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ESTABLISHMENT OF A REGENERATION AND TRANSFORMATION SYSTEM FOR ABIOTIC STRESS TOLERANCE IN SESAME

By

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B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., ٢٠٠٥

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Department of Agricultural Botany
Faculty of Agriculture
Cairo University
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ABSTRACT

Sesame (*Sesamum indicum* L.) is an important oil crop in many tropical and sub-tropical regions of the world, yet has received little attention in applying modern biotechnology in its improvement due to regeneration and transformation difficulties. Here within, we report the successful production of transgenic fertile plants of sesame (cv. Sohag ١), after screening several cultivars. *Agrobacterium tumefaciens* strain LBA٤٤٠٤ harbouring binary vector pBI١٢١ carrying *nptII* and *uidA* genes was used in all experiments. Recovery of transgenic sesame shoots was achieved using shoot induction medium (Murashige and Skoog MS basal salt mixture + Gamborg's B٥ vitamins + ٢,٠ mg/l BA + ١,٠ mg/l IAA + ٥,٠ mg/l $AgNO_3$ + ٣٠,٠ g/l sucrose + ٧,٠ g/l agar + ٢٠٠ mg/l cefotaxime and ٢٥ mg/l kanamycin) and shoots were rooted on MS medium + B٥ vitamins + ١,٠ mg/l IAA + ١٠,٠ g/l sucrose and ٧,٠ g/l agar. Rooted shoots were transplanted into pots and grown to maturity in greenhouse. Incorporation and expression of the GUS gene into T٠ sesame plants was confirmed using polymerase chain reaction (PCR), reverse transcriptase-PCR (RT-PCR) and GUS histochemical assay. Several factors were found to be important for regeneration and transformation in sesame. The most effective factors were plant genotype and the presence of $AgNO_3$ for successful recovery of sesame shoots. Co-cultivation time and optical density of the *Agrobacterium* suspension were also critical for sesame transformation. This work is an attempt to open the door for further genetic improvement of sesame using important agronomic traits.

Key words: Sesame, *Agrobacterium tumefaciens*, silver nitrate, *In vitro* plant regeneration, *Sesamum indicum*.

DEDICATION

I dedicate this work to whom my heartfelt thanks; to my father and my mother for their patience and help, as well as to my brothers and sister for all the support they lovely offered along the period of my post graduation.

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ARABIC SUMMARY

اسم الطالب: أمـل فاروق عبد الحميد أحمد الشافعي **الدرجة:** ماجستير

عنوان الرسالة: استحداث نظام للإستيلاد والتحوير الوراثي لتحمل الظروف البيئية المعاكسة في نبات السمسم

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المستخلص العربي

يعتبر السمسم من أهم المحاصيل الزيتية في العديد من مناطق العالم الاستوائية وشبه الاستوائية وعمومًا لم يلقى نبات السمسم إلا القليل من الاهتمام من تطبيقات التقنيات الحيوية الحديثة في تحسينه وذلك لصعوبة إستيلاد وتجديد خلاياه ولذلك كان الهدف الرئيسي من هذا البحث هو إنتاج نباتات سمسم خصبة من خلال زراعة الأنسجة النباتية ومحورة وراثيًا مع العديد من الأصناف المنزرعة محليًا. تم استخدام بكتريا الأجروباكتريم المحورة وراثيًا في جميع التجارب والمحتوية على الجين المسئول عن مقاومة المضاد الحيوي كانامايسين (neomycin phosphotransferase gene, *NPTII)* و (β- glucuronidase gene, *gus-A*) الجين المسئول الانتخاب عن طريق التفاعل الإنزيمي معطيًا دلالة لونية.

تم دراسة العديد من العوامل المؤثرة على نظام التخليق الخاص بنبات السمسم وكذلك نظام النقل الجيني ومن أهم تلك العوامل هو الصنف الوراثي للنبات، إضافة نترات الفضة و أيضًا كلاً من وقت التحضين وكثافة معلق بكتريا الأجروباكتريم والتي كانت من العوامل المهمة في نجاح عملية النقل الجيني لنبات السمسم.

تم تخليق نباتات السمسم على البيئة النباتية (Murashige and Skoog MS basal salt mixture) والمحتوية على فيتامينات بي٥ (Gamborg's B٥ vitamins)، ٢ملجرام/ لتر BA ، ١ مليجرام / لتر من IAA و ٥ مليجرام للتر من نترات الفضة، ٣٠ جرام / لتر سكروز، ٧ جرام آجار ويضاف كلا من المضاد الحيوي سيفوتاكسيم وكانامايسين وذلك لانتخاب النباتات المحورة وراثيًا فقط والحاملة لجين مقاومة المضاد الحيوي.

تم نقل النباتات التي نجحت في إنتاج الجذور إلى Conviron. تم اختبار اندماج الجين (GUS) في النباتات التام تجديدها معمليًا وذلك من خلال تفاعل البلمرة المتسلسل (polymerase chain reaction) وذلك للكشف عن وجود الجين وأيضًا تم استخدام تفاعل البلمرة المتسلسل المنعكس (reverse transcriptase-PCR) والذي يتم على مستوى الحامض النووي الريبوزي (mRNA) والذي يفيد حدوث عملية النسخ أيضًا تم اختبار الاندماج الجيني من خلال التفاعل اللوني الإنزيمي (GUS histochemical assay).

هذا العمل محاولة لفتح الباب للتحسين الوراثي بشكل أكبر في نبات السمسم لإنتاج ميزات زراعية هامة.

الكلمات الدالة: السمسم، الأجروباكتريم، نترات الفضة، إستيلاد وتجديد الخلايا معمليًا

استحداث نظام للإستيلاد والتحوير الوراثي لتحمل الظروف البيئية المعاكسة في نبات السمسم

رسالة ماجستير
في العلوم الزراعية
(فسيولوجيا النبات)

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INTRODUCTION

Sesame *(Sesamum indicum* L.) belongs to family *Pedaliaceae*, is an erect herbaceous annual crop. It is described as a queen of vegetable oil. The seeds contained high nutrients quatities such as ١٧,٧٣٪ protein with almost ٢٣,٤٥٪ carbohydrates and considered as a good source of the minerals, especially copper, manganese, magnesium, calcium, iron, phosphorus and zinc. In addition, sesame seeds are a good source of both dietary fiber and monounsaturated fats (Nzikou *et al.*, ٢٠٠٩). Sesame oil has excellent stability due to the presence of natural antioxidants such as sesamol, sesamin and sesamolin which has medicinal and pharmaceutical value (Jeng and Hou**,** ٢٠٠٥& Anilakumar *et al*., ٢٠١٠). Moreover, sesame has a relatively superior oil quality ranges from ٣٤٪ to ٦٠٪. Oleic and linoleic acids occur in nearly equal amounts, constituting about ٨٥٪ of the total fatty acids (Mondal *et al*., ٢٠١١).

Sesame is grown in tropical and subtropical areas with poor soils of limited fertility and inadequate moisture, India is the world's largest producer of sesame followed by China, Sudan and Ethiopia. In ٢٠٠٩, it was cultivated worldwide on a total area of over ٧,٥ million hectares with total production of ٣,٥ million tons of which ٣٣,٢١٪ was produced in Africa. Sesame currently ranked the sixth in the world production of edible oil seeds and twelfth for vegetable oil seeds production. Egypt ranked the fifths country in sesame production (FAO STAT, ٢٠٠٩).