

Molecular Gene Transfer for the Generation of salt tolerant rapeseed (*Brassica campestris*, *Brassica napus*) varieties in Bangladesh

Lutful Hassan

Department of Genetics & Plant Breeding, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, E-mail: LH@royalten.net

ABSTRACT

In Bangladesh, almost one-third of the country is located at the southern coastal part of the country. Salinity is a big problem for crop production in these areas. This research aims at production of transgenic Brassica varieties for salt tolerance. We developed a protocol for efficient, rapid and stable transformation of the local varieties of *B. campestris* and *B. napus*. After germination, the hypocotyls were cut into 1 cm segments, co-cultivated with the bacterial strain along with the plasmids and placed on shoot regeneration medium. For *Agrobacterium rhizogenes*, strain LBA 9402 was used for the production of hairy roots. For cotransformation experiments, the strain LBA 9402 with the binary vector pBIN19 with the p35S GUS INT gene was used. For plant regeneration 0.5 mm sections of the roots were excised and treated with a liquid callus-inducing medium (C23 γ) along with a control for three days. After that they were placed on N5 medium with antibiotics (500 mg/l carbenicillin and 200 mg/l claforan). The GUS staining was carried out according to Jefferson *et al.* (1987). PCR and southern analysis using the rolC gene as a probe will be applied to confirm the presence of the Ri-TL-DNA in transformed plants. For *Agrobacterium tumefaciens*-mediated transformation strains GV3101 with the vir plasmid pMP90, the strain C58C1 ATHV with the vir-plasmid pTiBo542 (=pEHA101; Hood *et al.* 1986) was used. As selectable marker genes, the *nptII* gene was used. As reporter gene the β -Glucuronidase-gene under control of the Ubi and the 35S-Promotor and with an Intron was used. For the confirmation of transgenic plants, leaf material will be taken from the growing plants for DNA isolation. PCR- and Southern analysis will be performed to determine the integration and the copy number of the transgene. The GUS-test will be performed to prove the β -glucuronidase expression and Northern analysis will be done to test the expression of the inserted genes.

Key words: *Brassica*, regeneration, transformation, *Agrobacterium*, salt tolerance

INTRODUCTION

Rapeseed, *Brassica campestris* L. and *Brassica napus* are important oil-yielding crops in Bangladesh. In addition to its cultivation for edible oil, some cultivars of these species are also has industrial applications in plastics, lubricants, lacquers and detergents. Approximately 70% of total *Brassica* under cultivation is *B. campestris*. *Brassica napus* varieties are also getting popularity due to its higher yield. But due to a remarkable crop loss of 30-100% in these species farmers of the coastal areas are reluctant to cultivate these species in this country. Conventional plant breeding methods alone are insufficient to solve this problem. However, the application of genetic engineering (gene technology) will be able to contribute significantly to combat the situation. Therefore, it is essential to develop a DNA delivery system for salt tolerant characteristics in the local varieties of these cultivated species.

Rapeseed has consistently proven to be one of the most recalcitrant members of the *Brassicaceae* in tissue culture. In spite of this problem there is great interest in the genetic transformation of this species for the production of transgenic plants. Therefore, efficient gene transfer and plant regeneration systems are necessary for the development of transgenic plants. Due to availability of low regeneration frequency and very few transformants the production of transgenic plants have not yet been established. Therefore, the aim of the project will be to establish an efficient method of plant regeneration using the right explants of *B. campestris* and

B. napus via the *Agrobacterium*-mediated transformation. Furthermore, this DNA delivery system will be utilized for the genetic transformation of these species for salt tolerance.

MATERIALS AND METHODS

A protocol was developed for efficient, rapid and stable transformation of the local varieties of *B. campestris*. Ten local varieties were taken as plant materials. Seeds were submerged in 70% ethanol for 3 minutes and then for 30 minutes in 0.1% mercuric chloride. These seeds were rinsed three times in sterile distilled water and germinated on ½ -strength MS medium without phytohormones for 6 days. After germination the hypocotyls were cut into 1 cm segments, co-cultivated with the bacterial strain along with the plasmids and placed on shoot regeneration medium. MS media were supplemented with ten various combinations of phytohormones for shoot regeneration. Calli were initiated from the hypocotyl segments on MS medium supplemented with phytohormones, solidified with 5 g/l of agar after adjusting the pH to 5.7 under continuous dark and at 30°C.

Agrobacterium strains: The following *Agrobacterium* strains along with different plasmids for transformation were used: *Agrobacterium rhizogenes* strain LBA 9402 was used for the production of hairy roots. For cotransformation experiments the strain LBA 9402 with the binary vector pBIN19 with the p35S GUS INT gene (Vancanneyt *et al.*, 1990) were used. For plant regeneration 0.5 mm sections of the roots were excised and treated with a liquid callus-inducing medium (C23γ) (Guerche *et al.* 1987) along with a control for three days. After that they were placed on N5 medium with antibiotics (500 mg/l carbenicillin and 200 mg/l claforan). The GUS staining was carried out according to Jefferson *et al.* (1987) (Figure 1). PCR and southern analysis using the rolC gene as a probe will be applied to confirm the presence of the Ri-TL-DNA in transformed plants.

Agrobacterium tumefaciens strains: 1) GV3101 with the vir plasmid pMP90 the strain C58C1 ATHV with the vir-plasmid pTiBo542 (=pEHA101; Hood *et al.* 1986), a strain similar to EHA101 which has been shown to be highly virulent for many of the important leguminous crops (Jin *et al.* 1997, Hood *et al.* 1987) was used.

As selectable marker genes the *nptII* gene (neomycin phosphotransferase) were used. As reporter gene the β-Glucuronidase-gene (GUS-Gene: Jefferson *et al.* 1987) under control of the Ubi and the 35S-Promotor and with an Intron (Vancanneyt *et al.* 1990) were used.

For the confirmation of transgenic plants, leaf material will be taken from the growing plants for DNA isolation. PCR- and Southern analysis will be performed to determine the integration and the copy number of the transgene. The GUS-test will be performed to prove the β-glucuronidase expression and Northern analysis will be done to test the expression of the inserted genes.



Figure 1: Gus expression in hairy root

RESULTS

An efficient, stable and reproducible *Agrobacterium tumefaciens*-mediated transformation protocol was developed for local varieties of *Brassica campestris*. Regeneration itself does not represent any problem. Stem segments proved to be the best explants. Shoot regeneration in *Agrobacterium rhizogenes*-mediated transformation was not possible till now (six months). In *Agrobacterium tumefaciens*-mediated transformation successful shoots regeneration was obtained from the transformed hypocotyls. MS media supplemented with 2 mg/l BAP+ 0.5 mg/l NAA showed the best results in regenerating the transformants. The transformed shoots were kept at controlled environment for hardening for two weeks. After that they were kept in a net house and grown in plants (Figure 2). Successful flowering occurred and finally the seeds were harvested from the mature plants.



Figure 2: Regenerated plants

DISCUSSION

The transformation protocol will be utilised for the delivery of gene construct with salt tolerance in local varieties of rapeseed. The rapeseed varieties will be used by the farmers of the coastal wetland of Bangladesh that will play important role in poverty alleviation.

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