Progress in producing a genetic transformation sytem for salt tolerant rapeseed in Bangladesh

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Abstract

We attempted genetic transformation of the local varieties of *Brassica campestris* and *B. napus* using *Agrobacterium*-mediated transformation. *Agrobacterium rhizogenes* strain LBA 9402 was used for the production of hairy roots. For co-transformation experiments the strain LBA 9402 with the binary vector pBIN19 containing the p35S GUS INT gene (Vancanneyt *et al*., 1990) was used. For plant regeneration 0.5 mm sections of root material were excised and treated with a liquid callus-inducing medium (C23γ) (Guerche *et al.* 1987) for three days. After that they were placed on N5 medium with antibiotics. The GUS staining was carried out according to Jefferson *et al*. (1987). *Agrobacterium tumefaciens* strains: I) GV3101 with the vir plasmid pMP90, II) the strain C58C1 ATHV with the vir-plasmid pTiBo542 (=pEHA101; Hood *et al*. 1986), a strain similar to EHA101 (Jin *et al.* 1997, Hood *et al*. 1987) were used. The selectable marker gene, *npt*II (*neomycin phosphotransferase*) was used. The reporter gene *β-Glucuronidase* (*GUS*: Jefferson *et al*. 1987) under control of the Ubi and the 35S-Promotor and with an Intron (Vancanneyt *et al*. 1990) was used. Stem segments proved to be the best explant. Shoot regeneration in *Agrobacterium rhizogenes* transformation experiments was not succesful. Regeneration from *Agrobacterium tumefaciens* mediated transformation proved to be successful. Insertion of salt tolerant genes (AtNHX1) from *Arabidopsis thaliana* in the popular varieties of *Brassica* genotypes is in progress. The transformed rapeseed varieties will be used by the farmers of the coastal wetland of Bangladesh.

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

Introduction

Agricultural productivity is severely affected by soil salinity. One possible mechanism by which plants could survive salt stress is to compartmentalize sodium ions away from the cytosol. Overexpression of a vacuolar Na+/H+ antiport from *Arabidopsis thaliana* that promotes sustained growth and development in soil watered with upto 200 millimolar sodium chloride. This salinity tolerance was correlated with higher-than-normal levels of *AtNHX1* transcripts, protein, and vacuolar Na+/H+ (sodium/proton) antiport activity. These results demonstrate the feasibility of engineering salt tolerance in plants. Keeping this view in mind, we attempted the generation of salt tolerant rapeseed (*Brassica campestris* and *Brassica napus*) varieties in Bangladesh by the application of genetic transformation. To achieve the goal, the salt tolerant gene *AtNHX1* from *Arabidopsis* is to be inserted in Rapeseed varieties.

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 also have industrial applications in plastics, lubricants, lacquers and detergents. Approximately 70% of total *Brassica* under cultivation is *B. campestris*. *Brassica napus* varieties are also becoming popular due to its high yield potential. However, due to a remarkable crop loss of 30-100% in these species, caused by intolerance to high salt levels, farmers of the coastal areas of Bangladesh are reluctant to cultivate them. Conventional plant breeding methods alone are insufficient to solve this problem. However, the application of genetic engineering will be able to contribute significantly to combat the situation. Therefore, it is essential to develop a DNA delivery system for the transfer of salt tolerant characteristics to 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 low regeneration frequency and very few transformants the production of transgenic plants have not yet been established in Bangladesh. 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 *Agrobacterium*-mediated transformation. Furthermore, this DNA delivery system will be utilized for the genetic transformation of these species for salt tolerance.

Material and methods

A protocol was developed for efficient, rapid and stable transformation of the local varieties of *B. campestri and B. napus*. 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 half-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 darkness and at 30ºC.

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 (Figure 1). 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 2). 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: I) 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.

The selectable marker gene *npt*II gene (neomycin phosphotransferase) was 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) wasused.

Figure 1: Stem segments with hairy roots

Figure 2: 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. In *Agrobacterium tumefaciens*-mediated transformation successful shoot regeneration was obtained from the transformed hypocotyls (Figure 3 & 4). 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 plant. Successful flowering occurred and finally the seeds were harvested from the mature plants.

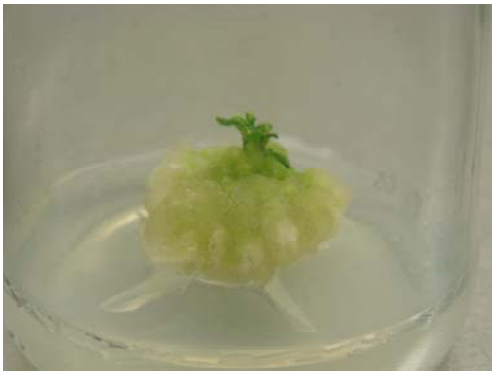

Figure 3: Regenerated shoot on selection media

Figure 4: Growing shoots

Discussion

For the confirmation of putative 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 *ß-glucuronidase* expression and Northern analysis will be done to test the expression

of the inserted genes.

The transformation protocol will be utilised for the delivery of a 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 an important role in poverty alleviation.

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