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DIRECCIÓN DE NUEVAS CREACIONES**

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Abogado
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ASUNTO	Radicación	09-111673
	Trámite	011
	Evento	378
	Actuación	519
	Oficio	
	Folios	006

16515

Patente de Invención	☒	Patente de Modelo de Utilidad	☐
Vía Nacional	☒	Cap. I	☒
Vía PCT (fase nacional)	☒	Cap. II	☐

No. Solicitud Internacional PCT/IB2007/053412
Fecha de Presentación Internacional 27/Agosto/2007

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 40 a 63	08/10/2009
Reivindicaciones:	Folios 65 a 67	08/10/2009
Dibujos:	Folios 69 a 70	08/10/2009
Prioridad:	RM2007A000129	14/03/2007

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486), y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Planta de tabaco mutagenizada como cultivo de semillas para la producción de aceite para usos energéticos, industriales y alimenticios."

El problema planteado en esta solicitud consiste en suministrar materia prima eficiente para la obtención de biodiesel y otros suplentes a partir de semillas de tabaco

Para resolver este problema técnico la solicitud en estudio proporciona plantas

Expediente 09-111673 Primer Artículo 45

capaces de producir grandes cantidades de semillas para obtener aceite.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C12N 15/82; A01H 1/06

4. Excepción a la Patentabilidad (Art 20 D 486 literal (c))

El objeto de las reivindicaciones 1 a 6, 12 no es patentable de acuerdo con el Art citado.

El objeto de las reivindicaciones 9 a 10 no es patentable de acuerdo con el Art citado, las etapas de los métodos reclamados corresponden a procesos esencialmente biológicos como son la obtención de plantas a través de cruces y la germinación de semillas

5. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicaciones 7, 8 y 13 no es patentable, de acuerdo con el Art 14.

6. De la Solicitud de Patente

Título

Se sugiere al solicitante modificar el título ya que las plantas no son objeto de patente según el Art. 20 literal c, ni tampoco los usos bajo el Art 14 y 21 D 486.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 11 no es clara por que el método no especifica la estructura de los vectores de transformación. El solicitante debe proporcionar el tipo de vector y la secuencia exacta recombinante que es introducida en dicho vector. Además, se informa al solicitante que el literal g corresponde a un proceso esencialmente biológico por lo que no es patentable según Art 20 D 486 literal c.

Las reivindicaciones 18 a 23 no son claras por que no caracterizan la materia a ser patentada, los aceites extraídos, complementos alimenticios y biodiesel deben ser caracterizados por sus componentes y proporciones, de manera que sea claro cual es el aporte del inventor.

Descripción (Art 28 D 486)

La Descripción no divulga la invención de manera suficientemente completa para que el la persona del oficio normalmente versada en la materia pueda ejecutarla (ó reproducirla), la descripción hace referencia a el biodiesel caracterizado por ser totalmente compuesto por aceite de tabaco (folio 53) sin embargo para que dicha materia sea protegida debe encontrarse correctamente caracterizada, por sus componentes y proporciones. De igual manera deben ser caracterizados los complementos alimenticios de los folio 53 y 54.

Por otra parte la descripción menciona plantas transformadas con vectores que codifican enzimas de interés para modificar la composición y cantidad de aceite producido por la semilla de la planta (folio 48) sin embargo no divulga cuales son los vectores usados ni cuales las secuencias recombinantes introducidas, de manera que la invención carece de divulgación suficiente y sustento.

7. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en fecha de presentación de la solicitud de prioridad: 14/03/2007, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	EP0257993	Nucleic acid fragment encoding herbicide resistant plant acetolactate synthase.	02/Marzo/1988
D2	Plant Cell Physiol. 43(12): 1518-1525	Chloroplast Transformation with Modified accD Operon Increases Acetyl-CoA Carboxylase and Causes Extension of Leaf Longevity and Increase in Seed Yield in Tobacco	2002
D3	Howtopedia	How to Process Oilseed on a Small Scale	2006
D4	Biomass and Bioenergy 28 77-86	Use of tobacco seed oil methyl ester in a turbocharged indirect injection diesel engine	2005

D1 divulga un fragmento de ácido nucleico que codifica una acetolactato sintasa que proporciona resistencia a herbicidas. Este fragmento de ácido nucleico contiene al menos una mutación de nucleótidos que resulta en un cambio de aminoácido en una de las siete regiones de homología conservadas sustancialmente. Esta mutación da como resultado la producción de una proteína acetolactato sintasa que confiere mayor resistencia a los compuestos herbicidas de sulfonilurea en comparación con la proteína de tipo silvestre.

<http://www.freepatentsonline.com/EP0257993.pdf>

D2 da a conocer plantas de tabaco que contienen genes que codifican para Acetyl-CoA Carboxilasa que se encuentra relacionado con el metabolismo de ácidos grasos de la semilla, y evalúa la producción de dichos compuestos en la planta transformada y en su progenie.

<http://pcp.oxfordjournals.org/content/43/12/1518.full.pdf>

D3 divulga el proceso de extracción de aceite de semillas <http://en.howtopedia.org/?title=How to Process Oilseed on a Small Scale&oldid=2035>

D4 divulga las características y producción de biodisel que procede de aceite de semillas de tabaco que ha sido trans-esterificado con metanol

<http://www.sciencedirect.com/science/article/pii/S0961953404001217>

8. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La reivindicación 11 se refiere a un "Método según las reivindicaciones 9 o 10 que

comprende también los siguientes pasos:

e) la transformación de las plantas obtenidas en el paso d) con vectores que incluyen casetes de expresión que expresan los genes de la planta para resistencia a enfermedades de insectos, herbicidas y / o hongos y la selección de las plantas si transformadas en las generaciones T0-T4 para resistencia a enfermedades de insectos, herbicidas y / o hongos y / o;

f) la transformación de las plantas obtenidas en el paso d) con uno o varios vectores que comprenden casetes de expresión que expresan en los genes de semillas del metabolismo de ácidos grasos seguido de la selección de plantas transformadas en las generaciones T0-T4 para características tales como el contenido total de aceite de las semillas y la composición de ácidos grasos de las mismas;

g) cruce de las plantas obtenidas en las etapas e) y / o f). y la selección de la progenie resultante para una o mas de las características seleccionadas en el grupo: alta capacidad de producción de semillas en una cantidad superior a 40 quintales por hectárea. Contenido de aceite de semillas mayor al 42%, el título de yodo en el aceite obtenido de las semillas de dicha progenie inferior a 120, composición de ácidos grasos del aceite contenido en dichas semillas variable con respecto a la planta de tipo silvestre, la resistencia a insectos, resistencia a los herbicidas, la resistencia a los hongos.

Para los literales e y g:

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1
Plantas de tabaco transformadas	Plantas de tabaco transformadas
Vectores que expresan los genes de la planta para resistencia a enfermedades de insectos, herbicidas y / o hongos	Contienen genes de resistencia a herbicidas introducido mediante vectores
Obtención de la progenie de dichas plantas transformadas	Evaluación de dicha resistencia en la progenie obtenida de las plantas transformadas

Las características **esenciales** de la reivindicación 11 habían sido divulgadas previamente en D1, el cual da a conocer plantas de tabaco que contienen genes de resistencia a herbicidas introducido mediante vectores, y la evaluación de dicha resistencia en la progenie obtenida de las plantas transformadas (ver resumen y pagina 40 línea 26 a 28). En consecuencia, la reivindicación 11, literales e y g, no es nueva según lo divulgado en el documento D1.

Para los literales f y g:

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D2
Plantas de tabaco transformadas	Plantas de tabaco transformadas
Vectores que expresan los genes relacionados al metabolismo de ácidos grasos	Contienen genes que codifican para Acetyl-CoA Carboxilasa
Obtención de la progenie de dichas plantas transformadas	Evalúa la producción de dichos compuestos en la planta transformada y en su progenie

Las características **esenciales** de la reivindicación 11 habían sido divulgadas previamente en D2, el cual da a conocer plantas de tabaco que contienen genes que codifican para Acetyl-CoA Carboxilasa que se encuentra relacionado con el metabolismo de ácidos grasos de la semilla, y evalúa la producción de dichos compuestos en la planta transformada y en su progenie (ver página 1519 columna

izquierda primer párrafo). En consecuencia, la reivindicación 11, literales f y g, no es nueva según lo divulgado en el documento D2.

La reivindicación 14 se refiere a un "Un método para la extracción de aceite de las semillas de tabaco en el que el rendimiento de aceite se encuentra entre el 70 y el 95% del aceite contenido en dichas semillas, que comprende los siguientes pasos:
a) la extracción de dicho aceite mecánicamente presionando con la prensa helicoidal y la obtención de aceite y una torta de aceite residual;
b) la filtración de dicho aceite extraído en el paso a) con filtros de papel o tela."

Comparación de las características técnicas esenciales, con las del Estado de la Técnica:

Características esenciales	D3
Método de extracción de aceite de semillas	Método de extracción de aceite de semillas
Extracción por presionar la semilla en una prensa	Extracción por presionar la semilla en una prensa
Filtración del aceite	Filtración del aceite

Las características esenciales de la reivindicación 14 habían sido divulgadas previamente en D3, el cual da a conocer un método para la extracción de aceite de semillas, que hace parte del saber popular, en el cual las semillas pasan a través de una prensa donde el aceite es exprimido y recolectado por una perforación en una cubeta bajo la maquina y la torta residual sale por el otro extremo del eje (ver página 3 ultimo párrafo). El aceite crudo expulsado contiene partículas sólidas, dichas partículas pueden ser eliminadas permitiendo que el aceite en repose y luego se filtre a través de una tela fina (ver página 4 párrafos 2 y 3). Además, D2 divulga la extracción por solvente. Los aceites de semillas o la torta restante de expulsión se extracción con disolventes del aceite remanente de la torta residual, y después el aceite es recuperado por una destilación al vacío (página 3 primer párrafo literal e), por ultimo divulga la refinación de el aceite obtenido para hacerlo apto para el consumo humano (página 2 último párrafo). En consecuencia, la reivindicación 14, no es nueva según lo divulgado en el documento D3. Las reivindicaciones dependientes 15 a 18 no revelan características esenciales nuevas para el método, por lo cual no se consideran novedosas.

La reivindicación 19 se refiere a un "Biodiesel que comprende el aceite de semillas de tabaco, según la reivindicación 12 trans-esterificado con metanol.

Comparación de las características técnicas esenciales, con las del Estado de la Técnica:

Características esenciales	D4
Biodiesel que comprende el aceite de semillas de tabaco trans-esterificado con metanol	Biodiesel que comprende el aceite de semillas de tabaco trans-esterificado con metanol

Las características esenciales de la reivindicación 19 habían sido divulgadas previamente en D3, el cual da a conocer biodisel que procede de aceite de semillas de tabaco que ha sido trans-esterificado con metanol (página 79 columna derecha párrafo dos). En consecuencia, la reivindicación 19, no es nueva según lo divulgado en el documento D3.

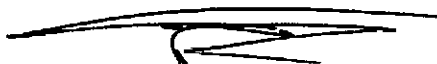
9. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	EP0257993	11	Novedad
D2	Plant Cell Physiol. 43(12): 1518-1526	11	Novedad
D3	Howtopedia	14 a 18	Novedad
D4	Biomass and Bioenergy 28 77- 86	19	Novedad

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha
18 SET. 2012

Elaborado por: Sandra Carolina Crespo Cárdenas
Revisado Por: Consuelo Leguizamón Leguizamón
Aprobado por: José Luis Salazar López

Chloroplast Transformation with Modified *accD* Operon Increases Acetyl-CoA Carboxylase and Causes Extension of Leaf Longevity and Increase in Seed Yield in Tobacco

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Acetyl-CoA carboxylase (ACCase) in plastids is a key enzyme regulating the rate of *de novo* fatty acid biosynthesis in plants. Plastidic ACCase is composed of three nuclear-encoded subunits and one plastid-encoded *accD* subunit. To boost ACCase levels, we examined whether overexpression of *accD* elevates ACCase production. Using homologous recombination, we replaced the promoter of the *accD* operon in the tobacco plastid genome with a plastid rRNA-operon (*rrn*) promoter that directs enhanced expression in photosynthetic and non-photosynthetic organs, and successfully raised the total ACCase levels in plastids. This result suggests that the level of the *accD* subunit is a determinant of ACCase levels, and that enzyme levels are in part controlled post-transcriptionally at the level of subunit assembly. The resultant transformants grew normally and the fatty acid content was significantly increased in leaves, but not significantly in seeds. However, the transformants displayed extended leaf longevity and a twofold increase of seed yield over the control value, which eventually almost doubled the fatty acid production per plant of the transformants relative to control and wild-type plants. These findings offer a potential method for raising plant productivity and oil production.

Keywords: Acetyl-CoA carboxylase (EC 6.4.1.2) — Fatty acid synthesis — Leaf longevity — Post-transcriptional regulation — Seed yield — Tobacco.

Abbreviations: ACCase, acetyl-CoA carboxylase; NEP, nuclear-encoded RNA polymerase; PEP, plastid-encoded RNA polymerase.

Introduction

Plant oil is one of the most important products of photosynthetic carbon assimilation and has a variety of industrial applications. One possible way to boost oil production is to manipulate a key enzyme involved in fatty acid biosynthesis. In plants, *de novo* fatty acid synthesis occurs in plastids, and plastidic acetyl-CoA carboxylase (ACCase; EC 6.4.1.2) catalyzes

the first committed, rate-limiting step of fatty acid biosynthesis: the formation of malonyl-CoA. Therefore, increasing ACCase levels may result in an increase in fatty acid production. Plants have two forms of ACCase: a heteromeric ACCase in plastids that is similar to *Escherichia coli*-type ACCase, and a homomeric ACCase in the cytosol that is similar to animal-type ACCase (Sasaki et al. 1993, Konishi and Sasaki 1994). Because membranes are impermeable to acyl-CoAs including malonyl-CoA (Jacobson and Stumpf 1972, Banhegyi et al. 1996), and the plastidic ACCase plays an exclusive role in the biosynthesis of malonyl-CoA in plastids. A recent attempt to target *Arabidopsis* animal-type ACCase into the plastids of transgenic oilseed rape achieved an approximately 5% increase in the total seed-oil content (Roesler et al. 1997). However, successful manipulation of the *E. coli*-type plastidic ACCase is yet to be achieved.

Plastidic ACCase is composed of biotin carboxylase, the biotin carboxyl carrier protein, and the alpha and beta subunits of carboxyltransferase, the genes of which are designated *accC*, *accB*, *accA*, and *accD*, respectively (Sasaki et al. 1995, Ohlrogge and Browse 1995). The *accD* gene is located in the plastid genome and the other three genes occur in the nuclear genome. Neither antisense-expression nor overexpression of *accC* significantly affects ACCase levels, although a moderate decrease and an elevation in *accC* mRNA levels were achieved with these techniques, respectively (Shintani et al. 1997). We hypothesized that the expression of *accD* in plastids might limit the total levels of plastidic ACCase, and that the overexpression of *accD* might increase ACCase expression, and hence fatty acid production, in plants. Recent progress in plastid transformation by homologous recombination (Svab and Maliga 1993, Bock 2001, Maliga 2002) provided us with an opportunity to examine our hypothesis in tobacco.

In plastids, two types of RNA polymerases are involved in transcription: nuclear-encoded RNA polymerase (NEP) and plastid-encoded RNA polymerase (PEP) (Hajdukiewicz et al. 1997). In general, housekeeping genes such as *accD* have NEP promoter(s), whereas photosynthetic genes have PEP promoter(s). Interestingly, the rRNA operon (*rrn*), which is abundantly expressed in both photosynthetic and non-

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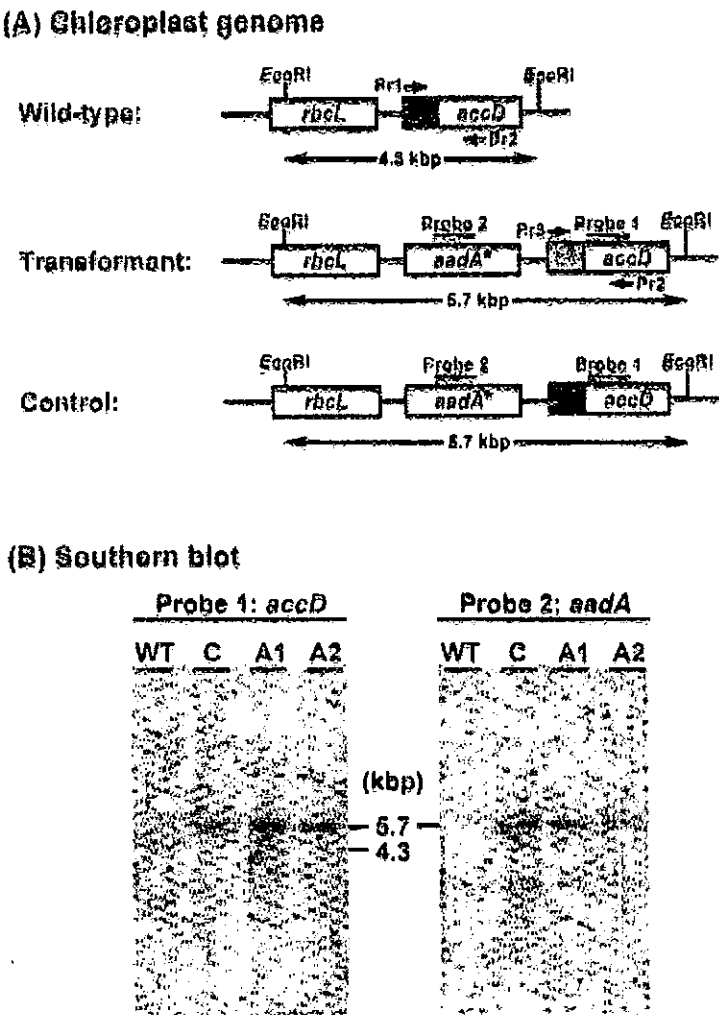


Fig. 1 Southern blot analysis. (A) Chloroplast genome structure in the wild-type, transformant, and control plants. Pr1, Pr2, and Pr3 indicate the positions of the PCR primers used for the selection of homoplasmic transformants (see text). Bars indicate the positions of probes 1 and 2 used in (B). (B) Total cellular DNA extracted from leaves of the wild-type and T₁ plants was digested with *Eco*RI and subjected to Southern blot analysis. WT, the wild type; C, control (with *aadA** insertion); A1 and A2, transgenic plants (with insertion of an *aadA**-*rrn* cassette). The sizes of the detected bands were the same as those predicted in (A).

photosynthetic organs, has both PEP and NEP promoters (Vera and Sugiura 1995). In the plastid genome of tobacco, *accD* forms an operon with *psaI*, *yef4*, *oemA*, and *peta* (Shinozaki et al. 1986) and these genes are polycistronically transcribed under the control of the NEP-type promoter of *accD* (Shinozaki et al. 1986, Nagano et al. 1991, Hajdukiewicz et al. 1997). Therefore, to overexpress *accD* in all tissues, we replaced the promoter of *accD* with that of *rrn* in the tobacco plastid genome by homologous recombination, and examined effects of the modification on ACCase level and fatty acids. Our results show that the promoter replacement not only increased the expression of *accD* but also enhanced the level of ACCase and fatty acid content in leaves of the resultant transgenic tobacco. Furthermore, the transformants displayed significantly extended leaf longevity, and improved seed production and hence seed oil per plant.

Results

Production of transgenic tobacco plants overexpressing plastidic ACCase

To replace the promoter of *accD* with that of *rrn* by homologous recombination, the plasmid *prbcL-aadA-rrn-accD* was constructed, in which the *accD* promoter was replaced with a *rrn* promoter, and a chimeric gene (*aadA**) (Svab and Maliga 1993) was inserted upstream of the *accD* promoter. *aadA** is a selectable marker gene that contains a modified *rrn* promoter and a *psbA* terminator, so that only cells with *aadA** inserted into the plastid genome can be selected for spectinomycin resistance (Svab and Maliga 1993). A control plasmid was constructed, with an *aadA** insertion upstream of the *accD* promoter. These plasmids were inserted into the tobacco plastid genome by particle bombardment (Fig. 1A)



Fig. 2 Northern blot analysis. Total cellular RNA extracted from young leaves (leaf maximum width 3 cm) of 12-week-old plants (see Fig. 5A) was subjected to Northern blot analysis. Tobacco cDNAs for *accD* and *accC* mRNAs were labelled as hybridization probes. *rRNA* was used as the loading control. Abbreviations are the same as in Fig. 1B.

(Svab and Maliga 1993, Book 2001). Because tobacco leaves have 500–10,000 copies of the plastid genome per cell (Bendich 1987), all transformants were made homoplasmic with respect to the transgenes.

Two independent T_0 transformants, in which the *aadA**-*rrn* cassette was inserted into the desired position, were selected by polymerase chain reaction (PCR) and designated A1 and A2. These transformants produced no wild-type *accD* band (data not shown) by PCR with primers Pr1 and Pr2 (see Fig. 1A), verifying that they are homoplasmic. Similarly, a control T_0 transformant, in which only the *aadA** was inserted into the wild-type genome, was established. All transformants grew normally under our growth conditions and their seeds germinated normally. Southern blot analysis verified that all T_1 progeny inherited the respective transgenes (Fig. 1B). Inheritance of the inserted *rrn* promoter in the T_2 progeny of the A1 and A2 transformants was also confirmed by PCR with primers Pr3 and Pr2 (data not shown).

Effects of *accD* overexpression on ACCase levels

Northern blot analysis of total cellular RNA from T_1 leaves (Fig. 2) showed that the control plants accumulated not only high levels of *aadA* mRNA but also significant levels of *accD* mRNA when compared with wild-type plants. Therefore, the insertion of *aadA** upstream from *accD* seems to slightly enhance *accD* mRNA levels, because the *rrn* promoter of *aadA* may affect expression of *accD*. *accD* mRNA accumulated significantly more in A1 and A2 plants compared with control and wild-type plants, indicating that the replaced *rrn* promoter functions well. However, the mRNA levels of nuclear-encoded *accC* were similar in the wild-type and transformant plants, indicating that *accC* mRNA levels were not affected under these conditions.

Immunoblot analysis showed that the levels of the four subunits comprising plastidic ACCase in total leaf proteins increased significantly in the control, and more extensively in

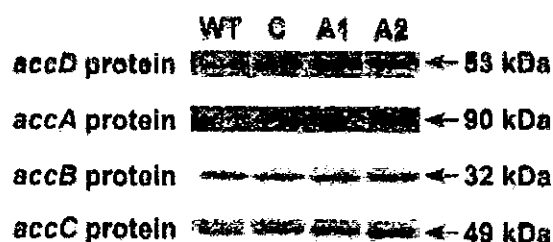


Fig. 3 Immunoblot analysis. The same amounts of total leaf protein (10 µg) from young leaves (leaf maximum width 3 cm) of 12-week-old plants shown in Fig. 5A were loaded in each lane. Abbreviations are the same as in Fig. 1B.

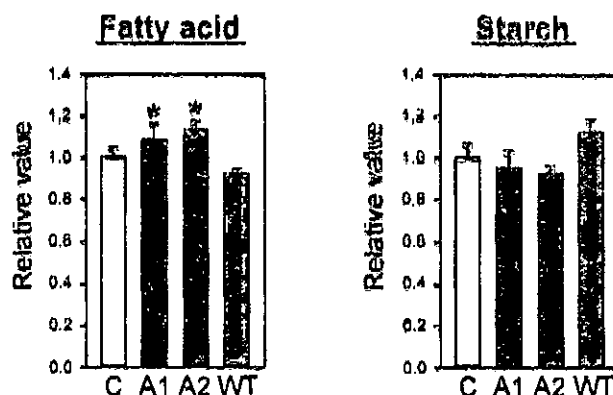


Fig. 4 Fatty acid and starch contents in leaves. Mature green leaves (leaf maximum width 7 cm) from the 12-week-old plant shown in Fig. 5A were used for analysis. Values are based on fresh weight and are given as the averages ($\pm$ SE) of four determinations made from two plants. An asterisk indicates that the values for A1 and A2 are significantly different ($P < 0.05$) from those of the control and wild-type plants, according to a *t*-test. Fatty acid and starch contents were 3.9 and 182 µg (mg FW)⁻¹ of leaves, respectively, for the control. Abbreviations are the same as in Fig. 1B.

the A1 and A2 transformants, compared with the wild-type (Fig. 3). The level of plastid-encoded subunit increased with its transcript level, but the levels of nuclear-encoded subunits increased without a corresponding increase in their transcripts, which suggests involvement of post-transcriptional regulation in expression of the nuclear-encoded genes. The specific activity of ACCase in the chloroplasts of young leaves of A1 and wild-type plants was 63 and 17 pmol min⁻¹ (mg protein)⁻¹, respectively, indicating that the four increased subunits were assembled into an active enzyme in the chloroplasts.

Effects on fatty acid contents in leaves

Lipid analysis showed that the fatty acid content of A1 and A2 leaves increased by approximately 5–10% over that of the control (Fig. 4), and these differences are statistically significant according to a *t*-test ($P \leq 0.05$). In A2 leaves, the content of monogalactosyldiacylglycerol, the major chloroplast membrane lipid, increased by about 30% above that of the control,

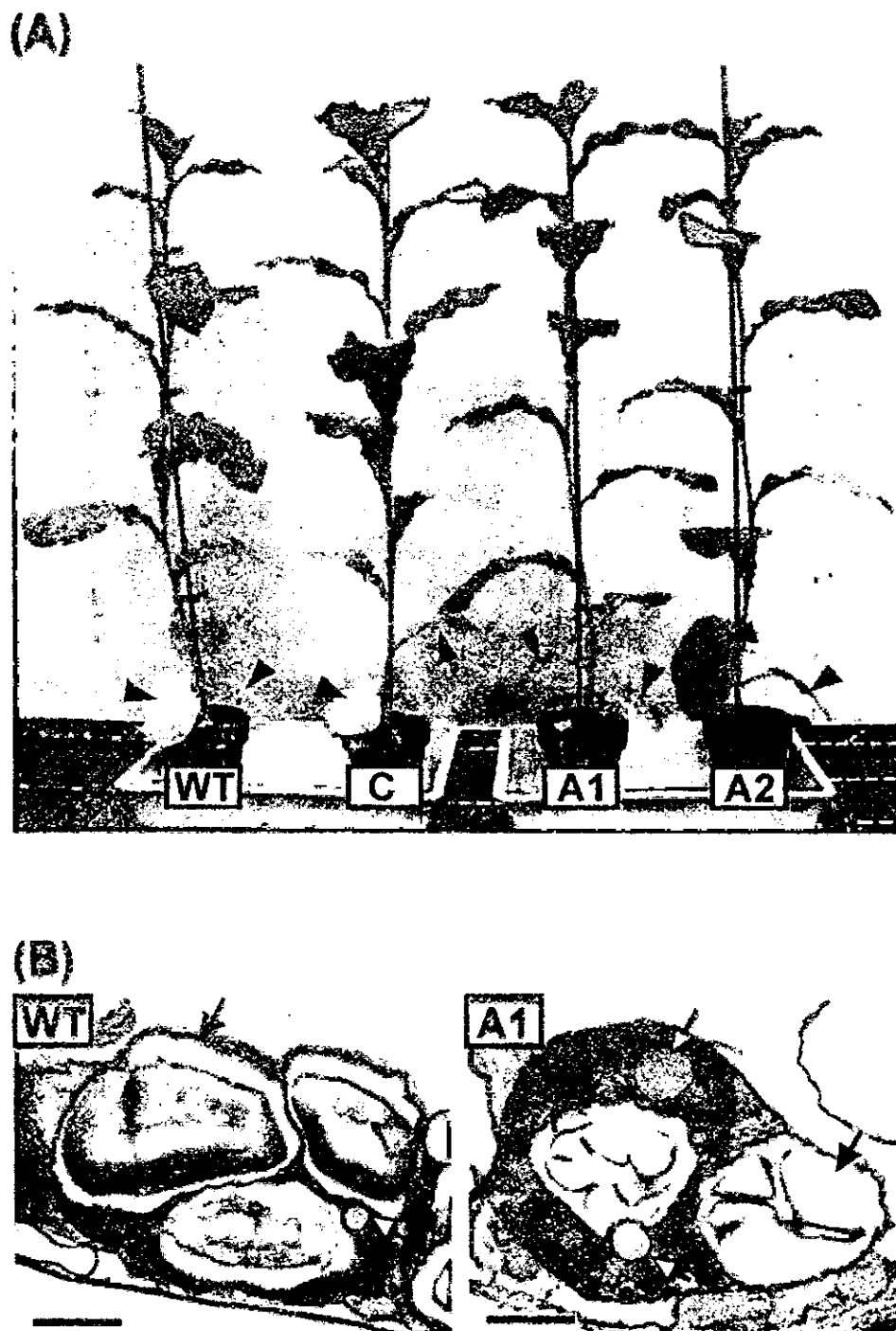
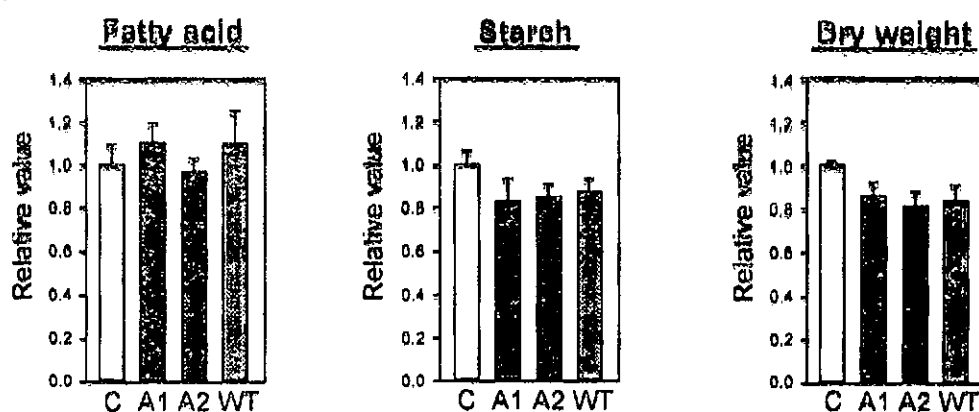


Fig. 5 Wild-type and T_1 plants after 12 weeks of culture. (A) Seeds were germinated and grown for 5 weeks on RMOP medium supplemented with 0.5 mg ml^{-1} spectinomycin dihydrochloride (only for T_1 transformants) at 25°C under a 12-h light/12-h dark regimen at a photon flux density of $40 \mu\text{mol m}^{-2} \text{ s}^{-1}$ during the light period. The spectinomycin resistant seedlings were transplanted into soil in pots ($700 \text{ ml plant}^{-1}$) and grown for a further 7 weeks in a growth chamber, as described in the Materials and Methods. Plants were fertilized with diluted (1/2,000) Hyponex (Osaka, Japan) every 2 d. Abbreviations are the same as in Fig. 1B. (B) Electron microscopy was carried out on mature green leaves (leaf maximum width 7 cm) harvested from the 12-week-old plant shown in Fig. 5A after illumination with white light for 1 h. Black and white arrows indicate starch granules and lipid droplets, respectively. Bar = $1 \mu\text{m}$. Abbreviations are the same as in Fig. 1B.

A) per seed



B) per plant

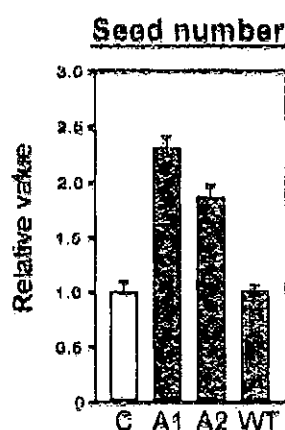


Fig. 6 Fatty acid and starch contents in seeds, and seed yields per plant. (A) All the seeds from a plant were pooled, and fatty acids and dry weight were measured in duplicate for aliquots of 50 seeds. The average value for fatty acid content per dry seed weight was calculated. Data obtained from 2–3 plants were averaged and presented as values relative to the control. Fatty acid and starch contents of 1,000 dry seeds from control plants were 4.6 mg and 0.4 mg, respectively. The weight of 1,000 dry seeds from the control plant was 103 mg. Abbreviations are the same as in Fig. 1B. (B) The number of seeds per plant was counted for 2–3 plants and the value relative to the control was calculated for each experiment. The average values from four independent series of experiments are shown. The seed number per plant for the control group was 1.2×10^4 . Abbreviations are the same as in Fig. 1B.

whereas no other leaf glycerolipid including neutral lipids differed significantly between the A2 and control plants (data not shown). It is noteworthy that the levels of trienoic fatty acids, hexadecatrienoic acid (16:3) and linolenic acid (18:3), increased in A2 leaves by 32% and 14%, respectively, relative to the control, with concomitant decreases in the levels of dienoic fatty acids. Starch analysis showed that the starch content of the control and the transformants decreased compared with those of the wild type.

Other phenotypes of transgenic plants

The photosynthetic activity of the fully expanded leaves of T₁ plants (11th leaves from the bottom in Fig. 5A) did not differ significantly among the A1, control, and wild-type plants (data not shown). The transgenic plants were indistinguishable

from the wild-type except that A1 and A2 senesced later than the wild-type and control plants (Fig. 5A). The oldest leaves of 12-week-old wild-type and the control plants (blue arrowheads) were yellow, whereas A1 and A2 leaves of a similar age were green (red arrowheads) and turned yellow 7–10 d later. Indeed, the chlorophyll content of fully expanded leaves (8th leaves from the bottom) in A1 plants was 1.3-fold higher than that of the wild-type plants.

Transformant leaves were compared with those of the wild type by electron microscopy. Both the A1 transformant and the wild-type chloroplasts contained starch granules and opaque droplets, but the transformant appeared to contain more droplets and fewer granules than the wild-type plants (Fig. 5B). Microscopic survey showed that the starch granules became smaller and the droplets became larger in the transformants.

Thus, apparent alteration of chloroplast constituents was observed.

Effects on seeds

The fatty acid content of the seeds (Fig. 6A) and the fatty acid composition (data not shown) were very similar in the wild-type and all transformant plants. The starch content of A1 and A2 plants was less than that of the control plants. Seed dry weight was also similar in the wild-type, A1 and A2 plants, but this value was slightly less than that of the control. However, the number of seeds per plant in the A1 and A2 transformants (Fig. 6B) increased about 2-fold over the control and wild-type plants. The number of seeds per capsule increased considerably in A1 and A2 transformants compared with the wild-type and control plants, although the numbers of flowers and capsules were similar among all plants. Ultimately, the increase in seed yield doubled the fatty acid production per plant.

Discussion

Promoter replacement enhanced *accD* expression and ACCase levels

In this work, we found that replacement of the *accD* promoter with the *rriI* promoter in the tobacco plastid genome increased the levels of *accD* transcripts as well as *accD* protein, in plastids. Therefore, the expression of *accD* is, at least partly, regulated at the level of transcription. The overexpression of *accD* increased the three nuclear-encoded subunits of ACCase and increased the activity of ACCase in plastids. This finding supports our hypothesis that the expression of *accD* in plastids limits total levels of plastidic ACCase, and implies that *accD* protein level is a determinant for enzyme level. In this way, we succeeded in raising heteromeric ACCase levels.

Post-transcriptional regulation and the apparent coordination of protein synthesis of nuclei and plastids

Our finding that the levels of nuclear-encoded subunits increased without a corresponding increase in their transcripts suggests that post-transcriptional regulation is involved in ACCase synthesis, probably at the level of subunit assembly. We infer that a molar excess of nuclear-encoded ACCase subunits is synthesized in the cytoplasm and enters into the plastids, to be assembled with the plastid-encoded ACCase subunit. Conversely, unassembled nuclear-encoded ACCase subunits are probably rapidly degraded for lack of partner *accD* subunit in plastids. Therefore, only assembled subunits are detected by immunoblotting, and an apparent coordination of nuclear-encoded and plastid-encoded proteins is observed. Most proteins encoded by the plastid genome assemble with the nuclear-encoded proteins to form a functional complex, which was thought to require the coordinated synthesis of nuclear-encoded and plastid-encoded proteins. However, our results suggest that such coordinated synthesis is not always necessary, at least in the case of ACCase. By overexpressing

the plastid-encoded subunit, we showed that post-transcriptional regulation is involved in the formation of a functional complex in plastids.

Relative amounts of mRNAs for four ACCase are known to co-ordinately change during leaf and seed development (Ke et al. 2000). This result suggests that the four transcript levels are regulated at least in part by coordination between nucleus and plastids during development. However, our results that overexpression of *accD* did not affect the levels of *accC* mRNA suggests that no close coordination occurs between the nucleus and plastids in the regulation of the transcript levels. Taken together, we propose that although the rates of synthesis of nuclear- and plastid-encoded subunits are regulated at the levels of transcripts in the cytoplasm and plastids, respectively, the level of each ACCase subunit is ultimately regulated at the level of protein assembly in plastids.

Effects on leaves

An increase of ACCase in transgenic tobacco chloroplasts resulted in an increase of total fatty acid content in leaves, and extended leaf longevity. Lipid analysis showed that the major chloroplast membrane lipid, monogalactosyldiacylglycerol, increased and the trienoic fatty acids also increased. These changes probably affect membrane properties, which might affect leaf longevity, although the kinds of membrane properties that influence leaf longevity have not been identified. It is also possible that membrane repair might be enhanced in our transgenic tobacco plants, which might delay leaf senescence, because older leaves approaching senescence are believed to require increased repair activity in the thylakoid membranes (Hellgren and Sandelius 2001).

Alternatively, decreased starch content in A1 and A2 might in turn delay leaf senescence (Miller et al. 1997). Furthermore, we do not exclude the possibility that altered fatty acid composition might play a role in delaying leaf senescence, because fatty acids are important regulators of plant growth and differentiation (Ohlrogge and Browse 1995, Topfer et al. 1995). Our transgenic tobacco plants may provide a useful experimental system for studying the mechanism of senescence in relation to lipid and carbohydrate metabolism.

Effects on seeds

We did not observe significant changes in fatty acid composition or content per seed. As described in the legend to Fig. 4 and Fig. 6, the ratio of fatty acid content to starch content is more than 11 in seeds, whereas it is 0.02 in leaves. This suggests that the biosynthesis of fatty acids is more active in developing seeds than in leaves in tobacco. Indeed, oil accounts for about 30% of seed dry weight. We are not successful in determining the level of ACCase in developing seeds because the seeds did not synchronously mature in the seed capsule. However, if the biosynthesis of fatty acids in seeds is fully activated in the wild type, further enhancement of the biosynthesis per seed may be difficult by the present method.

To our surprise, a remarkable increase in seed number was observed in transgenic plants. It is reasonable to ascribe the increased seed number per plant to increased leaf longevity, because extended longevity brings about abundant production of photoassimilates. Although further experiments are required to elucidate the mechanisms underlying this phenomenon, the outcome has potential utility in crop improvement. Improvements in seed yield should circumvent the limitation of fatty acid production per seed and provide a basis for developing a new strategy for improving commercially important oil-producing crops, such as oilseed rape and soybean. Enhanced production of seed oil should accelerate our efforts towards engineering plants as factories for industrial materials (Sommerville and Bonetta 2001).

The replacement of the *accD* promoter described here enhanced the primary transcripts of the *accD* operon that contains *accD*, *psaI*, *ycf4*, *cemA*, and *petA*. We did not survey the gene products for *psaI*, *ycf4*, *cemA*, and *petA*, and do not exclude the possibility that some of these gene products except for *accD* protein might have positive effects on the prolonged leaf longevity and the increased seed yield.

Promoter engineering

We succeeded in engineering the promoter of the plastid genome, using the *rrn* promoter that contains the NEP and PEP promoters. There are various possible promoters to direct the expression of a target gene in a specific organ at an appropriate time. Studies of promoter dissection may provide more powerful tools for plastid gene expression. The technique of plastid promoter engineering presented here offers a new method to improve oil production and increase plant yields.

Materials and Methods

Construction of plasmid DNA

PCR was performed with tobacco (cv. Xanthi) chloroplast DNA (Shinozaki et al. 1986) as template. The primers P1–P6, with added restriction sites, were used for PCR and are shown in Fig. 1; P1, 5'-CCCGGGGGGGGGGGGGGGTGTGATAGGAAATAAG-3'; P2, 5'-GTCGACGTGCTCTACTTGATTTTGC-3'; P3, 5'-GTCGAGTAGACCTTGTTGTGTGAG-3'; P4, 5'-CCCGGGGGGGGGGGGGAACCCCTT-TATATAT-3'; P5, 5'-GTCGACGCTCCCGGGGGGTCGTTTC-3'; and P6, 5'-GGTACCCGGGTATTCGCCGGAGTTTCG-3'. To replace the promoter of *accD* with the *rrn* promoter in tobacco plastids, the plasmid *prbcL-aadA-rrn-accD* was constructed as follows. The plasmid *paacD* contained PCR-amplified (using P1 and P2), promoter-deleted *accD* (nucleotides 59,645–59,748 were deleted, accession number Z00044) between the *SmaI*–*SalI* sites of pUC18. The plasmid *prbcL-accD* contained a PCR-amplified (using P3 and P4) *rbcL* fragment and the 3' non-coding region of *rbcL* (nucleotides 59,561–59,644, accession number Z00044) between the *EcoRI*–*SmaI* sites of *paacD*. The plasmid *paadA-rrn* contained a PCR-amplified (using P5 and P6) *rrn* promoter (nucleotides 102,563–102,716, accession number Z00044) between the *SalI*–*KpnI* sites of a pBluescript II SK+ (Stratagene) vector containing the *aadA** gene (Svab and Maliga 1993). Finally, an *aadA**–*rrn* cassette from the plasmid *paadA-rrn* was inserted into the *SmaI*–*NofI* sites of the plasmid *prbcL-accD* to produce the plasmid

prbcL-aadA-rrn-accD. For a control vector, *aadA** alone was inserted upstream from the genuine promoter of *accD* (at nucleotide 59,645, accession number Z00044).

Production and growth of transgenic tobacco

Tobacco plants (*Nicotiana tabacum* cv. Xanthi) were grown aseptically on agar-solidified MS medium (Murashige and Skoog 1962) supplemented with sucrose (30 g liter⁻¹), and aseptic leaves were subjected to particle bombardment for homologous recombination, as described previously (Svab and Maliga 1993). Homoplastic transgenic shoots were generated by repeated shoot regeneration on RMOP medium (Klein et al. 1988) containing 0.5 mg ml⁻¹ spectinomycin dihydrochloride, and were then rooted on MS medium. The resultant T₀ plants were transplanted into soil in pots (700 ml plant⁻¹) and cultured in a growth chamber at 25°C under a 12-h light/12-h dark regimen at a photon flux density of 300 µmol m⁻² s⁻¹ during the regulated light period. The homoplastic T₁ generation was used in all experiments.

Southern and Northern blot analyses

Total cellular DNA and total cellular RNA were isolated, and Southern and Northern blot analyses were performed as described previously (Madoka and Mori 2000).

Immunoblot analysis

Total leaf protein from young leaves (leaf width 3 cm) was extracted with a sample loading buffer for sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli 1970). Proteins (10 µg) were separated by SDS-PAGE and transferred onto a nitrocellulose membrane. The blots were probed with antibodies, as described previously (Sasaki et al. 1993). Antibodies were prepared against recombinant proteins for tobacco *accD*, pea *accA*, and pea *accC*, all of which were expressed as glutathione *S*-transferase fusion proteins, and purified as described previously (Kozaki et al. 2000).

Measurement of ACCase activity

ACCase activity in chloroplast extracts was measured as described previously (Sasaki et al. 1997).

Fatty acid and lipid analysis

Fatty acid content was analysed according to the method described previously (Lemieux et al. 1990). Fatty acid methyl esters were quantified with a gas chromatograph (GC-353, GL Sciences, Tokyo, Japan) operated at 190°C using a CP-Sil 88 (Chrompack, Tokyo, Japan) column (50 m × 0.25 mm). Lipids were analysed as described previously (Rotein et al. 2001).

Determination of starch content

Starch content was analysed according to a previously described method (Focks and Benning 1998). Leaves (3 mg) or 100 seeds were crushed and incubated in 1 ml 80% (v/v) aqueous ethanol at 80°C for 1 h. After centrifugation, the pellet was extracted again with the above solution and the residual pellet was used for starch analysis with a starch assay kit (Sigma).

Electron microscopic analysis

Leaf tissue was fixed with 5% (v/v) glutaraldehyde in 50 mM phosphate buffer (pH 7.2) for 8 h, and postfixed in 2% (v/v) osmium tetroxide for 12 h. The tissue was then dehydrated in a graded series of acetone and embedded in Epon 812 resin. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined under an electron microscope (Hitachi, H-600, Tokyo, Japan).

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Use of tobacco seed oil methyl ester in a turbocharged indirect injection diesel engine

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Abstract

Vegetable oils and their methyl/ethyl esters are alternative renewable fuels for compression ignition engines. Different kinds of vegetable oils and their methyl/ethyl esters have been tested in diesel engines. However, tobacco seed oil and tobacco seed oil methyl ester have not been tested in diesel engines, yet. Tobacco seed oil is a non-edible vegetable oil and a by-product of tobacco leaves production. To the author's best knowledge, this is the first study on tobacco seed oil methyl ester as a fuel in diesel engines.

In this study, potential tobacco seed production throughout the world, the oil extraction process from tobacco seed and the transesterification process for biodiesel production were examined. The produced tobacco seed oil methyl ester was characterized by exposing its major properties. The effects of tobacco seed oil methyl ester addition to diesel No. 2 on the performance and emissions of a four cycle, four cylinder turbocharged indirect injection (IDI) diesel engine were examined at both full and partial loads. Experimental results showed that tobacco seed oil methyl ester can be partially substituted for the diesel fuel at most operating conditions in terms of performance parameters and emissions without any engine modification and preheating of the blends.

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Keywords: Tobacco seed oil; Methyl ester; Diesel engine; Performance; Emission

1. Introduction

The gradual depletion of world petroleum reserves, increases in prices of petroleum-based fuels and environmental pollution due to exhaust emissions have encouraged studies to search for

alternative fuels. In view of these, vegetable oils have been considered as alternative fuels for compression ignition engines. Vegetable oils are renewable, non-toxic, biodegradable, and have low emission profiles [1–5]. However, there are some drawbacks related to the use of straight vegetable oils in diesel engines primarily due to their high viscosity, lower volatility and lower heat content [6–8].

The high viscosity causes some problems in atomization of injector systems and combustion in

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cylinders of diesel engines. Also, in long term operations, high viscosity of vegetable oils may lead to ring sticking, formation of injector deposits, development of gumming, as well as incompatibility with lubricating oils [1,9]. Different techniques have been developed to solve their high viscosity and low volatility problems of vegetable oils, such as preheating oils, blending or dilution with other fuels, transesterification and thermal cracking/pyrolysis [1,2,10–12]. Transesterification appears to be the most promising technique which is a chemical process of converting vegetable oil into biodiesel fuel [5,11,13,14]. Biodiesel can be used as a blend in diesel engines without modification. Detailed reviews about transesterification process are available in the literature [2,12].

The important compositional difference between biodiesel and the diesel fuel is concerned with oxygen content. Biodiesel contains 10–12% oxygen in weight basis and this lowers the energy content. The lower energy content causes reductions in engine torque and power [7,15–17]. Biodiesel containing oxygen reduces exhaust emissions such as HC, smoke and CO mainly due to the effect of complete combustion [4,5,18–23]. Since biodiesel contains little sulphur compared to the diesel fuel, a significant reduction in SO₂ emission was obtained [24]. There are four possible reasons for reduction in particulate emissions. First is the presence of oxygen in fuel rich regions of the combustion fuel spray [25–28]. Second is the modification of radical pool, which inhibits key soot formation reactions and provides OH radicals for oxidation of soot precursors [27,29,30]. Third is the removing carbon from soot formation process via C–O bonding within molecule due to oxygen, thereby sequestering carbon from the soot formation process [26,31–33]. Fourth is the low level of sulphur content of biodiesel [5].

NO_x emissions mainly depend on the engine fuelling system, engine type and engine loading. Two different observations can be seen in the literature. First, higher NO_x emissions may be due to higher temperatures of combustion chamber using biodiesel [18]. This is also evident from higher exhaust gas temperatures from biodiesel fuelled engines [11]. However, some studies

showed lower NO_x emissions [4,5,24,34,35]. This is because higher cetane numbers of bio-diesel shortens the ignition delay. The amount of premixed fuel and peak burning temperature are reduced, leading to the reduction in NO_x emissions [5].

The price of edible vegetable oils is higher than that of the diesel fuel. Therefore, instead of using such oils, use of waste vegetable oils [18,24,36–39] and non-edible crude vegetable oils [11,40,41] have been considered as potential alternative fuels.

In the present investigation, tobacco seed oil, that is a non-edible oil, was considered as a potential alternative fuel for compression ignition engines. Apart from Giannelos et al. [42], there has been no study reported in the literature on tobacco seed oil as a biodiesel. They investigated the specifications of tobacco seed oil and compared these specifications with other vegetable oils. They suggested that tobacco seed oil may be an appropriate substitute for the diesel fuel. However, tobacco seed oil methyl or ethyl esters have not been searched and have not been tested in any engine yet. This was the basic motivation behind the research in this paper.

This study has investigated the use of tobacco seed oil for biodiesel production. Along with discussion on potential tobacco seed production throughout the world, the oil extraction process from tobacco seed oil and the transesterification process for biodiesel production were considered. To assess its use as a diesel engine fuel, the produced tobacco seed oil methyl ester as a biodiesel was characterized by exposing its major physical–chemical properties. The engine tests were carried out on a turbocharged indirect injection diesel engine fuelled with diesel fuel No. 2 and tobacco seed oil methyl ester (10%, 17.5% and 25% in volume) and diesel fuel No. 2 blends. The results were summarized.

2. Materials and methods

2.1. Tobacco plant

Tobacco plant is a beautiful plant growing to a height of 90–180 cm with large oval leaves. It has

pink flowers and green capsules containing numerous very small seeds. The seeds can be stored in dry conditions at ordinary temperatures and can be resistant to high humidity. The average mass of 1000 seeds is in the range of 0.08–0.09 g. The oil content of the seed ranges from 36% to 41% by weight [42]. The remaining part consists of protein, crude fiber, carbohydrate and inorganic material.

The tobacco plant is grown in 119 countries in the world [43]. The leaf of the plant is a commercial product and used in the production of cigarettes in the tobacco processing industries. Therefore, statistical information on tobacco harvesting area and leaves production is available and easily accessible [43].

Tobacco seeds are a by-product of tobacco leaves production. Since tobacco seed oil is a non-edible oil, it is not used as a commercial product in food industry. Only a small amount of tobacco seeds is collected from fields for next year production, most of them are left unused in fields. There is no statistical information on potential tobacco seed and seed oil production in the literature to the best knowledge of the author. However, they may be estimated using the harvesting area. Regarding the present study, the seeds used were collected from Acipayam and Buldan Districts of Denizli City in Turkey.

2.2. Tobacco seed oil extraction and biodiesel production

Since tobacco seed oil is not available in markets, the oil was initially extracted from the collected seeds in laboratory conditions. The extraction procedure is as follows. Tobacco seeds were dried for 2 h at 110 °C and were ground to a fine powder. A soxhlet extraction apparatus was used to extract the oil from seeds. Diethyl ether was used as a solvent in extraction process. The oil was separated from the diethyl ether using a Buchi R114 rotary vacuum evaporator.

The oil was converted into methyl ester by means of transesterification process which involves making the triglycerides of tobacco seed oil react with methyl alcohol in the presence of a catalyst (NaOH) to produce glycerol and fatty acid ester. Although 3/1 molar ratio of alcohol to triglycer-

ides is needed to complete a transesterification process stoichiometrically, in practice, the ratio should be higher to drive the equilibrium to a maximum ester yield. However, large quantities of methanol slow down the phase separation of the two phases produced [2,11,17,44]. It was reported that the largest ester yield is obtained for 6/1 methanol/triglyceride molar ratio which is the twice of stoichiometric ratio [38,39,45]. Therefore, the molar ratio of methyl alcohol to glycerides was used as 6/1. NaOH was preferred as a catalyst due to its high activity [46]. The amount of NaOH was determined using a titration process as advised in [47]. It was found that 7.5 g NaOH was necessary per liter of the oil. This is within the recommended range of catalyst in the literature [2,39]. It has been reported that high catalyst concentrations greater than 1.5% lead to the production of large amounts of soaps [39,46].

Transesterification process was carried out using the rotary evaporator including a condenser unit, a thermostatic bath and a flask of 1000 ml volume. The flask is rotated in a thermostatic bath to keep the temperature constant throughout the reaction. The rate of transesterification rises with the increase of reaction temperature [44]. However, the maximum operating temperature cannot exceed the boiling point of the alcohol used in the process. For this reason, the temperature chosen for the reactions was 55 °C which is below the boiling temperature of methanol. The process can be summarized as follows: The tobacco seed oil was heated to 55 °C, NaOH was dissolved in methanol to produce the methoxide. Then the prepared methoxide was poured into the oil. The solution was stirred for 90 min holding the temperature at 55 °C. Then, the heater was turned off and stirring was being continued for 90 minutes without heating. The mixture was put in a separation funnel and was allowed to form two layers overnight. The bottom layer was glycerol while the upper layer was the ester. After settling is complete, the glycerin was taken out.

The ester was washed with pure water three times. Small amount of phosphoric acid (2.5 ml/l of the oil) was used in the first washing. At the end of the process the oil was heated to 90 °C at vacuum to remove any water from the oil left in

the ester. The final ester became clear straw yellow. The tobacco seed oil and tobacco seed oil methyl ester will be referred to as TSO and TSOME, respectively throughout the paper. Also diesel fuel No. 2 will be referred to as D.

2.3. Experimental apparatus for engine tests and exhaust emissions

The experimental set-up consists of a turbo-charged indirect injection (IDI) diesel engine, an engine test bed and two gas analyzers. The specifications of the diesel engine are given in Table 1. A Cussons-P8681 type engine test bed consists of a hydraulic dynamometer, measurement instruments and a control panel. The hydraulic dynamometer is water cooled and rated for 112 kW (150 bhp) power absorption at 9000 rpm maximum operating speed. The load on the dynamometer was measured by using a strain gauge load sensor. Inductive pickup speed sensor was used to measure the speed of the engine. The fuel consumption was measured with a burette with 50 and 100 ml volumes and a stopwatch. Air inlet, fuel, engine coolant inlet-outlet, lubricating oil, exhaust gas temperatures were measured by K type thermocouples. There are two fuel tanks. One is for the diesel fuel and the other for an alternative fuel. A Sun MGA 1200 gas analyzer was used to measure CO emission. A MRU 95/CD gas analyzer was used to measure NO_x and SO₂ emissions.

Table 1
Specifications of the diesel engine

Type	Ford XLD 418 T turbocharged 4 stroke, water cooled, IDI
Number of cylinder	4
Stroke	82.0 mm
Bore	82.5 mm
Compression ratio	21.5:1
Displacement	1.753 l
Maximum torque	182 Nm at 2200 rpm
Maximum power	55 kW at 4500 rpm
Type of injection pump	Rotary distributor

Table 2
The accuracies of the measurements and the uncertainties in the calculated results

Measurements	Accuracy
Load	±2 N
Speed	±2 rpm
Time	±0.5%
Temperatures	±1 °C
CO	±20 ppm
NO _x	±20 ppm
SO ₂	±20 ppm
Calculated results	Uncertainty
Torque	±2%
Power	±2%
hefu	±3.5%
Thermal efficiency	±3.5%

The four fuels tested in the experiments were diesel fuel No. 2 and blends containing TSOME in 10%, 17.5% and 25% proportions by volume. In this study, the legend D100 represents 100% the diesel fuel No. 2, while D90/TSOME10 is 90% the diesel fuel mixed with 10% TSOME, D82.5/TSOME17.5 is 82.5% the diesel fuel mixed with 17.5% TSOME and D75/TSOME25 is 75% the diesel fuel mixed with 25% TSOME in volume basis.

Experiments were carried out at different loads (Full-100%, 75% and 50%). The blends were prepared just before the experiments. The fuels were tested in the engine running at 150 bar original fuel injection pressure. The accuracies of the measurements and the uncertainties in the calculated results are shown in Table 2.

3. Results and discussion

3.1. Potential tobacco seed oil production, oil extraction, TSOME production and its characterization

The seeds were collected with a special care to find the seed yield of the harvesting areas. The seed yield was determined as 617 kg (±142 kg) per

hectare area of tobacco plant in Denizli. It should be emphasized that the seed yield may vary considerably depending on the type of the tobacco plant and area. For example, Patel et al. [48] determined that a type of tobacco plant in India can give the seed yield as 1171 kg/ha. The oil content of the collected seeds was found approximately 38% ($\pm 2\%$) in weight using diethyl ether as an extraction solvent.

The estimation values of potential tobacco seed production in the world shown in Table 3 is based on the seed yield obtained in harvesting areas in Denizli as stated above. Also, Table 3 includes the estimation values of the seed oil production.

Ten major countries having harvesting areas greater than 50000 hectares hold approximately, 75% of the potential production in the world. The other 109 countries have approximately, 25% of the potential production. It can be concluded that the tobacco seed oil may be a great source of biodiesel production in the world, especially in ten countries shown in the table.

Another point is the feasibility of the production of this source on large scale. Since seeds are collected after leaves are collected, this process does not interrupt leaves production. Using seeds for biodiesel production will also bring an extra

income for farmers. However, there is one thing to be noted: unfortunately there are some difficulties in collecting seeds from fields. This may be easily eliminated by designing special machines similar to wheat combine harvester. In addition, the tobacco seed cake constituting the rest of the oil extraction is known to be rich in nitrogen (around 6%), therefore could serve as an animal feed supplement.

The other interesting aspect is that cigarettes are made of leaves of tobacco plants and they are harmful to human health. By means of biodiesel production from the tobacco seed oil, tobacco plant will probably become something useful. Instead of using its leaves, maybe one day only its seed will be used.

The fatty acid composition of the tobacco seed oil and the other vegetable oils are compared in Table 4. The fatty acid composition of tobacco seed oil is dominated by linoleic, oleic, palmitic, stearic fatty acids plus much less proportions of linolenic, eicosanoic, palmitoleic, eicosenoic, heptadecanoic and behenic acids [42,49]. The major fatty acid in the oil is linoleic (67.75–69.49%), similar to soybean, sunflower, cotton and corn oils. The fatty acid composition of the oil would allow prediction of important properties of this material [50–53].

Table 3
Statistical information about tobacco [43]

Country	Harvesting area (hectares)		Estimated ^a seed (kg)	Estimated ^b oil (kg)
	2001	2002 ^c	2002	2002
China	1,339,600	1,330,408	820,861,736	311,927,460
India	393,500	425,000	262,225,000	99,645,500
Brazil	328,000	377,000	232,609,000	88,391,420
Turkey	237,318	207,140	127,805,380	48,566,044
Indonesia	205,000	205,000	126,485,000	48,064,300
USA	174,992	173,782	107,223,494	40,744,928
Malawi	100,200	100,200	61,823,400	23,492,892
Zimbabwe	77,558	77,558	47,853,286	18,184,249
Argentina	60,000	65,500	40,413,500	15,357,130
Greece	57,000	57,000	35,169,000	13,364,220
The rest of the world ^d	1,326,776	1,047,246	646,150,782	243,537,297
World Total	4,201,944	4,065,834	2,508,619,578	953,275,440

^aEstimated seed yield: 617 kg seed per hectare.

^bEstimated oil yield: 38% (in weight) of seed.

^cPreliminary.

^d109 countries which has the harvesting area less than 50,000 hectares.

Table 4
Fatty acid compositions of common vegetable oils (% by weight)

Fatty acid	Tobacco		Soybean [12]	Rapeseed [12]	Sunflower [12]	Cotton [12]	Palm [12]	Corn [12]
	[42]	[49]						
Myristic (14:0)	0.00	0.17	0.0	0.0	0.0	0.0	0.0	0.0
Palmitic (16:0)	10.96	8.87	13.9	3.5	6.4	28.7	42.6	11.8
Palmitoleic (16:1)	0.2	0.0	0.3	0.0	0.1	0.0	0.3	0.0
Stearic (18:0)	3.34	3.49	2.1	0.9	2.9	0.9	4.4	2.0
Oleic (18:1)	14.84	12.4	23.2	64.1	17.7	13.0	40.5	24.8
Linoleic (18:2)	69.49	67.75	56.2	22.3	72.9	57.4	10.1	61.3
Linolenic (18:3)	0.69	4.20	4.3	8.2	0.0	0.0	0.2	0.0
Others	0.69	2.12	0.0	1.0	0.0	0.0	1.0	0.1

In this study, 86% of the oil was converted to biodiesel using the transesterification process described in the previous section. This is an acceptable yield for a crude oil [54]. However, an ongoing research in our laboratory is being carried out to be able to increase this percentage.

The dynamic viscosity values of TSO, TSOME and the diesel fuels used in the experiments were measured at different temperatures using Brookfield Rotary viscometers RVDVII+. Fig. 1 shows the variations of the dynamic viscosities with temperature range from 5 to 45 °C. It is shown in the figure that the transesterification of TSO provided a significant reduction in viscosity, especially at low temperatures. The variation of TSOME viscosity is very close to the diesel fuel. The addition of TSOME slightly increased the viscosities of blends.

The properties of biodiesel produced are very important and should be taken into consideration before testing it in the engine [55,56]. The properties of TSOME and Diesel fuel No. 2 on the basis of DIN EN 14214 standards are given in Table 5. It can be seen that the properties of TSOME are in the acceptable ranges.

3.2. Effects of TSOME addition on performance and emissions of the engine at different loading conditions

The results of engine tests carried out on a turbocharged indirect injection diesel engine fuelled with the diesel fuel No. 2 and the three blends are summarized here.

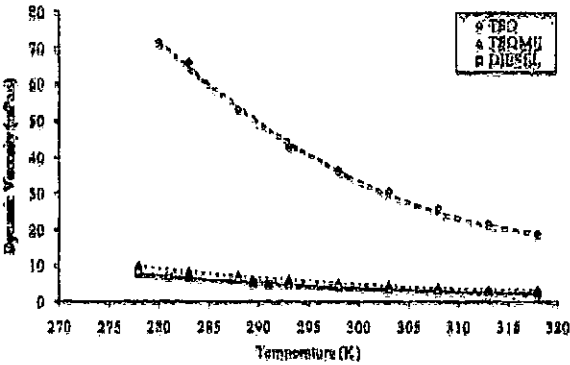


Fig. 1. The change of dynamic viscosities of TSO (tobacco seed oil), TSOME (tobacco seed oil methyl ester) and diesel fuel No. 2 with temperature.

TSOME addition did not show any appreciable variation in the engine performance. However, although the heating value of the TSOME is lower than that of the diesel fuel, in most cases TSOME blends resulted in slightly higher torque and power relative to the diesel fuel at full load. The higher torque and power by the blends may be explained with several reasons. First of all, the fuel is pumped to the cylinders on volumetric basis, therefore higher density of the blends provides larger mass flow rate for the same fuel volume. Meanwhile more viscous blend means less internal leakage in the fuel pump [56–58]. Also, TSOME contains approximately 11.5% (in weight) oxygen that takes part in combustion and this may be a possible reason for more complete combustion, thereby increasing the torque and power [4,11]. The higher power with the blends is consistent

Table 5
Specifications of TSOME and Diesel fuel No. 2.

Property	Unit	DIN EN 14214 limits		TSOME	Diesel No. 2	Testing method
		Min.	Max.			
Ester content	% (m/m)	96.5		98.15		pr EN 14103
Density at 15 °C	kg/m ³	860	900	886.8	841.5	EN ISO 3675
Viscosity at 40 °C	mm ² /s	3.5	5.0	3.5	2.9	EN ISO 3104
Sulfur content	mg/kg	—	10	6	6800	ISO 14596
Cetane number		51		51	51.5	EN ISO 5165
Water content	mg/kg	—	300	366	169	EN ISO 12937
Acid value	MgKOH /g		0.5	0.45	—	pr EN 14104
Iodine value			120	112	—	pr EN 14111
Heating value	kJ/kg			39811	44631	

with the thermal efficiency trends. The efficiency of the blends is slightly higher than the corresponding value of the diesel fuel. This means that the combustion of the blends was completed more efficiently than the combustion of the diesel fuel [18,41,58].

Among the blends, D82.5/TSOME17.5 provided the maximum increase in the torque, power and thermal efficiency. The maximum increase in power was occurred at 2000 rpm as 28.74 kW. This value is 3.13% higher than the power 27.84 kW obtained with the diesel fuel. Furthermore, the peak thermal efficiency was observed at 2500 rpm as 29.8%. This is approximately 2.02% higher than thermal efficiency of the diesel fuel.

Although D75/TSOME25 provided slightly higher power than that of diesel fuel, the power of this blend is lower than that of D82.5/TSOME17.5. It should be emphasized that after a certain amount of TSOME, the advantage of TSOME may be lost partly due to the lower heating value and the higher viscosity. Although TSOME viscosity is fairly close to the diesel fuel viscosity, the reduction in power and efficiency with higher content of TSOME in the blend was inevitable due to lower heating value [4]. If the TSOME content is continued to increase, the power and efficiency of the blend will be lower than those of diesel fuel.

Diesel No. 2 fuel and D82.5/TSOME17.5 were also tested at partial loads (75% and 50%). All the performance parameters were found to be very

close to each other. At the partial loads, the overall mixture was further leaned out. Therefore addition of TSOME had only a slight beneficial effect as an oxygenated fuel on performance [8]. Since the heating value of TSOME is lower than that of the diesel fuel, there were slight reductions in the engine torque and power. Although, the thermal efficiency of D82.5/TSOME17.5 is higher than that of the diesel fuel at the full load, at the partial loads the blend presented lower thermal efficiency than the diesel fuel [16]. This is consistent with the power reduction. This indicates that the addition of oxygenated fuel is most effective in rich combustions [59–61].

Generally, CO level decreased with the addition of TSOME mainly due to the complete combustion [4,58,62]. The lowest CO was obtained with D75/TSOME25. It is very likely that this is due to the highest oxygen content of this blend. CO and soot compete for the available oxygen in the rich combustion regime at the full engine load. At the partial loads, TSOME fuel blends have only a slight effect on the CO emissions due to the dominant premixed lean combustion with excess oxygen under these conditions [59,60]. CO level decreased sharply with speed at the full load. However, the level is more stable at the partial loads.

TSOME contains fairly low sulfur compared to Diesel fuel [42,63], therefore SO₂ emissions of the blends were lower than those of the diesel fuel as expected. For example, D82.5/TSOME17.5

showed a significant reduction in SO_2 (up to 45%) at full load, similar to the result found by Dorado et al. [24]. This amount of reduction was also determined at partial loads.

The NO_x emissions of the TSOME blends were slightly higher than those of the diesel fuel at both full and partial loads. There are mainly three factors affecting NO_x emission; oxygen concentration, combustion temperature and time. The higher temperatures of combustion chamber and the presence of fuel oxygen with TSOME caused higher NO_x emissions, especially at full load [18,24,64]. The results showed that the increase was around 5% at full load. However, this increase was negligible at partial loads, especially at 50% load. Although the oxygen concentration is higher at partial loads, the combustion temperature and time decrease, leading low NO_x . Also, it is known that the external oxygen supplied with air is less effective than the fuel-borne oxygen in the production of NO_x .

4. Conclusions

In this study, tobacco seed oil methyl ester has been produced and tested in a diesel engine as an alternative diesel fuel. The experimental results are described as follows:

- (1) There is remarkable tobacco seed oil potential for biodiesel production in the world.
- (2) The seed yield was determined as 617 kg (± 142 kg)/ha and the oil extraction yield of tobacco seed was found as 38% ($\pm 2\%$) in weight basis with diethyl ether as a solvent.
- (3) In transesterification process of TSO, the molar ratio of methyl alcohol to glycerides was used as 6/1 with 7.5 g NaOH per litre of the oil as a catalyst and the reaction temperature was kept at 55 °C. The 86% of the crude oil could be converted to biodiesel.
- (4) The determined properties of the TSOME are suitable for DIN EN 14214 standards.
- (5) TSOME addition did not show any considerable variation in the engine performance.
- (6) The addition of TSOME decreased CO emission due to the fact that TSOME contains

about 11.4% oxygen by weight, and this oxygen helps to oxidize the combustion products in the cylinder. There was a significant SO_2 reduction with blends due to lower sulphur content of the biodiesel. NO_x emissions slightly increased due to higher combustion temperature and the presence of fuel oxygen with blend at full load. However, the increasing amount of NO_x emission slowed down with decreasing load.

As a result of all of the findings mentioned above, it can be concluded that tobacco seed oil methyl ester can be partially (up to 25–30%) substituted for diesel fuel No. 2 at most operating conditions in terms of performance parameters and emissions without any engine modification and preheating of the blends.

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How to Process Oilseed on a Small Scale

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Small Scale Oilseed Processing - Technical Brief

PRACTICAL ACTION Technology challenging poverty

Most countries in the world have large refineries producing cooking oil from a variety of raw materials including maize, sunflower, soya, coconut, mustard seed and groundnuts. These large centralised plants have the advantage of high efficiency and reduced costs due to the economy of scale. Despite this, in many situations smaller scale decentralised oil extraction can prove to be economic and provide opportunities for income generation. Most commonly, opportunities exist where:

- oil produced in the large refineries does not find its way out to more remote and distant rural areas.
- high transport costs are involved in wide distribution of cooking oil so increasing the price of oil.
- small farmers produce oilseeds such as groundnuts for sale to the large refineries which they then buy back, at high cost, in the form of cooking oil but without the valuable high protein oil cake.
- the crude oil is used to produce added value products, most commonly soap.
- more unusual, high value oilseeds are available; examples include Brazil Nuts, Macadamia Nuts.

File:P01.jpg

Figure 1: Sunflowers grown in Kenya ©Practical Action/Morris Keyonzo

The main risks that need to be considered are:

The policy environment

In many countries the oil processing sector is highly politicised and regulations exist which make entering the market difficult and tend to support the monopoly of the large processors. Large refineries may, for example, insist that farmers sell all their seed under a contract. In other cases seed has to be sold to a central Government marketing board, the board also supplying seed for planting. To determine whether small scale processing is likely to be economic it is most important to first investigate the local situation and regulations.

Raw material supply

Clearly there must be sufficient raw material available locally. One factor that will greatly influence the viability of the enterprise will be the amount of credit needed to purchase a stock of seed. The enterprise should aim to keep the minimum stock of seed but always have enough to continue operating throughout the year. This requires considerable working capital.

Marketing

In general more profit will be made if the cooking oil is packed into retail size bottles. In many countries glass or plastic bottles are difficult to obtain and need to be purchased in large quantities so tying up capital. The possibility of using second-hand bottles should be examined as well as selling in drums to local stores. A

survey need to be carried out to make sure that the packaging used meets the demands of customers. (eg size of pack, type of container)

The viability of any oil extraction enterprise depends to a considerable extent on the sale of the oil cake for use in animal feeds and other sub-products. Markets for oil cake must be investigated and demand established before processing starts.

Health and safety

As oil processing is classified as a food processing enterprise it will be subject to local legislation. Care should be taken that standards are understood and met. The particular problem of aflatoxins will require attention. Aflatoxins are natural poisons produced by certain moulds that grow on seeds and nuts. They are of considerable concern to oil seed processors and groundnuts are particularly susceptible to contamination. The growth of aflatoxin producing moulds can be prevented by correct drying and by preventing moisture pickup by raw materials in store. It is most important that those considering establishing an edible oil enterprise should be able to recognise aflatoxin producing moulds and understand how to correctly select and store raw materials. If aflatoxin is present very little passes to the extracted oil, the majority will be found in the cake remaining after extraction. Aflatoxin contaminated cake presents a real danger if incorporated in livestock feeds. As aflatoxin is difficult to remove good practice is essential to prevent any mould growth and so prevent problems.

Not all oilseed crops are suitable for processing at small scale, the most common types that are suitable include groundnut, sunflower, coconut, sesame, mustard seed, oil palm and shea nuts. Typical oil contents of common raw materials are shown in Table 1.

Table 1 Common oils and their uses

Oilseeds		
Castor	35-55	Paints, lubricants
Cotton	15-25	Cooking oil, soaps
Linseed	35-44	Paints
Rape/mustard	40-45	Cooking oil
Sesame	35-50	Cooking oil
Sunflower	25-40	Cooking oil
Nuts		
Coconut	35 (Fresh)	Cooking oil, soaps
Groundnuts	38-50	Cooking oil, soaps
Palm Kernel	46-57	Cooking oil, cosmetics
Shea nuts	34-44	Cooking oil, soaps
Mesocarp Palm fruit	av. 56	Cooking oil, soap

In many cases the crude extracted oil is not suitable for human consumption until it has been refined to remove undesirable free fatty acids that taste rancid, dark colours and waxes. Refining involves considerable extra equipment costs. The most suitable oils for small and medium scale extraction are those that need little or no refining eg. mustard, sesame, groundnut, sunflower, palm and palm kernel .

Methods of extraction

Five common methods are used to extract oil:

a) Water assisted. Here the finely ground oilseed is either boiled in water and the oil that floats to the surface is skimmed off or ground kernels are mixed with water and squeezed and mixed by hand to release the oil. b) Manual pressing. Here oilseeds, usually pre-ground, are pressed in manual screw presses. A typical press is shown in diagram 1. c) Expelling. An expeller consists of a motor driven screw turning in a perforated cage. The screw pushes the material against a small outlet, the "choke". Great pressure is exerted on the oilseed fed through the machine to extract the oil. Expelling is a continuous method unlike the previous two batch systems. d) Ghani. A ghani consists of a large pestle and mortar rotated either by animal power or by a motor. Seed is fed slowly into the mortar and the pressure exerted by the pestle breaks the cells and releases the oil. Ghani technology is mainly restricted to the Indian sub-continent. e) Solvent extraction. Oils from seeds or the cake remaining from expelling is extracted with solvents and the oil is recovered after distilling off the solvent under vacuum.

Most small enterprises will find that small expellers are the best technology choice. Methods such as water extraction and manual pressing only produce small amounts of oil per day, the extraction efficiencies are low and labour requirements high. Solvent extraction while highly efficient involves very substantial capital cost and is only economic at large scale. There are also health and safety risk from using inflammable solvents.

The basic steps involved in processing oilseeds by expeller are shown in the flow diagram below.

File:P03a.jpg

Equipment required

The equipment needed to set up a small or medium scale oil extraction enterprise falls into three main categories:

- pre-extraction equipment; eg dehullers, seed/kernel crackers, roasters, mills.
- extraction equipment; manual presses,ghanis, expellers
- equipment for basic refining of the oil; filters, settling tanks.

File:P03b.gif

Figure 2: Decortating machine

The specific equipment required will depend on the particular crop being processed, the final oil quality required and the scale of operation. In a small guide it is impossible to cover both the whole range of technical options and possible crops the following section concentrates on one example; the extraction of sunflower and groundnut oil by expeller.

Shelling or dehulling

Most oil bearing seed need to be separated from outer husk or shell. This is referred to as shelling, hulling or decortication. Shelling increases the oil extraction efficiency and reduces wear in the expeller as the husks are abrasive. In general some 10% of husk is added back prior to expelling as the fibre allows the machine to grip or bite on the material.

A wide range of manual and mechanical decorticators are available and typical examples are shown in Figure 2.

After decortication the shell may have to be separated from the kernels by winnowing. At small scale this can be done by throwing the material into the air and allowing the air to blow away the husk. At larger scale mechanical winnowers and seed cleaners are available

Heating or conditioning

Pre-heating the seeds prior to expelling speeds up the release of the oil. Pre-heating is generally carried out in a steam heated kettle mounted above the expeller.

File:P04a.gif

Figure 3: Two screen Grain/seed cleaner cum grader

Expelling

A wide range of makes and sizes of expellers are available. In India in particular a number of efficient small or "baby" expellers are available. A typical example with a capacity of up to 100 kg/hr is shown in figure.3. This machine has a central cylinder or cage fitted with eight separate sections or "worms". This flexible system allows single or double-reverse use and spreads wear more evenly along the screw. When the screw becomes worn only individual sections require repair thus reducing maintenance costs. As the material passes through the expeller the oil is squeezed out, exits through the perforated cage and is collected in a trough under the machine. The solid residue, oil cake, exits from the end of the expeller shaft where it is bagged.

File:P04b.jpg

Figure 4: Tinytech oil expeller in operation in Zimbabwe ©Practical Action/Kolih Mashell

Filtration

The crude expelled oil contains solid particles. These can be removed by allowing the oil to stand and then filtering the clear oil by gravity through fine cloth. A better but more expensive method is pumping the crude oil through a filter press.

The typical system described above can be obtained for about \$US 4500 (1992 price) and consists of:

- Decorticator with blower to remove shell, 150 kg/hr
- Boiler, 50 kg steam/hr at 30 psi
- Cooker
- Expeiler 75 - 100 kg/hr
- Filter pump and press.

File:PO5.gif
Figure 5: A filter press

If filling into retail packs is planned a simple manual piston filter would be required.

Production capacity

A small mill as described above has the following capacity (based on single shift, 8 hrs/day, 24 days/month)

kg/ day	kg/annum	
Groundnut Seed	864	248,832
oil litres	260	74,076
oilcake	566	163,164
Sunflower Seed	720	207,360
oil litres	150	45,888
oilcake	522	150,444

Economic viability will be greatly increased if the mill works double shift.

In terms of employment a small oil mill would provide work for the owner/manager and four workers.

Equipment suppliers

Note: This is a selective list of suppliers and does not imply endorsement by Practical Action.

Agro Industrial Agency
Near Malaviya Vadi
Gondal Road
Rajkot - 360 002
India
Tel: +91 281 461134/462079/451214
Fax: +91 281 461770
Email: jagdishindia@hotmail.com (mailto:jagdishindia@hotmail.com)

- "Jagdish" Tiny Oil Press
Used for the extraction of vegetable oil from oilseeds. The reverse worm system in the pressing chamber results in the highest recovery of oil.
Food Groups: Oilseeds. Capacity: 50 kg/hour
Power: Diesel/Electric

TinyTech Plants Tagore Road
 Rajkot - 360 002
 India
 Tel: +91 281 2480166, 2468485, 2431086
 Fax: +91 281 2467552
 Email: tinytech@tinytechindia.com (mailto:tinytech@tinytechindia.com)
 Website: <http://www.tinytechindia.com/>

- Oil expellers

Capacity: 125 kg/hour. Power: Electric

- Filter Press

This is suitable for the filtration of oil. It gives transparent, very clean oil without any solid particles. Food Groups: Oilseeds
 Capacity: 100-200 litres/hour

Alan Blanch Chekworth
 Malmesbury
 Wiltshire
 SN16 9BG
 UK
 Tel: +44 (0) 666 577333
 Fax: +44 (0) 666 577339

- Expellers / Oil Screw Press - model mini 50

Barrel constructed from separate cast rings spaced apart by shims. The barrels are 60mm diameter by 155mm drainage length with a single piece worm shaft driven from the discharge end through a totally enclosed intermediate chain. The unit is complete with feed hopper, manual feed chute, oil discharge spout and base plate. A versatile, low cost machine, designed to suit village communities or small industries. Suitable for oil and cattle cake. Food Groups: Oilseeds
 Capacity: 50 kg/hour input.
 Power: Diesel/Electric

C S Bell Co
 170 West Davis Street
 PO Box 291
 Tiffin
 Ohio
 USA
 Tel: +1 419 448 0791
 Fax: +1 419 448 1203

- No.60 Power Grist Mill

This mill is suitable for use on large and small farms. Adjustable for grinding texture. Includes hopper, feed regulator slide, coarse and fine grinding burrs and 12 inch diameter pulley. Food Groups: Cereals/Oilseeds/Herbs/spices
 Capacity: 150 kg/hour
 Power: Electric/Manual

Foraberg Agritech India PVT Ltd
 123, GIDC Estate
 Makarpura
 Vadodra - 390 010
 India
 Tel: +91 (0) 265 645752
 Fax: +91 (0) 265 641683
 Email: Forabagri@ad1.vsnl.net.in (<mailto:Forabagri@ad1.vsnl.net.in>)

- Pneumatic Aspirator / Winnower

This machine is used for "air washing" of the material to remove all dust, lights and impurities which are lighter than the seed. Winnowing of harvested and threshed seeds.
 Food Groups: Cereals/Herbs/spices/Oilseeds

Ashoka Industries Kiruna
 Wajananwulla
 Sri Lanka
 Tel: +94 71 764725

- Oil Expeller

Food Groups: Oilseeds
 Capacity: 3 litres/hour. Power: Electric
 Goldin India Equipment PVT Limited

F-29, B.I.D.C. Industrial
Estate
Gorwa
Vadodra - 390 016
Gujarat
India
Tel: +91 (0) 265 3801 168/380 461
Fax: +91 (0) 265 3801 168/380 461
Email: goldin@EQUIP.XEEDDQ.xoemail.com (mailto:goldin@EQUIP.XEEDDQ.xoemail.com)

• Screen Air Separator / Grain Cleaners

Most suited as precleaner before storage of seeds. Variable speed drive ensures accurate quality separations at high capacity. Totally enclosed aspiration system with cyclone provides dust free working in the plant.
Food Groups: Cereals/Herbs/spices/Oilseeds/Vegetables Capacity: 100 - 2000 kg/hour. Power: Battery/Electric

Flower Food Company
91/38 Ram Indra Road,
Sai 10, Km 4
Bangkok
Thailand
Tel: +66 2 5212203 / 5523420

• Seed Decorticator Machine
Food Groups: Oilseeds
Capacity: 500 kg/hour. Power: Electric

Nigerian Oil Mills Ltd
P.O.Box 264
Atta
Owerri
Imo-State
Nigeria

• Milling Machine / Mills and Grinders
This machine is used for crushing palm kernels, groundnut seeds and moringa seeds to extract oil.
Food Groups: Oilseeds/Nuts/Herbs/spices
Power: Electric/Manual

• Oil Expeller
This machine is used for crushing palm kernels, groundnut seeds and moringa seeds to extract oil. Food Groups: Oilseeds/Nuts/Herbs/spices
Power: Electric/Manual

References and further reading

- *The Manual Screw Press: For small-scale oil extraction* Kathryn H. Potts and Keith Machell, ITDG Publications 1995
- *Small Scale Vegetable Oil Extraction* S.W. Hend et al, NRI, ISBN 085954 387 0

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English

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French

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Spanish

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Please provide your feedback

[[gsdf?c=d-00gtzint-000--1-0--010---4---0--0-10]-1en-5000--50-about-0---01131-00117%2cCNJnXc0a8012f0000057844f1611d-OutfZz-8-0-0&c=CL2.1.5&d=Js7695c&az=A>=2&gc=&lls=0&f=es&az=A&p=about

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