypes of calli obtained from the protoplast fusion elucidated the hybridity of some of the calli. Plant regeneration from the asymmetric hybrid calli was also successful, but regenerated plants did not show the hybridity as a result of analysis of peroxidase isozyme. This may be caused by the complete elimination of soybean chromosomes. The morphology of the regenerated plants resembled that of *L. corniculatus* derived from parent calli. However, the regenerated plants were usually teratologically an erect type in contrast to the creeping type of normal *L. corniculatus*. In the other experiment, donor protoplasts of alfalfa were given lethal dose of X-irradiation and recipient protoplasts of *L. corniculatus* were inactivated with IOA. Donor and recipient protoplast were fused with polyethylen glycol (PEG) (KAIMORI *et al.* 1998; NIIZEKI 2001). Fusion products initiated cell division and resulted in calli, some of which have a high capacity for plant regeneration. Many hybrid calli cultured for 1 month were found to have isozymes indicating the banding pattern of one parent in some isozymes and that of another parent in others. These facts may suggest that chromosomes or chromosome segments of both parents may be eliminated randomly at the early stage of callus culture. However, most of the banding patterns had altered to those of the *L. corniculatus* in 2 months of culture. Therefore, the number of calli having the chromosomes of both parents seem to decrease and most of the calli had chromosomes of *L. corniculatus*. This may indicate that callus cells with *L. corniculatus* genomes rapidly came to be selected as dominant. Shoot regeneration did not occur from the symmetric somatic hybrid calli of *L. corniculatus* and alfalfa (NIIZEKI and SAITO 1989; NIIZEKI *et al.* 1989). This fact might be attributed to the imbalance of *L. corniculatus* and alfalfa nuclear genomes or incomplete genome of alfalfa, some of which chromosomes were eliminated after protoplast fusion. These results indicate that most of the alfalfa chromosomes irradiated by X-rays degenerated during subcultures. Accordingly, it may be possible to regenerate the shoots from the calli derived from asymmetric hybrids with X-ray-irradiated donor protoplasts, while regeneration of novel shoots is not likely from symmetrical somatic hybrids carrying complete chromosomes of both parents or some chromosomes of one parent. Isozyme and Southern blot analysis indicated that some hybrid calli had nuclei of *L. corniculatus* and chloroplast genomes of alfalfa. This fact proved that the calli obtained were real asymmetric hybrids. The recalcitrance in shoot regeneration by the calli may be caused by the imbalance in morphogenetic potential of the nucleus and chloroplast genome of the two species concerned. However, improvement or modification of the culture media may provide a breakthrough for regeneration of novel plants.

Somatic hybrid cell lines between two leguminous species, soybean and hyacinth bean, were obtained by

SANO *et al.* (1988). In attempts to use the leguminous species for protoplast fusion, hybrids obtained between *L. corniculatus* and soybean or alfalfa as mentioned above. These results, which may represent one of the few successful cases of wide hybridization of leguminous species, show the possibility of obtaining somatic hybrids in leguminous species in addition to those reported in the Solanaceae and Cruciferae species. However, even in species in the Cruciferae, male sterility or low fertility has often been found in interspecific somatic hybridization (KIRTI *et al.* 1992 ; LELIVELT and KRENS 1992). HAUSEN and EARLE (1997) suggested that one reason for low fertility may be alloplasmic male sterility caused by incompatibility between the nucleus and the cytoplasmic genome, whereas NOTHNAGEL *et al.* (1997) indicated that backcrosses with one parent may become a useful tool in overcoming the male sterility or low fertility in some cases of Cruciferae. Somatic hybrids of *L. corniculatus* and another leguminous species also showed male sterility, so that no seed was obtained. Thus, from the point of view of plant breeding it may be also worthwhile to attempt to backcross somatic hybrid with one parent in these leguminous species. Indeed, progeny of a somatic hybrid of birdsfoot trefoil and rice was obtained when the somatic hybrid was backcrossed with birdsfoot trefoil as pollen parent (unpublished data).

Genetic Transformation

L. corniculatus is herbage legume which readily regenerates plants in culture and amenable to transformation by *Agrobacterium tumefaciens* and *A. rhizogenes* which induce crown gall and hairy root, respectively. These qualities make it particularly suitable species for testing genetic manipulation strategies (ARMSTEAD and WEBB 1987 ; CHRISTOU 1994 ; TABAEIZADEH 1989 ; AKASHI *et al.* 1998b). Bacterial genes of *A. rhizogenes* transferred into the plant induce the growth of distinctive roots at the infection site (TEPFER 1984). In culture, these roots exhibit characteristic traits. They continue to grow without a supply of exogenous hormone supplements, they are negatively geotropic, they produce opines and they are cytologically stable (AIRD 1988). Regenerated transformed plants from hairy roots had the associated hairy root phenotype. In *L. corniculatus*, this transformation resulted in only minor morphological effects, mainly in flower morphology, but this significantly reduced fertility (PETIT *et al.* 1987 ; WEBB *et al.* 1990, 1994a, c). No adverse effects, however, have been found on the plant nitrogenase levels or on their ability to fix nitrogen (PETIT *et al.* 1987 ; WEBB *et al.* 1990). Thus, such regenerated plants have proved especially of genes involved in nodulation.

Recently, a new continuous culture system of super-growing roots (super roots) has been reported in *L. corniculatus* (AKASHI *et al.* 1998). More than three years after initiation, the super roots continue to grow at the initial high rate, are readily cloned from secondary tips and easily regenerate plants upon transfer to light. The complete system, from primary root culture to plant formation, works without a need for exogenous hormones. AKASHI *et al.* (2000) reported the isolation and culture of protoplasts from the long-term culture and the regeneration of plants from super root-derived protoplasts. Regenerated plants appeared morphologically normal and were able to undergo nodulation when infected with *Rhizobium loti*. Transformation with nod genes, combined with the possibility of re-establishing super growing root cultures from transformed tissues and regenerating plants under hormone-free conditions may provide a perspective for future nodulation research.

Successful genetic transformation of any plants involve not only the production of primary transformants showing stable expression of inserted genes but also the inheritance and continued expression of those genes in subsequent generation. However, there is an increasing awareness that newly inserted genes can be silenced, not only during the life time of the primary transformants, but also in their progeny (FINNEGAN and McELROY 1994 ; ULIAN *et al.* 1994). *A. rhizogenes* was assessed as a vehicle for transformation of *L. corniculatus* (WEBB *et al.* 1994a, b). Plants were co-transformed using *A. rhizogenes* strain LBA9402 harboring the bacterial plasmid pRi1855 and the binary transformation vector pJit73. pRi1855 transfers both T_L and T_R sequences, while pJit73 encodes -glucuronidase (GUS) and also two selectable marker genes giving resistance to the antibiotics, kanamycin (*nptII* gene) and hygromycin (*aphIV* gene). Two primary transformants (line 6 and 12) were

resistant to hygromycin and showed a significantly lower GUS activity in line 6 than in line 12 in various tissues. Genetical analysis of progeny produced by lines 6 and 12 indicated that line 6 had one dose of the *uid* gene (GUS gene), while line 12 had two or more independently segregating doses of the gene. Both line 6 and 12 contained multiple copies of T_L-DNA, while only line 6 was T_R positive. In the progeny of lines 6 and 12 there was no evidence for linkage of T_L-DNA with *uid*, while in the progeny of line 6, T_R-DNA was under-represented. GUS-positive progeny which were free of both T_L and T_R sequences were identified from both lines. Two out of six progenies of line 6 contain *uid* gene but do not have detectable GUS activity, although in one of them the tissue are resistant to hygromycine. This suggests that there is silencing of GUS activity in some of the progeny of line 6. The other case of gene silencing was found in *L. corniculatus* plants transformed with a maize cDNA (GIL) encoding a sulphur-rich γ -zein obtained by using two fusion genes: one with the CaMV 35S promoter, the other with the RuBisCO small subunit (*rbcS*) promoter (Belluci *et al.* 1999). The highest expression of GIL mRNA was found in plants transformed with GIL under the *rbcS* promoter. The steady level of GIL mRNA in the leaves was generally directly correlated with the GIL copy number. However, due to a transcriptional block, no GIL mRNA was detected in some of the 35S-GIL multicopy transformants. Analysis with methylation-sensitive restriction enzymes revealed that the T-DNA of the silenced 35S-GIL transformants was methylated. The presence of the transgenes is important, but the goal is the predictable and reliable expression of the introduced genes in the required tissues and those of progeny. Up to now, even the influence of the number of copies of the transgenes and their positions in the *Lotus* genome on their expression is not yet clear.

End products of the phenylpropanoid pathway are important characters in forage crop quality. One example of this class of compound is condensed tannins which are characterized by their ability to react with proteins to form stable tannin-protein complexes. Condensed tannins are considered to be the effective anti-bloat agents in forage legumes of *L. corniculatus* (SARKAR and HOWARTH 1976; JONES and LITTLETON 1971). Root culture of *L. corniculatus* transformed with *A. rhizogenes* grew rapidly in liquid medium when cultured in the dark and produced large numbers of shoots when illuminated (MORRIS and ROBBINS 1992). The shoots, which could be regenerated to produce some fertile plants were maintained in liquid medium as shoot-organ cultures. The accumulation and cellular distribution of condensed tannins increased at ratio equivalent to control plants. Condensed tannin accumulation was linearly related to root growth and had a similar spatial distribution in tannin cells in roots and leaves compared to control plants.

On the contrary, it is also important to explore the possibility of decreasing the levels of condensed tannins in crop species using antisense technology (ROBBINS *et al.* 1998). Lowering the amounts of condensed tannins in animal feedstuff is important both for ruminant and nonruminant (monogastric) livestock. At high levels (> 3 - 4% dry weight) tannins in forage and fodder are deleterious for use with ruminants and such levels reduce both palatability and nutritive value. Transgenic *L. corniculatus* plants harboring antisense dihydroflavonol reductase (AS-DFR) sequences have produced and analyzed (CARRON *et al.* 1994; ROBBINS *et al.* 1994; ROBBINS *et al.* 1998). In initial experiments the effect of introducing three different antisense *Antirrhinum majus* L. DFR constructs into a single recipient genotype (S50) was assessed. There were no obvious effects on plant biomass, but levels of condensed tannins showed a statistical reduction in leaf, stem, and root tissues of some of the antisense lines. In subsequent experiments a detailed study of AS-DFR phenotypes was carried out in genotype S33 using pMAJ2 (an antisense construct comprising the 5' half of the *A. majus* cDNA). In this case, reduced tannin levels were found in leaf and stem tissues and in juvenile shoot tissues. Analysis of soluble flavonoids and isoflavonoids in tannin down-regulated shoot tissues indicated few obvious default products. However, when two S33 AS-DFR lines were outcrossed, there was an underrepresentation of transgene sequences in progeny plants and no examples of inheritance of an antisense phenotype were observed.

Legume species characteristically accumulate phenylpropanoid phytoalexins under conditions of biological stress such as pathogen attack, wounding, etc. A number of groups have used legume callus and cell suspension

culture systems as models to study mechanisms controlling phytoalexin biosynthesis. Several species have been studied including alfalfa (DALKIN *et al.* 1990), white clover (GUSTINE 1981) and French bean (ROBBINS *et al.* 1985). When *A. rhizogenes* transformed root culture of *L. corniculatus* were treated with glutathione, isoflavan phytoalexins accumulated in both tissue and culture medium (ROBBINS *et al.* 1991; ROBBINS *et al.* 1995). This accumulation of phytoalexins was preceded by a transient increase in the activity of phenylalanine ammonia lyase (PAL). Elicitation of PAL occurred throughout the growth curve of *Lotus* hairy roots and in different sectors of transformed root material. While some workers have studied the action of elicitors in experiments on whole plant material, experiments using disorganized legume tissue cultures are undoubtedly of value.

A high frequency of transformation and regeneration protocol for *L. japonicus* has been achieved by utilizing *Agrobacterium*-mediated hypocotyl transformation (HANDBERG and STOUGAARD 1992; STILLER *et al.* 1997; THYKJAER *et al.* 1995). Transgenic plants of *L. japonicus* were regenerated by hypocotyl transformation using a *bar* gene as a selectable marker (Lohar *et al.* 2001). The *bar* encodes for phosphinothricin acetyl transferase that detoxifies phosphinothricin (PPT), the active ingredient of herbicides such as Ignite (AgrEvo) and Basta (Hoechst). Transgenic *L. japonicus* plants resistant to PPT were positive upon PCR by *bar* gene-specific primers. In five out seven independent lines tested, PPT resistance segregated as a single dominant allele indicating a single T-DNA insertion into the plant genome. All regenerated plants were fertile and void of visible somaclonal abnormalities contrary to 14% infertility when antibiotic selectable markers were used. The production of PPT herbicide-resistant *L. japonicus* plants may have significant commercial application in crop production.

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ロータス属植物の生物工学

新 関 稔

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要 約

マメ科の *Lotus* 属植物は地中海沿岸を起源とし、200近い種が世界各地に広がっている。その中で *L. corniculatus* L.(パーズフット・トレフォイル)はヨーロッパ、アジア、北アメリカに生育し、栄養価の高い牧草として栽培されている。また、地中に深く根を張ることから、ハイウェイの土手等のエロージョン防止に使用されたり、最近では黄色の花が一面に咲くことから花き植物としても用いられている。しかし、初期生育が悪く雑草との競争に弱く、さやが裂開しやすく採種が困難で

あったり、HCN を含む等の欠点を持つ。このような形質を改良するために交雑育種を中心とした改良が試みられてきたが、最近では生物工学的手法が用いられてきた。このような形質に対するソマクローナル変異や細胞融合技術の適用で成功した例は未だわずかであるが、将来展望は明るい。*L. japonicus* L. は生育期間が短く、ゲノムサイズも小さいので、マメ科のアラビドプシスと呼ばれ、*L. corniculatus* L. と共に形質転換の研究に用いられるようになり、さらに根粒菌との共生のメカニズム解明に盛んに用いられるようになってきた。そこで、ここでは我々のデータを入れて、この分野の最近の進歩を論じた。