Developing Herbicide Resistant Lentil (*Lens culinaris* Medikus subsp. *culinaris*) through *Agrobacterium* Mediated Transformation

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Abstract

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Lentil (*Lens culinaris* Medikus sub sp. *culinaris*) is an important food legume and is valued for its seeds with high protein content. Lentil is a weak competitor to weeds. High percentage of losses in seed yield is due to infestation. The production of herbicide-tolerant genetically modified crops has become a common practice. Weed transgenic soybean resistant to broad-spectrum herbicides was developed. Only very few attempts were undertaken to produce transgenic lentil. This study attempted to investigate the utility of introducing a herbicide tolerance gene into lentil. The plasmid construct pCGP1258, harboring the *bar* gene conferring resistance to the herbicide ammonium *glufosinate* and the *gus*A reporter gene, was inserted in *Agrobacterium tumefaciens* strain AgL0. Three lentil lines: ILL 5582, ILL 5883 and ILL 5588 were used for transformation. Experiments were carried out in a sterile culture (*In vitro*). High selection pressure of 20 mg/l of glufosinate was applied to the explants for 18 weeks. Survived shoots were grafted on non-transgenic rootstock. Plantlets were transferred to soil and acclimatized. The presence of the transgene was confirmed by the polymerase chain reaction (PCR) using specific primers. The functions of *bar* and *gus*A genes were assayed by painting with the herbicide and tissue staining, respectively. In this study we report the integration of *bar* gene in lentil and the production of transgenic plants resistant to ammonium glufosinate.

Key words: *Agrobacterium tumefaciens*, herbicide resistance, transformation.

Introduction

Lentil (*Lens culinaris* Medikus sub sp. *culinaris*) is the fifth most important pulse crop in the world and is mainly grown in the semi-arid regions particularly in the Indian subcontinent and dry areas of the Middle East. Global production of lentil has increased by over 100% during the past two decades to 4.17 million tones. Major increase in lentil production has been recorded from developed countries, which at present contribute 38% to the global output. As many as 51 countries cultivate lentil but Canada has emerged as the largest producer followed by India, Turkey, Australia, Nepal, Syria, Iran and Bangladesh. These countries contribute to 87 % of the global production. Syria grows lentil on about 143,000 ha area and produces 154,000 ton of grains (1, 9).

Weeds continue to have a major impact on crop production in spite of efforts to manage them. Lentil plant is a poor weed competitor, and is characterized by having short shoot and does not form a dense canopy until after flowering. Most annual grass and broadleaf weed species can compete effectively with lentil throughout the growing season. Reduction in seed yield due to weed competition was estimated to be 20 -30 %, and the critical period lies between 30–60 days after sowing (30).

Most commonly used broad-spectrum herbicides are glufosinate, glyphosate, bromoxynil, sulfonamides and sulfonylurea. Resistance to these herbicides depends upon the genes that have been inserted into the crop plant (4). Agrobacterium –mediated transformation is one of the most commonly employed methods for gene transfer to dicot plants (3). *Agrobacterium* is a soil-borne bacterium that causes the crown gall disease of many dicots. Virulent *Agrobacterium* strains are harboring a large plasmid 250 kilo base pair (kbp) known as tumor –inducing (Ti) plasmid, which is necessary for tumor formation. Tumor cells contain a fragment of Ti plasmid called transfer DNA (T-DNA). The T-DNA is flanked by 25 base pair (bp) repeats, which are the left and right borders. The T-DNA carries several genes conferring special properties to the tumor cells. Genes conferring virulence (*vir*) are located on the Ti plasmid and are necessary for T-DNA transfer (11, 29). The principle underlying the use of the Agrobacterium plasmid as a vector is that any gene placed between the right and left borders will be transferred to the plant genome.

The *bar* gene which is isolated from *Streptomyces hygroscopicus* codes for the Phosphinothricin-N-acetyltransferase enzyme (PAT) (22). The PAT enzyme inactivates the natural product phosphinothricin (PPT) or its synthetic version ammonium glufosinate, which is the active ingredient in herbicide formulations such as Basta™, Liberty™ and Herbiace™. The herbicide is detoxified through the acetylation of the PPT free amino group using acetyl coenzyme A as a cofactor that prevents PPT binding to the glutamine synthetase enzyme (5). Many Glufosinate resistant crops have been obtained by introducing *bar* gene through *Agrobacterium tumefaceins* mediated transformation or by particles bombardment (7). Sarker *et al.* (26) reported on the development of transgenic lentil plants by Agrobacterium mediation and Gulati *et al*. (12) through particle bombardment.

The optimization of *Agrobacterium tumefaciens*-plant interaction is probably the most important aspect to be considered. It includes the integrity of the bacterial strain, its correct manipulation and the study of its reaction in wounded plant tissue, which may develop in to a necrotic process in the wounded tissue or can affect the interaction and release of inducers or repressors of Agrobacterium virulence system. The type of explant is also an important factor and it must be suitable for regeneration allowing the recovery of whole transgenic plants. The establishment of a method for efficient regeneration of one particular species is crucial for its transformation.

The objectives of this study were to optimize Agrobacterium-mediated transformation in lentil and to develop plants tolerant to the herbicide ammonium glufosinate.

Material and Methods

Plant material

Seeds of three lentil lines: ILL 5582, ILL 5883 and ILL 5588 were surface-sterilized for 1 minute in 70% ethanol, then for 7 min in 2.5% sodium hypochlorite, and then rinsed for three times in sterile water. Swollen and decolorized seeds were discarded, thereafter 16 seeds were blotted on sterile filter paper in Petri dishes and 7 ml of sterile water was added. Seeds were then incubated for germination under dark conditions at 22-24 °C for two days (Figure 1-A).

Agrobacterium strain and plasmid

The wild Agrobacterium strain AgL0 was transformed with the binary vector pCGP1258 which harbors the *bar gene* encoding for phosphinothricin acetyl transferase (PAT) and the *gus*A gene, encoding for *β*-glucuronidase (GUS) used as reporter genes. Agrobacterium cells were maintained on solid Luria Broth Agar (LB) medium and selected with 50 mg/l of tetracycline. The bacterial culture was incubated over night at 28°C and 150 rpm in MG bacterial induction medium (1 L of MG contains: 5 g manitol, 1 g glutamic acid, 0.25 g potassium phosphate, 0.1 g sodium chloride, 0.1 g magnesium sulphate, 5 g tryptone, 2.5 g yeast, 0.001 mg biotin, the pH was adjusted to 7.0 and 50 mg tetracycline was added after autoclaving).

Overnight bacteria culture was diluted 1:10 in fresh MG medium and grown for 2–3 hours until an OD_{550nm} of 0.4–0.8 was reached. The bacterial cells were collected by centrifugation at 13000 rpm for 10 min at 4°C. The supernatant was discarded and pellet was resuspended in 1 ml MS medium (23), pH 5.7 supplemented with 10 mM glucose and 20 µM acetosyringone for *vir* gene induction, to reach 5×10^8 final concentration.

Transformation, shoot regeneration and selection

Two transformation methods based on yellow lupin (*Lupinus luteus*) transformation system were used (21):

(i) Two day-old lentil explants (small seedlings) were rescued after seed coats were removed. A fine syringe needle dipped in the bacterial culture was used for stabbing the explant apices, the buds under the scale leaves and the cotyledonary buds for 3-4 times.

(ii) The same procedures in method (i) were followed with a small modification. It consists of adding 0.5 µl of the bacterial culture on the apex after the stabbing, with high precaution to avoid the medium contamination.

The treated explants were arranged on plates with twenty five explants per plate, then were grown on already autoclaved medium (Figure 1-B), containing MS basal salts supplemented with B5 vitamins (1 mg/l nicotinic acid, 10 mg/l thiamin- HCl and 1 mg/l pyridoxin-HCl), 10 mg 6-benzylaminopurine (BAP), 1 mg naphthaleneacetic acid (NAA), 30 g/l sucrose and solidified with 0.3 % phytagel. The pH was adjusted to 5.7 before autoclaving for 20 min. at 120°C. The explants were transferred to new plates after 2-3 days with the same medium type but supplemented with 1 mg BAP and 0.1 mg NAA for shoot regeneration (Figure 1-C) under three cool white florescent lights. For the next steps, of regeneration, elongation and selection media (Figure 1-D, 1-E), 150 mg of ticarcillin were added to stop *Agrobacterium* growth.

Two weeks later, explants were transferred to a new elongation medium containing 500 mg calcium nitrate, 370 mg magnesium sulfate, 1 g potassium nitrate, 1 g ammonium nitrate, 300 mg potassium phosphate, 65 mg potassium chloride, 14 mg manganese sulfate, 13.2 mg iron stock (Fe-Na-EDTA), 3.8 mg zinc sulfate, 1.6 mg/l boric acid, 0.8 mg/l potassium iodide, 0.1 mg/l ammonium molybdate, 0.427 mg/l cupper sulfate, 100 mg/l myo-inositol, 2 mg/l nicotinic acid, 0.8 mg/l thiamin- HCl and 0.8 mg/l pyridoxin-HCl, 0.1 mg/l BAP, 0.01 mg/l NAA, 30 g sucrose and 0.3% phytagel. The elongated explants were divided after 2 weeks into two segments and cultivated on elongation medium supplemented with 20 mg/l phosphinothricin for selection. Surviving explants were transferred to fresh selection medium for 9 rounds with two weeks intervals (Figure 1-F). The total number of explants survived was scored in each phase.

Micro-grafting

Non- transgenic seeds were germinated on water-agar medium under dark condition at 24°C for five days. A V shaped notch about 2 mm deep was cut in the inter-node region in the seedlings. Selected putative transgenic shoots (over of 0.5 cm in length) were similarly trimmed at the base and inserted between the split of the seedlings (Figure 1-G). Two weeks later, the surviving grafted materials were transferred to soft agar medium containing MS salts, B5 vitamins, 20 g/l sucrose, 1 mg/l indol butric acid (IBA), and 0.4% agar (pH 7.0) for 2 weeks, all secondary shoots arose from rootstock were removed, then plants were transferred to pots containing soil, sand and peat moss (2:1:1) in the growth room under 16/8 h photoperiod, 175 µEinstein light intensity at 21/18°C. Initially the plants were covered with polyethylene bags, which were punctured after one week to reduce the atmospheric humidity, then removed after another week (Figure 1-H).

Test of GUS activity

Reporter genes are necessary to identify transformed cells or plants grown on selective medium. The *uid*A (*gus*A) gene, which encodes for *β*-glucuronodase enzyme is one of

the common genes used for that purpose. This enzyme can cleave the substrate X-gluc (5-bromo-4-chloro-3-indolyl β-D-glucuronic acid) resulting in the production of an insoluble blue color in those plant cells displaying GUS activity (16). For the GUS assay, 4 ml X-gluc (1mg/ml) was mixed with 6 ml GUS buffer [100 mM phosphate buffer, 10 mM Na_2 EDTA, 0.5 mM K_3 (Fe $(CN)_6$), 0.5 mM K_4 (Fe $(CN)_6$). $_3H_2O$]. Random samples of the explants after co-cultivation, detached leaflets and flowers from T_0 plants were tested; tissues were immersed in 200 µl of the above solution and incubated for 16 h at 37ºC. Green tissues were cleared in 70% ethanol (17), and checked for their color.

Test of ammonium glufosinate resistance

The herbicidal activity of PPT (ammonium glufosinate) is based on its inhibition of glutamine synthetase (GS) resulting in the rapid accumulation of intercellular ammonia, cessation of photorespiration and photosynthesis, and chloroplast disruption; therefore, plants dies within few days (7).

Resistant plants to PPT are produced when the *bar* gene is integrated into the plant genome. The gene product phosphinothricin acetyl transferase (PAT) catalyses the acetylating of the free amino group of PPT to yield *N*-acetyl-L-phosphinothricin, a compound that does not inactivate glutamine synthetase. Transformants were tested for the expression of *bar* gene by painting the upper surface of T_0 plants (plants derived from the micro propagation) with 600 mg/l PPT containing 0.1% Tween 20 as surfactant. Result was scored after 7 days.

Polymerase Chain Reaction (PCR) analysis

Genomic DNA was isolated from 0.3 g young leaves for PCR analysis according to the cetyl trimethyl ammonium bromide (CTAB) method of Doyle and Doyle (6) with some modifications. Leaves were macerated in 800 µl of CTAB buffer (3% CTAB, 1.4 M NaCl, 0.2% β-mercapto-ethanol, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0), 0.5% PVP), mixed and incubated for 30 min. at 60ºC, followed by adding 800 µl chloroform-isoamyl alchohol (24:1); the tube contents were gently mixed to avoid shearing of genomic DNA. The samples were then centrifuged for 10 min at 14,000 rpm. The aqueous (upper phase) was transferred to a clean microfuge tube and precipitated with 2/3 volume of pre-cold isopropanol. The DNA was pelleted by centrifugation as mentioned above. The supernatant was discarded and the pellet washed in 200 µl washing buffer (76% ethanol, 10 mM ammonium acetate). The buffer was removed and the pellet resuspended in TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA) supplemented with 10 mg/ml RNAse A, incubated for 30 min at 37ºC. Aliquots of 100 µl of 7.5 mM ammonium acetate and 750 µl ethanol were then added and mixed gently. The supernatant was discarded and the pellet was dried and resuspended in 200 µl of distilled water.

The primers used for the amplification of a specific *bar* sequence (264 bp) were 5'- GCAGGAACCGCAGGAG TGGA-3' and 5'- AGCCCGATGACAGCGACCAC-3'. 0.1 µM of each primer was used with 0.25 µg/genomic DNA, 1x PCR buffer (100 mM Tris- HCl, 500 mM KCl, 15 mM $MgCl_2$), 200 µM dNTPs and 0.8 unit of Taq DNA polymerase. The reaction conditions were 4 min for initial denaturation at 94ºC, then followed by 30 cycles for 90 sec denaturation (94ºC), 90 sec annealing (62ºC), 30 sec extension (72ºC), and finally a 5 min extension step at 72ºC.

Results

Tissue culture and transformation

Two methods were followed to optimize the transformation system in lentil. The protocol and its modification led directly to shoot formation without intermediate callus phase. High selective pressure of 20 mg/liter PPT was applied to the regenerated shoots for 18 weeks: In method "i" a total of 3470 small seedlings isolated from 3 lines of lentil were used. Only 7 explants survived for 6 rounds of selection (MFS6), no one has reached to or developed after nine rounds (MFS9). Therefore, the transformation efficiency was after 9 rounds of selection 0% (Table 1).

In method "ii" 0.5 µl of the bacterial culture was added on the apex of the stabbed seedlings cultured in the co-cultivation medium by the micropipette. Total of 1672 small seedlings detached from the 3 lines mentioned were used. Our modification increased plants survival after six round of selection to 22 explants instead of 7 and eventually, produced 3 independent putative transgenic explants (3 clones: a.1, b.1 and c.1). The transformation efficiency was different between lines. It was 0%, 0.11% and 0.43% for the lines line ILL 5588, ILL 5582 and ILL 5883, respectively (Table 1).

Rooting

After nine rounds of selection, the putative transgenic explants were cultured on the regeneration medium to induce the buds and to increase the shoots number. 33 shoots derived from the clone (a.1/ILL 5582) were developed, whereas the previous treatment was not able to increase the shoots number from the clones (b.1 and c.1/ ILL 5883). Eventually, a total of 39 putative transgenic shoots derived from three clones were grafted, 33 grafts succeeded and transferred to soft agar medium; only 14 plantlets developed in the pots, which correspond to 84.6% and 35.9% recovery, respectively (Table 2).

Histochemical assay

Transformation efficiency was determined in early stage after co-cultivation by the detection of GUS activity. Total of 712 of the co-cultivated embryos in method "i" and "ii" representing approximately 14% treated embryos were randomly picked up from the plates and immersed in solution of X-gluc and GUS buffer over night at 37ºC then blue colored embryos were scored.

In transformation method "i" total of 300,145 and 120 embryos of the lines ILL 5582, ILL 5883 and ILL 5588 were treated for *gus* expression. The result revealed the presence of GUS activity in the stabbing sites as extended blue dots. The transformation efficiency was 45.3% (ILL 5582), 73.1% (ILL 5883) and 61.7% (ILL 5588).

In method "ii" total of 96 and 51 embryos of the lines ILL 5582 and ILL 5883 were also treated for GUS. Our modification by adding 0.5 µl bacterial culture has

improved the ratio of gus gene expression to 75 and 78.4% in the same lines (Table 3). The blue color was distributed as fine dots in the whole apical meristem, and the dots were only detectable under the microscope. The same procedures to detect gus expression in the detached leaves and flowers of T_0 plants showed deep blue color in the leaflets and flowers (Figure 1-I and 1-J).

Assessment of herbicide resistance in T_0 plants

To evaluate the *bar* gene activity and the accumulation of PAT enzymes in transformed lentil, leaflets of untransformed and 13 transgenic plants were painted with 600 mg/l PPT (Figure 1-K and 1-L). One week after the herbicide application, leaflets of 6 plants ILL 5582 and 2 plants ILL 5883 showed complete tolerance to herbicide. However, leaflets of 4 transgenic plants and the untransformed plant were completely necrotic (Table 4).

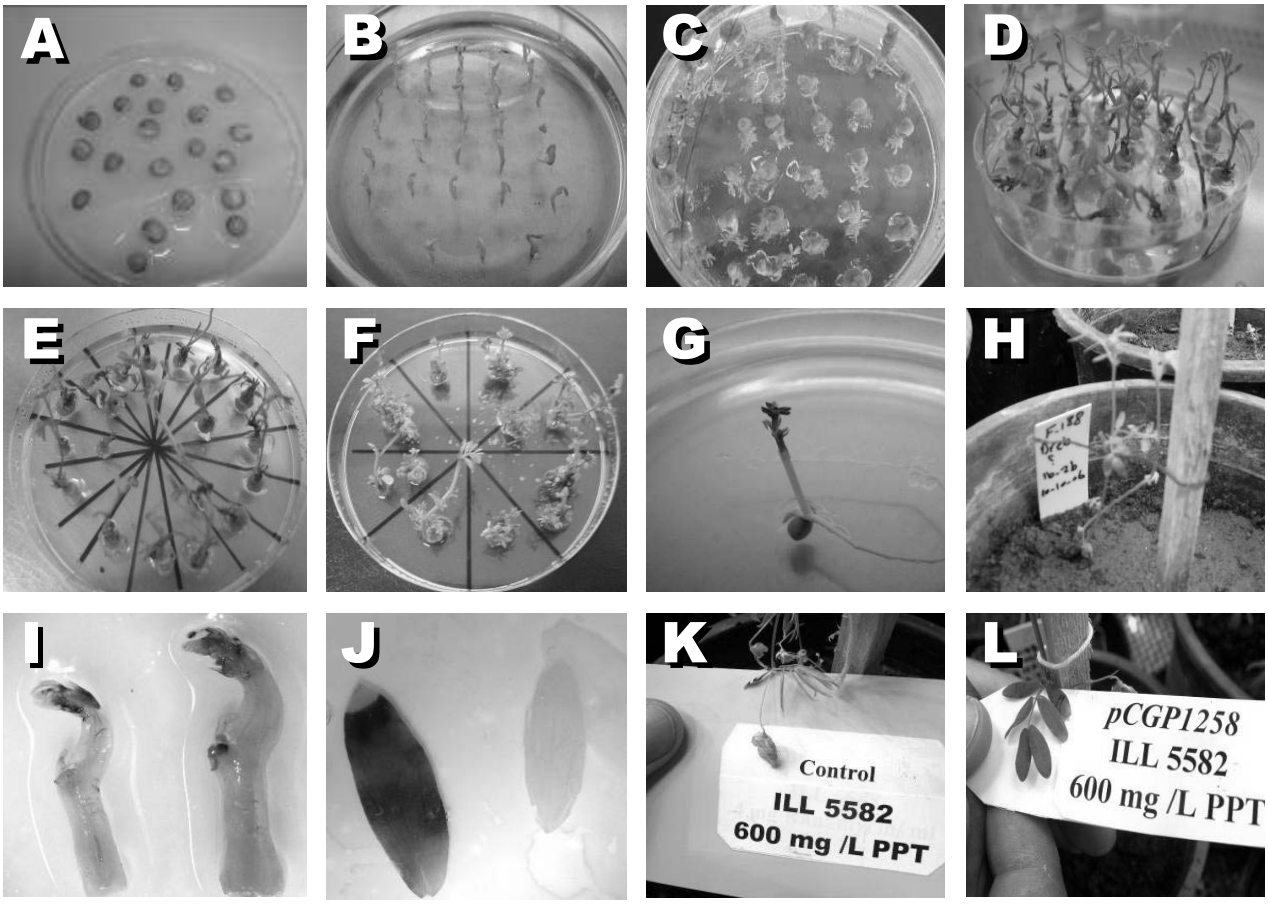

Figure 1. (A) Germination of lentil seeds, (B) Co- cultivated embryos, (C) Explants on regeneration medium, (D) Explants elongation on MF medium, (E) Selection on MFS medium supplemented with 20 mg/l phosphinothricin, (F) Putative transgenic explants, (G) Transformed shoots grafted on non-transgenic rootstocks, (H) Grafted shoots transferred to soil after acclimatization, (I and J) GUS activity in lentil embryos and leaflet, respectively, (K and L) Herbicide susceptible and resistant leaves, respectively.

Table 1. Total number of co-cultivated embryos and the transformation efficiency in three different lentil lines

Treatment	Line	Total number of co-cultivated embryos	MFS 6	No. of putative transgenic	Efficiency % **
Stabbing	ILL5582	1026	3	0	0.00
	ILL5883	611	1	0	0.00
	ILL5588	1833	3	1	0.01
Stabbing +A *	ILL5582	867	9	1	0.11
	ILL5883	456	13	2	0.43
	ILL5588	349	0	0	0.00

MF6 six rounds of selection

* Adding 0.5 µl of the bacterial suspension on the apex of the embryos

** The transformation efficiency was obtained by dividing the number of the independent events ×100 with the total number of co-cultivated embryos.

Table 2. Recovering T_0 plants from in vitro clones grafted on non-transgenic rootstock and confirmed by PCR.

Line	Clone	No. of grafted shoots	No. of successful grafts	No. of developed T_0 plants	No. of PCR positive plants for *bar*	No. of T_0 seeds
ILL 5582	a.1	33	27	9	6	58
ILL 5883	b.1	1	1	1	1	9
	c.1	5	5	4	1	75
ILL 5588	d.1	0	0	0	0	0
Total	4	39	33	14	8	142

Polymerase Chain Reaction (PCR)

All putative transgenic plants developed in the growth room were tested with the specific primers for the presence of the *bar* gene, represented by a DNA fragment of 294 bp (Table 2). Among the 14 plants, the expected 264 bp fragment was detected in 8 plants compatible with GUS-positive plants. Twenty three T_0 seeds of the clone a.1 and 11 of the clone b.1 were planted to analyze the T_1 progeny. PCR tests revealed the inheritance of the *bar* gene in 4 T_1 plants b.1/ILL 5883 but in none of the a.1/ILL 5582 and c.1/ILL 5883 clones (Figure 2).

Discussion

Generally legumes are considered recalcitrant to transformation (28) and this has slowed down the application of biotechnological tools in these crops. Nevertheless, transgenic soybean has been produced and *glyphosate* tolerant soybeans (Round up Ready) are successfully grown commercially (15).

Phosphinothricin (PPT) or ammonium glufosinate is a potent inhibitor of glutamine synthetase in plants (8, 19) and is available commercially as a non-selective herbicide. The *bar* gene which confers resistance to PPT, encodes the enzyme phosphinothricin acetyl transferase (PAT), which catalyzes the conversion of PPT to a nontoxic acetylated product (5). Furthermore, only few reports are available about the successful transfer of PPT resistance by *Agrobacterium*-mediated transformation to chickpea (20), bean (2), pea (25), faba bean (14) and soybean (31). A transgenic lentil resistant to sulfonylurea herbicides was produced by particle bombardment (12).

The transformation efficiency in lentil is still low. Only two papers described the recovery of transgenic shoots, one is based on *Agrobacterium* mediation (27), and the second on particles bombardment (12). The two methods proved to have similar transformation efficiency.

In this study, we have developed a transformation and regeneration systems for lentil and have introduced two foreign genes, *bar* and *gus*, in two lentil lines, ILL 5582 and ILL 5883. The protocol used in our first experiments was based on yellow lupin transformation procedures. This protocol was successful in yellow lupin and led to transgenic plants derived from six lines and showed a range of tolerance levels to PPT (21). But, the application of this procedure in lentil was not successful. We introduced some modifications consisted of adding 0.5 µl of bacteria culture of the transformed strain AgL0 to the apical meristem. This modification improved the transformation efficiency and was crucial to recover transgenic plants.

Table 3. Percentage of GUS – positive embryos after co-cultivation

Line	Treatment	Positive	Negative	Total	%
ILL 5582	Adding 0.5 µl b.c.	72	24	96	75
	Without adding	164	136	300	45.3
ILL 5883	Adding 0.5 µl b.c.	40	11	51	78.4
	Without adding	106	39	145	73.1
ILL 5588	Adding 0.5 µl b.c.	-	-	-	-
	Without adding	74	46	120	61.7
Total	-	428	284	712	-60.1

Table 4. Assessment of glufosinate resistance in T_0 plant leaves by painting with 600 mg/l PPT

Line	Clone	No. of tested T_0 plants	No. of resistant plants	No. of silent plants
ILL 5582	a.1	8	6	2
ILL 5883	b.1	1	1	0
	c.1	4	1	3
Total	3	13	8	5

The other problem limiting the production of transgenic plants is the rooting procedure. The *in vitro* multiplication of an individual lentil plant is limited by the difficulty to develop roots from the regenerated transformed shoots. Root induction in regenerated shoots has been conventionally achieved by increasing different auxins concentrations either alone or by a combination between auxins and cytokines. These treatments produced roots on 25% of the shoots, some times the roots were ineffective plantlets were transferred to soil (10, 18, 24, 27).

An alternative of the use of various hormone concentrations is the micrografting technique. Many plants can be produced from one regenerable clone. Micrografting was used successfully to produce roots in vitro in chickpea (20) and faba bean (14). High micrografting efficiency by using untransformed shoots of lentil was reported by Gulati *et al*. (13).

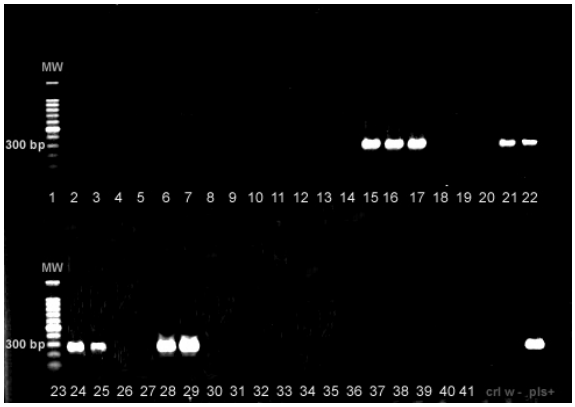

Figure 2. PCR analysis of some T_0 and T_1 transgenic plants: lanes 15 16, 17, 21, 22, 24, 25, 27 and 28: amplified 264 bp fragment of the bar gene, lanes 42, 43: negative control derived from wild type plant and water, the construct pCGP1258 served as positive control in lane 44.

In this study we optimized the micrografting of transgenic lentil shoots to be adequate to our system. A total of 39 putative transgenic shoots were grafted. About 85% of the regenerated shoots were developed successfully on rootstock.

14 plants (36%) derived from 4 clones survived and produced 142 seeds under growth room conditions. The histochemical assay revealed GUS expression in the co-cultivated embryos in the two transformation methods used during this study. In the method "I", based on stabbing, an extended blue spots were visualized in the stabbing sites, whereas in method "ii" fine blue dots were visible on the whole area of the apex, where bacteria was added to the meristem. In addition, the efficiency has increased when 0.5 µl of the bacterial culture was applied on the apical meristem. Eventually, the transformed shoots were only obtained from this modified method. Our finding is compatible with those of Sarker *et al*. (27).

The integration of the *bar* gene within the genomic DNA was confirmed by PCR. Specific primer pairs which amplify a 264 bp fragment were used for this purpose. All 14 putative transgenic plants were tested; only 7 plants were confirmed to be transgenic. *Bar* gene activity and accumulation of PAT enzyme was assessed by painting T_0 plant leaves with 600 mg/l PPT equivalent to 3 L/ha Basta; All plants assigned as PCR-positive proved to be herbicide resistant. Stable expression of GUS gene in T_0 plants was also visualized through histochemical staining in lentil leaves and flowers.

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الملخص

خطيب، فاتح، سمير قدسية، بيانكا غزال، جوان بارتون، هيساشي تسوجي موتو ومايكل باوم. 2007. تطوير نباتات عدس *Lens culinaris* Medikus sub sp. *culinaris* محورة وراثياً مقاومة لمبيد أعشاب غير اختياري باستخدام البكتيريا *Agrobacterium* كوسيط. مجلة وقاية النبات العربية، 25: 185-192.

يعدّ العدس *Lens culinaris* Medikus sub sp. *culinaris* أحد المحاصيل البقولية الحبية الغذائية المهمة، ويعود ذلك إلى احتواء بذوره على نسبة عالية من البروتين. تسبب الأعشاب الضارة فقداً كبيراً في غلة هذا المحصول نظراً لانخفاض قدرته التنافسية معها؛ إذ أنه لا يشكل غطاءً نباتياً كثيفاً إلا في مرحلة متأخرة من عمر النبات. نظراً لعدم وجود مبيدات أعشاب انتخابية على العدس، ولقلة البحوث المتعلقة بالتحوير الوراثي لهذا المحصول فقد هدف هذا البحث إلى إرساء طريقة مناسبة لتحوير العدس وراثياً وإكساب النباتات صفة المقاومة لمبيد الأعشاب ammonium glufosinate. تم استخدام البكتيريا *Agrobacterium tumefaciens* كوسيط في عملية التحوير، حيث حورت السلالة AgL0 من هذه البكتيريا لتحتوي على البلازميد المسمى pCGP1258 الذي يحمل المورثة *bar* التي تمنح صفة المقاومة لمبيد الأعشاب والمورثة *gus*A شائعة الاستخدام في الكشف عن الخلايا المحورة ولاحقاً عن النباتات المحورة وراثياً. استخدم في هذا البحث ثلاثة مدخلات من العدس وهي ILL 5588، ILL 5582 و ILL 5883، ونفذت التجارب بطريقة زراعة الأنسجة. تم انتخاب النباتات لمدة 18 أسبوعاً على وسط مغذي يحتوي الليتر منه على 20 مغ ammonium glufosinate. من ثم، تم تطعيم الأفرع الخضرية الناتجة على أصول بذرية غير محورة للحصول على جذورها، ثم نقلت النباتات إلى أصص تحتوي على تربة معقمة وتحت ظروف متحكم بها في غرفة الزراعة بهدف تقسيتها. استخدم التفاعل التسلسلي البوليميرازي PCR للتأكد من نجاح عملية انتقال المورثة *bar* إلى النباتات الناتجة (المحورة)، وتم تقييم عمل هذه المورثة وظيفياً من خلال دهن الأوراق بالمبيد ammonium glufosinate، حيث أبدت الأوراق مقاومة لهذا المبيد. كما تم تقويم نشاط المورثة *gus*A عن طريق تلون أنسجة الأوراق والأزهار باللون الأزرق الناتج عن تفاعل الأنزيم *β*-glucuronidas (GUS)، الذي تشفر له هذه المورثة مع مادة X-gluc التي تضاف لوسط التفاعل. لقد استطعنا أن نؤكد ادماج المورث *bar* في نبات العدس وانتاج نبات مقاوم لمبيد ammonium glufosinate.

كلمات مفتاحية: بكتيريا التدرن التاجي، التحوير الوراثي، مقاومة مبيدات الأعشاب.

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