

Development and Identification of Soybean Plants with an Increased Tolerance to Osmotic Stress (in progress...)



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BACKGROUND

Soybean (*Glycine max*) is a commercially significant crop worldwide that delivers a source of protein, oils, and carbohydrates as well as many vitamins and nutrients for human intake and animal forage. Adverse circumstances such as high salinity soils affect 19.5% of irrigated agriculture lands and are responsible for the loss of 2 million hectares (1% of total world agriculture land) each year (Ashraf & Foolad 2007). Irrigation with saline water is a top contributor to soil salinity, resulting in the loss of productive agriculture lands. Soybean is a crop most affected by this and, consequently, its production is not meeting consumer demands. In order to combat this agricultural issue, we are attempting to develop a salt tolerant soybean cultivar by overexpressing the *codA* gene.

ABSTRACT

Increased osmotic stress is a major inhibitor of plant growth worldwide. One of the ways in which plants combat increased osmotic stress is through the accumulation of glycine betaine (GB), which is produced through a two-step oxidation reaction. However, not all plants produce GB in large quantities or at all. In order to address this issue, genes from plants that synthesize GB and GB-biosynthetic genes from bacteria have been introduced into low producers/non-producers of GB to create transgenic lines (Chen & Murata 2011). A commonly used bacterial gene is *codA* which codes for choline oxidase A (COX) (Chen et al 2011). COX is the rate limiting step in the oxidation of choline to GB and by increasing expression of *codA*, GB production and the plant's tolerance to osmotic stress will also increase.

Soybeans are an example of low GB producers and one of the most economically important crops worldwide. Therefore, it would be beneficial to create transgenic soybeans with an increased tolerance to osmotic stress. In this experiment, we are attempting to insert the *codA* gene via *Agrobacterium* into soybeans using the cotyledonary node method.

MATERIALS & METHODS

In this project, the construct pHL093 (shown below) in *Agrobacterium* strain LBA4404 was used to transform *Glycine max* v. William 82. The *codA* gene in the construct is driven by the constitutive corn ubiquitin promoter. Following the expression cassette is the *bar* gene, which confers resistance to the herbicide glufosinate and allows for selection of transformants.

The cotyledonary node method is used to transform the soybeans. After germinating for 5 days the explants were wounded and infected with the *Agrobacterium*. The explants and *Agrobacterium* were then allowed to co-cultivate for an additional 5 days before transferring them to shoot induction medium. After 4 weeks on shoot induction medium the explants were transferred to shoot elongation for an additional 2-8 weeks. Once the shoots reached a length of about 1 in. they were transferred to rooting medium for two weeks before transplanting the shoots.

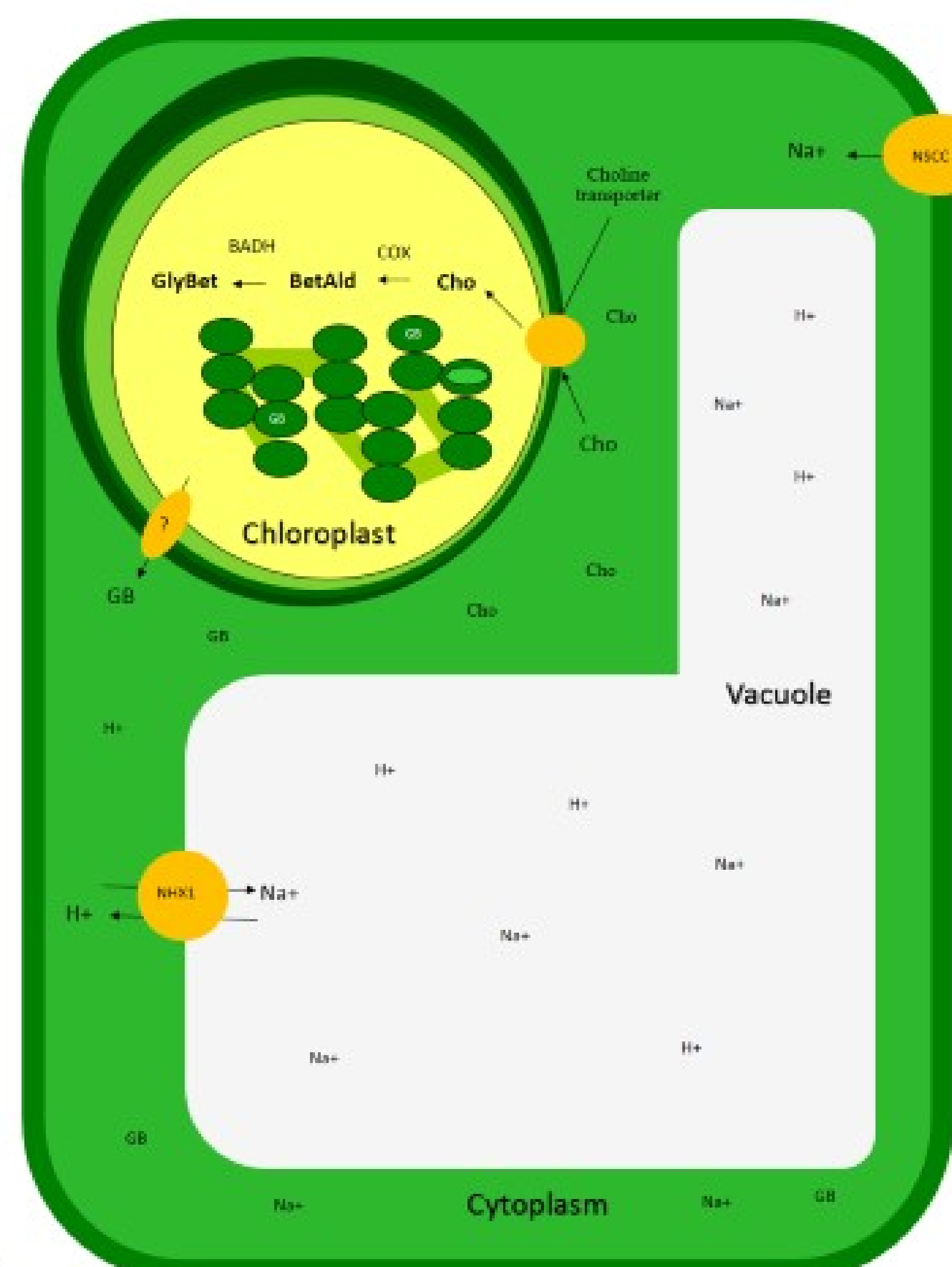
This is an ongoing project and transformed plants will be analyzed by Southern blotting. F₁ progeny will also undergo testing by herbicide leaf painting, Southern blotting and salt stress tests to determine level of increased salt tolerance.

GLYCINE BETAIN MECHANISM

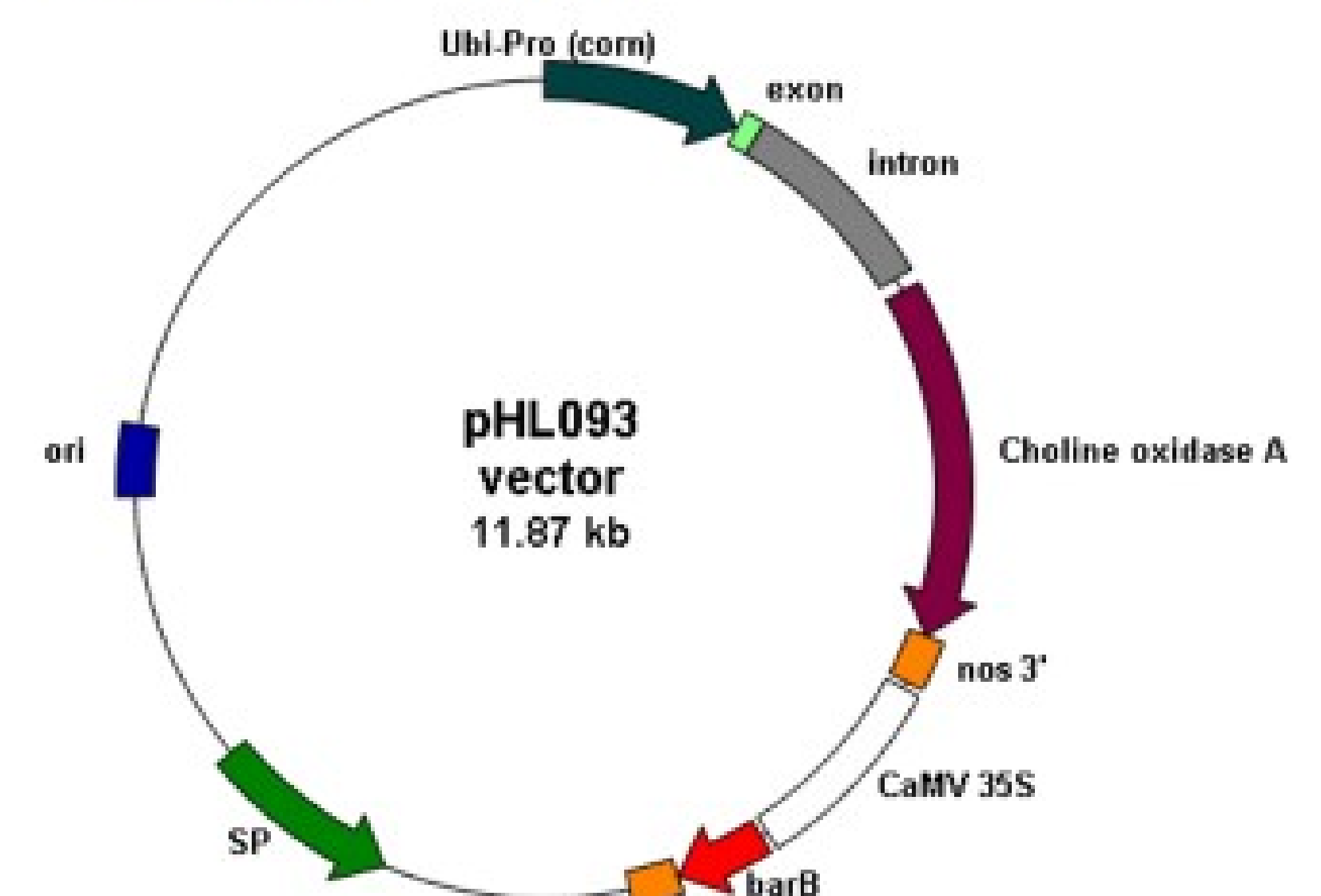
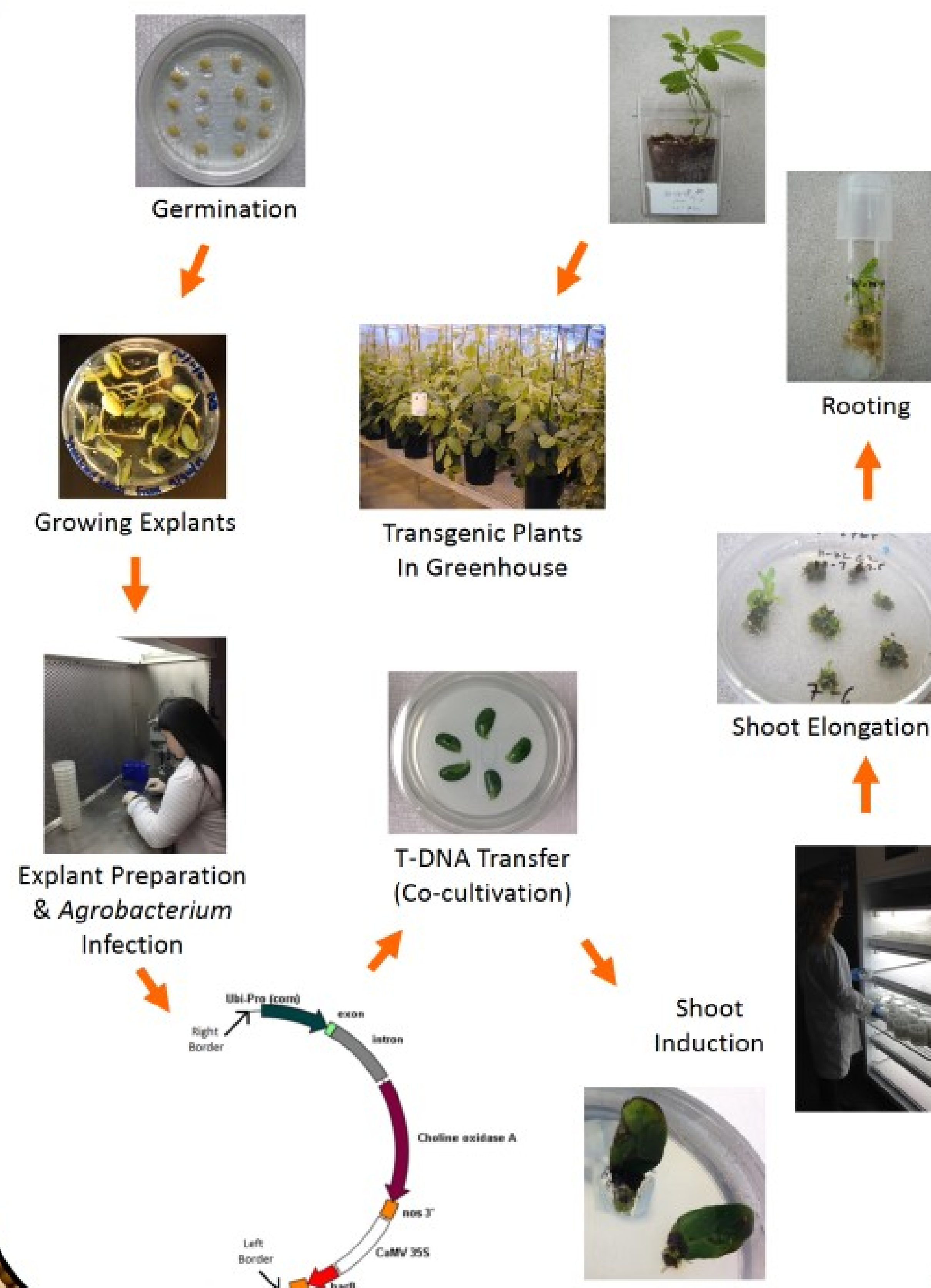
The mechanism by which GB protects the cell against osmotic stress is complex and not completely known. GB is synthesized in the chloroplasts when choline (Cho) is oxidized by COX to betaine aldehyde which is then further oxidized by betaine aldehyde dehydrogenase (BADH) to GB. After synthesis, some GB leaves the chloroplast and enters the cytosol.

Osmoregulation

- Maintaining osmotic balance in cytosol (Demiral & Turkan 2004)
- Protection of Photosystem II complex by protecting thylakoid membranes (Demiral et al 2004)
- Protection of quaternary protein structures by preventing dissociation caused by dehydration (Demiral et al 2004)
- Protection of RUBISCO (Demiral et al 2004)
- Maintaining structural integrity of plasma membrane by interacting with membrane proteins, maintaining turgor (Mansour 1198)



Agrobacterium-mediated Soybean Transformation



RESULTS

Four experiments were performed in which 1,988 explants were wounded and infected with *Agrobacterium* strain LBA4404 containing pHL093.

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