Scientific Research Publishing

American Journal of Plant Sciences, 2019, 10, 1-11
http://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Production of Transgenic *Camelina sativa* Plants via *Agrobacterium*-Mediated Transformation of Shoot Apical Meristems

Viji Sitther[1*], Behnam Tabatabai[1], Oluwatomisin Enitan[1], Somayeh Gharaie Fathabad[1], Sadanand Dhekney[2]

[1]Department of Biology, Morgan State University, Baltimore, MD, USA
[2]University of Wyoming, Sheridan Research and Extension Center, Sheridan, WY, USA
Email: *viji.sitther@morgan.edu

How to cite this paper: Sitther, V., Tabatabai, B., Enitan, O., Fathabad, S.G. and Dhekney, S. (2019) Production of Transgenic *Camelina sativa* Plants via *Agrobacterium*-Mediated Transformation of Shoot Apical Meristems. *American Journal of Plant Sciences*, **10**, 1-11.
https://doi.org/10.4236/ajps.2019.101001

Received: October 23, 2018
Accepted: January 1, 2019
Published: January 4, 2019

Abstract

A method to produce transgenic *Camelina sativa* plants in cvs. PI650159 and PI650161 was developed. Micropropagated shoot meristem cultures were established from *in vitro* germinated seedlings and used as target tissues for *Agrobacterium*-mediated transformation. A plasmid harboring enhanced green fluorescent protein, *β* glucuronidase and neomycin phosphotransferase II genes were used to optimize parameters for transgenic plant production. Kanamycin at 40 $mg \cdot l^{-1}$ was effective in suppression of non-transformed cells while permitting growth of transgenic tissues. Shoot apical meristems co-cultivated with *Agrobacterium* exhibited stable enhanced green fluorescence protein (EGFP) and *β* glucuronidase (GUS) expression after culture on plant regeneration medium. We observed transformation efficiencies of 53.33% in cv. PI650159 and 98.33% in cv. PI650161. The presence of transgenes in both cultivars was confirmed by PCR, while quantitative real-time PCR detected single copy integration in Pl650161 and two copy integration in Pl650159. Transgenic plants exhibited EGFP and GUS expression in all tissues including shoots, leaves, buds, floral organs, seeds, and pods. Our results demonstrate a simple and efficient technique using apical shoot meristems for production of transgenic *C. sativa* plants that can be used for transfer of desirable traits.

Keywords

Genetic Engineering, Green Fluorescent Protein, Micropropagation, Oilseed

1. Introduction

Of the various sources of renewable energy, oilseed crops have emerged as one

of the most promising platforms for biodiesel production during the past few decades. *Camelina sativa* (L.) Crantz, a member of the Brassicaceae family, has gained great importance as a biofuel crop due to its important agronomic attributes. With oil content in seeds ranging from 38% - 43%, and the vast majority of fatty acids (>90%) being polyunsaturated [1] [2], biodiesel derived from *C. sativa* is well described. Both seed oil and biodiesel produced from the species are extensively tested and the fuel used in engine trials with promising results [3] [4]. Since the plant is established as a potential biofuel feedstock, it has been extensively tested for fatty acid composition and oil profiles, as well as crop improvement efforts to enhance agronomic qualities such as drought resistance met with success [5] [6].

Genetic transformation provides an avenue to incorporate important traits for varietal improvement and requires efficient *in vitro* regeneration and gene delivery systems to generate large number of transformants from which improved individuals can be selected. This process serves as an important tool for studying gene function and expression in addition to enhancing single traits such as disease resistance and stress tolerance. Selection of transformants is a crucial step in genetic transformation. In positive selection systems, the selectable marker gene encodes for an enzyme conferring resistance to a specific toxic substrate that enable the growth of the transformed tissues while killing the non-transformed ones. Of the 50 selection marker genes described for genetic plant transformation, the genes neomycin phosphotransferase II (*nptII*) and hygromycin phosphotransferase (*hpt*), conferring resistance to kanamycin and hygromycin respectively, and bargene encoding resistance to herbicide phosphinothricin are commonly employed [7]. In addition, reporter or non-selectable marker genes used as components of the plasmid constructs allow detection of the putative transformed cells, which are visualized using β-Glucuronidase (GUS) expression [8].

The development of *in vitro* somatic hybridization [9] [10] and plant regeneration from leaf explants [11] have led to an *Agrobacterium*-mediated genetic transformation system in *C. sativa* [12]. Vacuum-infiltration of flowers to transform *C. sativa* [13] has been successful and this method has been adapted to increase lipid production in seeds [14] [15]. A number of tissue culture-based *Agrobacterium* transformation protocols using *C. sativa* leaf segments, petioles and hypocotyls as explants have been reported as well [16] [17]. We recently demonstrated the use of *C. sativa in vitro* shoots as suitable tissues for *Agrobacterium*-mediated transformation [18]. Such methods have been successfully used in *Vitis vinifera* for the development of transgenic plants [19] [20].

In this study, we describe a simple and efficient transformation system for *C. sativa* using apical meristems and nodal segments from micropropagated cultures. Enhanced Green Fluorescent Protein (EGFP) and GUS assays were used for selection of transgenic explants. Transformation efficiency in the cultivars was tested and stable integration of the gene was confirmed by polymerase chain

reaction (PCR). Finally, copy number of the gene in selected clones was confirmed using quantitative real-time PCR.

2. Materials and Methods

2.1 Establishment of Micropropagation Cultures

Camelina regeneration medium (hereafter known as CR medium) was used for micropropagation of cultures and consisted of Murashige and Skoog (MS) salts and vitamins [21], 30 $g \cdot l^{-1}$ sucrose, 6.6 μM BAP and 2.6 μM NAA. Medium pH was adjusted to 5.8 before adding 7.0 $g \cdot l^{-1}$ TC Agar (Phytotechnology Laboratories LLC, Shawnee Mission, KS, USA). The medium was autoclaved at 121˚C and 15 psi for 20 min, cooled down to 55˚C and 25 ml medium was dispensed in each 100 × 16 mm petri dish. Micropropagated cultures of *C. sativa* cultivars PI650159 and PI650161 were established from seeds, which were briefly immersed in 70% ethanol and transferred 25% commercial bleach solution containing one drop Tween 20. Seeds were surface-sterilized by constant agitation for 15 min in the solution, followed by two 5 min rinses with sterile distilled water, blotted dry and transferred to CR medium. Plates were incubated in the dark at 25˚C for 48 h and then placed in an Adaptis plant growth chamber (Conviron, Winnipeg, CA, USA) under white light (75 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 25˚C ± 2˚C and 16 h light/8 h dark photoperiod. Following seed germination, shoot apical meristems from a single seedling were used to establish micropropagation cultures, which were increased in mass by transfer of proliferating shoot tips and nodes to fresh CR medium at 2 week-intervals.

2.2. Determination of Kanamycin Sensitivity

In order to optimize kanamycin concentrations that inhibited growth of non-transformed cells while allowing for selection of transgenic cells, cultures were tested in CR medium containing kanamycin concentrations of 5, 10, 15, 20, 40, 80, and 100 $mg \cdot l^{-1}$. Filter-sterilized kanamycin at various concentrations was added to autoclaved CR medium cooled to 55˚C. Additionally, 50 $mg \cdot l^{-1}$ cefotaxime was added to all treatments to study potential inhibitory effects of the antibiotic on culture regeneration. Four shoot tips (10 mm) were transferred to each petri dish, and each treatment replicated thrice for all antibiotic concentrations. Cultures grown in the absence of kanamycin served as control. Cultures were grown in conditions mentioned above, and explant weight and appearance recorded after 4 weeks on CR medium. The experiment was repeated once. Data was analyzed using analysis of variance (ANOVA) and Tukey's honest significant differences post hoc test to determine significance of mean separation between treatments. The single factor fixed effect ANOVA model, $Y_{ij} = \mu + \alpha K_i + \varepsilon_{ij}$, was used where Y is the explant weight when exposed to kanamycin concentration i and biological replicate j. The μ represents overall explant weight with adjustments from the effects of kanamycin concentration (αK), and ε_{ij} is the experimental error from concentration i and biological replicate j.

2.3. *Agrobacterium*-Mediated Transformation

A binary vector containing the enhanced green fluorescent protein (*egfp*), *β* glucuronidase and neomycin phosphotransferase II (*nptII*) genes under the control of a CaMV 35S promoter was used to optimize transformation parameters [18]. The binary plasmid was transferred to *Agrobacterium tumefaciens* "EHA 105" by freezing in dry ice, thawing at 25˚C and used in transformation studies (**Figure 1**). *Agrobacterium* containing the binary vector was cultured overnight on an orbital shaker (180 rpm) at 26˚C in 30 ml liquid MG/L medium [22] containing 20 $mg \cdot l^{-1}$ rifampicin and 100 $mg \cdot l^{-1}$ kanamycin. The bacterial solution was centrifuged at 2200 ×g for 8 min and the supernatant discarded. The pellet was resuspended in liquid CR medium, optical density adjusted to 0.2 at 600 nm (OD_{600}), cultured for an additional 4 h, and used for co-cultivation. Shoot apical meristems from rapidly growing micropropagation cultures were excised and immersed in *Agrobacterium* culture for 7 min. Explants were then transferred to solid CR medium and co-cultivated for 2 days at 26˚C in dark.

2.4. Growth of Transformed Cultures

After 2 days of co-cultivation, explants were transferred to petri dishes containing solid CRck40 (CR medium containing 50 $mg \cdot l^{-1}$ cefotaxime and 40 $mg \cdot l^{-1}$ kanamycin) and maintained in conditions mentioned above for 2 weeks. Transgenic cultures were identified on the basis of EGFP fluorescence, GUS histochemical staining assay and kanamycin resistance, and separated from non-transformed cultures. Resulting putative transgenic cultures were transferred to fresh CRck medium every 10 days for 8 weeks. Transient transgenic status of shoot cultures was screened for EGFP and GUS expression.

2.5. Analyses of Gene Expression

Transient EGFP expression in transformed cultures was detected using a Zeiss Stemi SV11 microscope (Carl Zeiss AG., Oberkochen, Germany) with an X-Cite 120 fluorescence illumination system (X-Cite, Quebec, Canada). Characteristic green fluorescent emission observed in co-cultivated explants was scored as transient expression. Transient GUS activity in co-cultivated explants was determined by an enzymatic reaction with X-Gluc (5-bromo-4-chloro-3-indolyl glucuronide) substrate. Co-cultivated explants were incubated in 1.5 ml microcentrifuge tubes containing 500 µl GUS staining solution (10 mM sodium phosphate pH 7.0, 10 mM EDTA, 10% Triton X-100, 1 mM potassium ferricyanide, and 2 mM X-Gluc) at 37˚C in dark for 24 h and transferred into 95% ethanol for

Figure 1. The transformation vector used in the study had a duplicated CaMV 35S promoter that drives the expression of a GUS and the GFP/NPT II genes in a divergent orientation.

24 h to remove chlorophyll. Non-transformed shoot tips were used as a negative control. Transient GUS expression was recorded as the percentage of explants that exhibited a blue stain versus the total number of explants detected by a Zeiss Stemi SV11 microscope (Carl Zeiss AG., Oberkochen, Germany). After 4 weeks of culture on regeneration medium, leaves from transgenic shoots were used to analyze EGFP and GUS expression as described above.

2.6. Transgenic Plant Recovery

Shoots 3.0 cm or longer were transferred to CR medium containing 0.4 $mg{\cdot}l^{-1}$ NAA and 16 $mg{\cdot}l^{-1}$ kanamycin for rooting (CRT). Plants with a well-developed shoot and root system were transferred to sterile Promix BX (A. H. Hummert Seed Co., St Louis, MO, USA) and acclimatized in a clear plastic dome under conditions of high humidity for 3 weeks. Plants were enriched with liquid 2:2:2 (NPK) fertilizer at weekly intervals and ultimately transferred to a greenhouse. Transgenic seeds were collected and EGFP fluorescence detected as mentioned above.

2.7. Analysis of Transgene Integration

Genomic DNA was extracted from six transgenic plant lines (three from each cultivar) using the Qiagen DNeasy plant extraction kit (Qiagen, Valencia, CA, USA). PCR and quantitative real-time PCR was performed to confirm transgene presence and copy number in transgenic plants. A forward primer EG-51 (5'-ATGGTGAGCAAGGGCGAGGAGCTGT-3') and a reverse primer EG-32 (5'-CTTGTACAGCTCGTCCATGCCGAGA-3') were used to amplify a 717 bp DNA fragment from the *egfp*/*nptII* fusion gene. Conditions for PCR reactions were: 95˚C for 4 min, 40 cycles at 94˚C for 1 min, 58˚C for 1 min, 72˚C for 1 min and a final cycle at 72˚C for 4 min [23]. DNA from a non-transformed plant was used as a negative control. PCR products were run on a 0.6% agarose gel and DNA bands were observed with a UV transilluminator.

Transgene copy number was determined using quantitative real-time PCR. Assays were carried out in an ABI QuantStudio 12K instrument equipped with a 96-well plate and CopyCaller™ Software (Life Technologies, Carlsbad, CA, USA). Oligonucleotide primers for amplification of a 340-bp target fragment from the *egfp* gene included a forward primer ERT-51 (5'-CCATCCTGGTCGAGCTGGAC-3'), a reverse primer ERT-32 (5'-TTCAGCTCGATGCGGTTCAC-3'), and a probe (5'-FAM-GCAAGCTGACCCTGAAGTTC-MGB-3'). All reactions were carried out in a 20 µl final volume containing 4 µl sample DNA (total of 20 ng), 10 µl of 2× Taqman genotyping master mix, 1 µl of 20× Taqman assay mix (containing primers and probe), 1 µl of 20× Taqman copy number reference assay and 4 µl sterile water. Real-time PCR reaction samples were replicated 4 times for each line tested. For accurate gene expression, the *C. sativa* actin gene primer-probe set (fp: 5'-ACAATTTCCCGCTCTGCTGTTGTG-3', rp: 5'-AGGGTTTCTCTCTTCCACATGCCA-3',

probe: 5'-VIC-TGTTTCAAACGCTCTATCCCTCGCTC–MGB-3') was used as reference. Quantitative real-time PCR conditions were as follows: 95˚C for 10 min followed by 40 thermal cycles of 95˚C for 15 s and 60˚C for 1 min. Fluorescence signals were analyzed by the method utilized by CopyCaller™ software (Life Technologies, Carlsbad, CA, USA). This analysis method utilized cycle threshold (Ct) values to extrapolate initial concentration of target DNA in each sample.

3. Results

3.1. Kanamycin Sensitivity

The effect of increasing kanamycin levels on growth and proliferation of transformed and non-transformed shoots was determined by measuring fresh weight after 2 weeks of cultivation. At 5 $mg \cdot l^{-1}$ and 10 $mg \cdot l^{-1}$ kanamycin, we observed an initial inhibitory effect, however, shoots eventually outgrew this effect after 2 weeks (data not shown). By contrast, kanamycin over a concentration of 40 $mg \cdot l^{-1}$ caused complete necrosis and inhibited growth of explants within 3 weeks, resulting in a significant decrease in average weight of explants in both the cultivars. While the weight of explants grown in the absence of kanamycin was 0.20 ± 0.029 g in cv. PI650159, explants grown in 40 $mg \cdot l^{-1}$ kanamycin measured 0.06 ± 0.005. Similar results were observed in cv. Pl650161 where control explants measured 0.17 ± 0.019 g compared to 0.05 ± 0.005 g in those exposed to 40 $mg \cdot l^{-1}$ kanamycin (Table 1).

3.2. Transformation of Apical Shoot Cultures and Seeds as Monitored by EGFP and GUS Expression

Shoot tips that expressed EGFP produced a bright green fluorescence when observed under a microscope equipped with epi-fluorescence illumination (Figure 2(a) and Figure 2(b)). We also observed distinct EGFP expression in all putatively transgenic seeds and pods (Figure 2(c) and Figure 2(d)). In addition, GUS staining of 60 transformed explants from each cultivar revealed transient transformation efficiencies of 53.33% and 98.33% for Pl650159 and Pl650161

Table 1. Effect of kanamycin concentrations on average explant tissue weight of *Camelina sativa* cultivars PI 650159 and PI650161.

Kanamycin concentration (mg/l)	Average explant fresh weight (g) of *Camelina sativa* culivars	
	Pl650159	Pl650161
0	0.19[a]	0.17[a]
20	0.11[b]	0.05[b]
40	0.09[b]	0.05[b]
80	0.09[b]	0.06[b]
100	0.07[b]	0.07[b]

[a]Mean weights for three independent biological replicates were calculated. Identical letters followed by values denote no significant difference between treatments within the same column ($P > 0.05$).

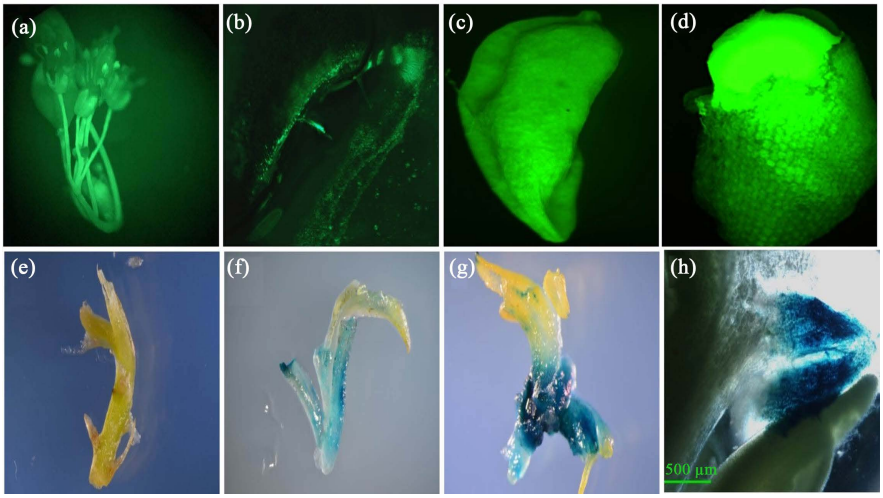

Figure 2. Overview of putative transgenic enhanced green fluorescence protein (EGFP) and *β*-glucuronidase (GUS) expression in *Camelina sativa* in plant tissues. (a) EGFP-positive shoot and floral organs; (b) EGFP-positive spots on the surface of leaf tissue; (c) EGFP-positive seed pods; (d) EGFP-positive expression in seeds; (e) GUS histochemical staining assay of untransformed shoot; (f) GUS-positive apical meristem; (g) GUS expression in apical meristems; (h) GUS expression in leaf tissue.

respectively (Table 2), represented by blue stain on explants (Figures 2(e)-(h)). Complete leaf areas including veins were substantially and uniformly stained in the transformed tissues. In addition, we detected significant GUS expression in the stems as well. On the contrary, no GUS activity was observed in non-transformed explants. Additionally, transgenic cells carrying these marker genes selectively grew on culture medium containing kanamycin while inhibiting the growth of non-transformed cells.

3.3. Molecular Analysis of Transformants

Genomic DNA extracted from the transgenic and control plant lines were amplified and tested using gene specific primers. PCR revealed the expected plasmid-encoding fragment of 717 bp in all EGFP positive transgenic plants, while DNA from the negative control did not. These results indicated that all lines expressing EGFP contained the gene (Figure 3). Molecular analysis using quantitative real-time PCR demonstrated variable copies of the *egfp* transgene. A single copy integration of the gene was confirmed in a cv.Pl650161 line. In cv. Pl650159 the lowest copy number detected was two, while another line of this cultivar had six copies and the remaining line contained a high copy number of the transgene (>10 copies) (Table 3).

3.4. Acclimatization

Explants of both cultivars in CRT medium produced roots in 3 - 4 weeks. All regenerated plantlets that were transferred to PROMIX survived. The *in vitro* propagated shoots started forming new leaves within 2 - 3 weeks after transferring them to pots containing PROMIX.

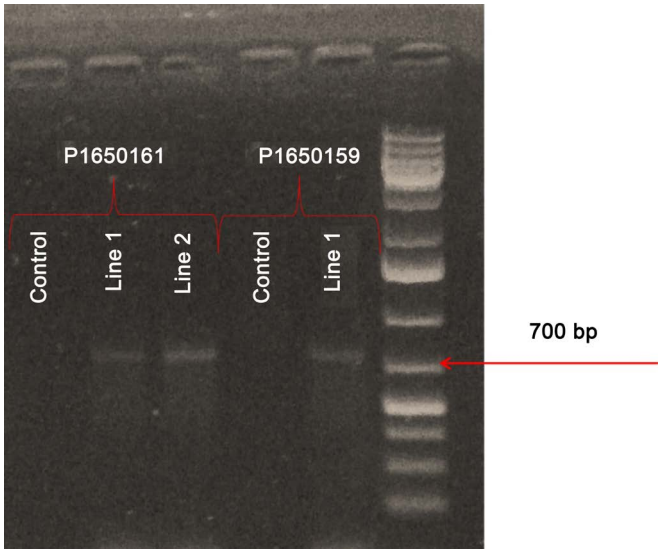

Figure 3. Detection of transgenic *Camelina sativa* by PCR analysis of genomic DNA containing the *egfp/nptII* fusion gene. Amplification was carried out using *egfp* specific primers for a 717 bp gene fragment. Genomic DNA from non-transformed explants served as negative control.

Table 2. *β*-Glucuronidase (GUS) expression in shoot tip explants of *Camelina sativa* cultivars PI 650159 and PI650161.

Cultivar	No. of explants tested	No. of GUS positive transgenic explants	Transformation efficiency (%)
Pl650159	60	32	53.33
Pl650161	60	59	98.33

Table 3. Transgene copy number in *Camelina sativa* cultivars PI 650159 and PI650161 determined by quantitative real-time PCR.

Cultivar	Line	Ref μC_T[a]	μC_T	$\mu\Delta C_T$	$\Delta\Delta C_T$	Copy Number[b]
Pl650159	1	22.136	20.774	−1.36233	−1.0613	2
	2	22.136	17.706	−4.42967	−4.12864	17.5
	3	22.136	19.305	−2.83067	−2.52964	6
Pl650161	1	22.136	24.219	2.083333	2.384363	0
	2	22.136	30.27	5.422333	5.723363	0
	3	22.136	21.755	−0.38133	−0.0803	1

[a]CSActin was used as the endogenous reference gene; [b]Average of three technical replicates.

4. Discussion

Biotechnological approaches that involve an effective gene transfer system, accurate selection of transformants, and recovery of transgenic plants are central requisites for crop improvement. It is imperative that efficient *in vitro* regeneration and gene delivery systems are well-established for the large-scale production of transformants from which improved individuals can be selected. While ex-

plant sources such as flowers [24] and cotyledons [25] are used for *C. sativa* genetic transformation, difficulty in regeneration and ease of transforming and testing several cultivars is a limitation. We report for the first time, the use of apical meristematic tissue as targets for *Agrobacterium*-mediated transformation in *C. sativa*. Rapid proliferation and generation of a large amount of starting material for transformation in a very short time was facilitated using shoot meristems as an explant source. Although, shoot apical meristem cells have been used for genetic transformation in other plant species [19] [26], there has been no previous report on apical shoot transformation of an *egfp*/*nptII* reporter-marker fusion in *C. sativa*. Active proliferation in these cells resulted in formation of transgenic plants in a relatively short period of time.

Our method to produce transgenic plants from apical meristematic explants was assisted by the inclusion of the *nptII* gene, which allowed the determination of optimal kanamycin concentrations since non-transformed cells die due to high sensitivity and lack of phosphotransferase to detoxify the antibiotic. Apical meristems screened in CR media amended with 40 $mg \cdot l^{-1}$ kanamycin aided in selection of successful transformants and reduced the percentage of escapes (**Table 1**). While we observed chlorophyll impairment in 20 $mg \cdot l^{-1}$ kanamycin, concentrations above 40 $mg \cdot l^{-1}$ proved effective in selection of transformed explants. Two weeks after exposure of cultures to kanamycin, non-transformed shoots were bleached resulting in death of tissues, while kanamycin resistant putative transgenic plants were green. The fresh weight of explants was also significantly reduced in kanamycin-containing media, indicating that a combination of the reporter gene *egfp* along with selectable marker *nptII* allowed for efficient screening and recovery of transformants. The *egfp*/*nptII* plasmid has been successfully employed in the transformation of several apical meristems of plant species including citrus [27] and embryogenic culture transformation in *Vitis rotundifolia* [23]. In the present study, we have established an improved protocol for enhancing culture growth and plant regeneration in transformed *C. sativa* explants by optimizing parameters. We observed that *Agrobacterium* culture adjusted to an OD_{600} of 0.2, co-cultivation interval of 48 h, and cefotaxime concentration of 50 $mg \cdot l^{-1}$ significantly enhanced explant survival.

Results of EGFP and GUS expression assays indicate high transient transformation efficiency in both cultivars (**Table 2**), while presence of the fusion gene in *C. sativa* genomic DNA was confirmed by PCR (**Figure 3**). Quantitative PCR confirmed stable, single copy integration of the transgene in cv. Pl650161 and two copy integration in cv. Pl650159 (**Table 3**). In addition, we also observed EGFP expression in the F1 generation seeds which indicates that integration of the gene is heritable (**Figure 2(c)** and **Figure 2(d)**). These results have paved the way to incorporation of value-added traits in the *C. sativa* genome. The ease in producing *in vitro* micropropagation cultures makes development of a shoot tip-based transformation system attractive, while the ability to transform *C. sativa* allows the rapid introduction of novel traits in this emerging crop. In subsequent studies, *C. sativa* will be transformed with genes for abiotic stress toler-

ance and qualitative traits for successful cultivation in marginal lands.

Acknowledgements

This work was supported in part by a grant from the USDA NIFA (Award # 2016-67032-25007) Research and Extension Experiences for Undergraduates (REEU) Program. We thank the Plant Introduction Unit at USDA-ARS for providing *Camelina sativa* seeds used in this study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this article.

References

[1] Budin, J., Breene, W. and Putnam, D. (1995) Some Compositional Properties of *Camelina* (*Camelina sativa* L. crantz) Seeds and Oils. *Journal of the American Oil Chemists' Society*, **72**, 309-315. https://doi.org/10.1007/BF02541088

[2] Gugel, R.K. and Falk, K.C. (2006) Agronomic and Seed Quality Evaluation of *Camelina sativa* in Western Canada. *Canadian Journal of Plant Science*, **86**, 1047-1058. https://doi.org/10.4141/P04-081

[3] Bernardo, A., Howard-Hildige, R., O'Connell, A., Nichol, R., Ryan J., Rice, B., Roche, E. and Leahy, J. (2003) *Camelina* Oil as a Fuel for Diesel Transport Engines. *Industrial Crop and Product*, **17**, 191-197. https://doi.org/10.1016/S0926-6690(02)00098-5

[4] Frohlich, A. and Rice, B. (2005) Evaluation of *Camelina sativa* Oil as a Feedstock for Biodiesel Production. *Industrial Crop and Product*, **21**, 25-31. https://doi.org/10.1016/j.indcrop.2003.12.004

[5] Choudhury, S.R., Riesselman, A.J. and Pandey, S. (2014) Constitutive or Seed-Specific Overexpression of Arabidopsis G-Protein γ Subunit 3 (AGG3) Results in Increased Seed and Oil Production and Improved Stress Tolerance in *Camelina sativa*. *Plant Biotechnology Journal*, **12**, 49-59. https://doi.org/10.1111/pbi.12115

[6] Lee, S.B., Kim, H., Kim, R.J., and Suh, M.C. (2014) Overexpression of *Arabidopsis* MYB96 Confers Drought Resistance in *Camelina sativa* via Cuticular Wax Accumulation. *Plant Cell Reports*, **33**, 1535-1546. https://doi.org/10.1007/s00299-014-1636-1

[7] Miki, B. and McHugh, S. (2004) Selectable Marker Genes in Transgenic Plants: Applications, Alternatives and Biosafety. *Journal of Biotechnology*, **107**, 193-232. https://doi.org/10.1016/j.jbiotec.2003.10.011

[8] Jefferson, R.A., Kavanagh, T.A. and Bevan, M.W. (1987) GUS Fusions: Beta-Glucuronidase as a Sensitive and Versatile Gene Fusion Marker in Higher Plants. *The EMBO Journal*, **6**, 3901-3907. https://doi.org/10.1002/j.1460-2075.1987.tb02730.x

[9] Narasimhulu, S.B., Kirti, P.B., Bhatt, S.R., Prakash, S. and Chopra, V.L. (1994) Intergeneric Protoplast Fusion between *Brassica carinata* and *Camelina sativa*. *Plant Cell Reports*, **13**, 657-660. https://doi.org/10.1007/BF00232940

[10] Sigareva, M.A. and Earle, E.D. (1999) Camalexin Induction in Intertribal Somatic hybrids between *Camelina sativa* and Rapid-Cycling *Brassica oleracea*. *Theoretical and Applied Genetics*, **98**, 164-170. https://doi.org/10.1007/s001220051053

[11] Tattersall, A. and Millam, S. (1998) Establishment and *in Vitro* Regeneration Studies of the Potential Oil Crop Species *Camelina sativa*. *Plant Cell Tissue Organ Culture*, **55**, 147-150. https://doi.org/10.1023/A:1006132407886

[12] Kuvshinov, V., Kanerva, A., Koivu, K., Kuvshinova, S. and Pehu, E. (2009) Transformation System for *Camelina sativa.* Patent # 12/290,379, USA.

[13] Liu, L., Liu, Z., Bai, H. and Sun, D.D. (2012) Concurrent Filtration and Solar Photocatalytic Disinfection/Degradation Using High-Performance Ag/TiO_2 Nanofiber Membrane. *Water Resources*, **46**, 1101-1112.

[14] Petrie, J.R., Shrestha, P., Belide, S., Kennedy, Y., Lester, G., Liu Q., Divi, U.K., Mulder, R. J., Mansour, M.P., Nichols, P.D. and Singh, S.P. (2014) Metabolic Engineering of *Camelina sativa* with Fish Oil-Like Levels of DHA. *PLoS ONE*, **9**, e85061. https://doi.org/10.1371/journal.pone.0085061

[15] Zhu, Y., Xie, L., Chen, G.Q., Lee, M.Y., Loque, D. and Scheller, H.V. (2018) A Transgene Design for Enhancing Oil Content in *Arabidopsis* and *Camelina* Seeds. *Biotechnology for Biofuels*, **11**, No. 1. https://doi.org/10.1186/s13068-018-1049-4

[16] Kanerva, A., Koivu. K., Kuvshinov. V., Kuvshinova, S. and Eija, P. (2011) Transformation in *Camelina sativa*. Patent # US7910803B2, USA.

[17] Yemets, A.I., Boychuk, Y.N., Shysha, E.N., Rakhmetov, D.B. and Blume, Y.B. (2013) Establishment of *in Vitro* Culture, Plant Regeneration, and Genetic Transformation of *Camelina sativa. Cytology and Genetics*, **47**, 14-20. https://doi.org/10.3103/S0095452713030031

[18] Sitther V., Tabatabai, B., Enitan, O. and Dhekney, S. (2018) *Agrobacterium*-Mediated Transformation of *Camelina sativa* for Production of Transgenic Plants. *Journal of Biological Methods*, **5**, e83. https://doi.org/10.14440/jbm.2018.208

[19] Dutt, M., Li, T., Dhekney, A. and Gray, J. (2007) Transgenic Plants from Shoot Apical Meristems of *Vitis vinifera* L. "Thompson Seedless" via *Agrobacterium*-Mediated Transformation. *Plant Cell Reports*, **26**, 2101-2110. https://doi.org/10.1007/s00299-007-0424-6

[20] Li, T., Dhekney, A., Dutt, M. and Gray, J. (2008) An Improved Protocol for *Agrobacterium*-Mediated Transformation of Grapevine (*Vitis vinifera* L.). *Plant Cell, Tissue and Organ Culture*, **93**, 311-321. https://doi.org/10.1007/s11240-008-9378-9

[21] Murashige, T. and Skoog, F. (1962) A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. *Physiologia Plantarum*, **15**, 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

[22] Dhekney, A., Li, T., Dutt, M. and Gray, J. (2012) Initiation and Transformation of Grapevine Embryogenic Cultures. In: *Transgenic Plants*, Humana Press, New York, 215-225. https://doi.org/10.1007/978-1-61779-558-9_18

[23] Dhekney, A., Li, T., Zimmerman, W. and Gray, J. (2009) Factors Influencing Genetic Transformation and Plant Regeneration of *Vitis*. *American Journal of Enology and Viticulture*, **60**, 285-292.

[24] Lu, C. and Kang, J. (2008) Generation of Transgenic Plants of a Potential Oilseed Crop *Camelina sativa* by *Agrobacterium*-Mediated Transformation. *Plant Cell Reports*, **27**, 273-278. https://doi.org/10.1007/s00299-007-0454-0

[25] Haggins, K.J., Potlakayala, S., Hussain, I., Josekutty, P.C. and Rudrabhatla, S. (2011) Improving Stress Tolerance in *Camelina sativa*. *NSF-REU Symposium*, Penn State Harrisburg.

[26] Barlass, M. and Skene, K.G.M. (1978) *In Vitro* Propagation of Grapevine (*Vitis vinifera* L) from Fragmented Shoot Apices. *Vitis*, **17**, 335-340.

[27] Dutt, M. and Grosser, J.W. (2009) Evaluation of Parameters Affecting *Agrobacterium*-Mediated Transformation of Citrus. *Plant Cell Tissue Organ Culture*, **98**, 331-340. https://doi.org/10.1007/s11240-009-9567-1