OHIO AGRICULTURAL RESEARCH AND DEVELOPMENT CENTER

Yield improvement in *Taraxacum kok-saghyz* by expression of deinhibited Rubisco

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ABSTRACT

Taraxacum kok-saghyz (TK), an important alternate natural rubber (NR) crop, is under commercial development for production of high quality, high molecular weight, rubber. TK can supplement the global NR supply and offer a partial sustainable replacement of petroleum-derived synthetic rubber.

TK's ability to assimilate carbon from atmospheric CO_2 is a key rate-limiting step of projected rubber yields. In all photosynthetic organisms, the rate of CO_2 uptake is reduced as O_2 builds up in active tissues and inhibits the enzyme responsible for carbon capture, Rubisco (Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase). Overcoming this limitation will increase carbon capture and may boost yield through increased plant size and/or root rubber concentration.

INTRODUCTION

New biotechnological tools may maximize CO_2 assimilation by the alternative rubber crop, TK. This will ensure rapid, environmentally and financially sustainable, expansion of rubber crops and rubber raw materials [1]. The Rhodophyta CO_2 assimilation enzyme Ribulose-1, 5-Bisphosphate Carboxylase/Oxygenase (RUBSICO) has been de-inhibited by Prof. Tabita of The Ohio State University, so that substantially higher rates of assimilation can be achieved than naturally occur [2]. This enzyme can be expressed in chloroplasts of the few species for which chloroplast transformation methods have been developed, along with the recently produced TK chloroplast genome sequence [3], which allows us to apply this method to TK. Since rubber is a sink for excess assimilate, expression of de-inhibited RUBISCO in this species may lead to both larger, more vigorous plants, and high rubber production. A successful outcome of this research would also provide the proof-of-concept needed to justify incorporation of deinhibited RUBISCO into broad-leaf food crops, to increase production with minimal increase in acreage.

MATERIALS AND METHODS

Biolistic plant transformation [4] was used to introduce the mutated native TK *rbcL* gene, A378V [2] and a plant promoter driven *bar* gene [5] to provide resistance to the herbicide phosphinothricin (glufosinate). The construct used for biolistic introductions of a mutated *rbcL* was built into the pUC19 multiple cloning site in an *E.coli* vector. The *aadA* gene, which encodes aminoglycoside 3′-adenyl-transferase (*aadA*) and confers resistance to spectinomycin, was cloned from a pYZ plasmid, with restriction sites ClaI and BmtI [6] added to the 5' and 3' ends of the product, respectively, then introduced downstream of the TK *rbcL* gene (Fig. 1).

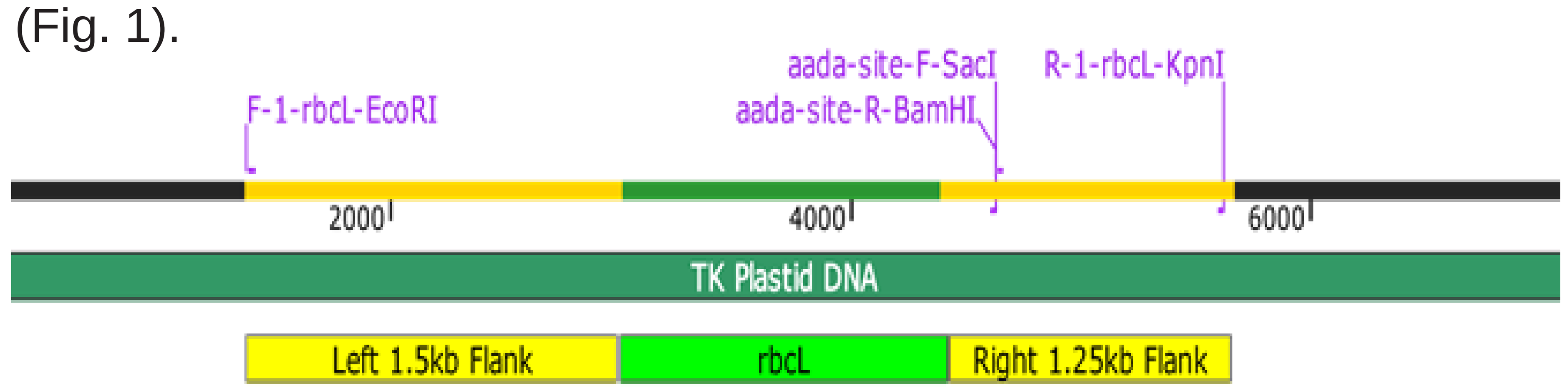

Figure 1. The aadA gene will be inserted downstream of the native rbcL gene. Along with the ~1 kb region of the rbcL gene, an additional ~1.5 kb of flanking region will be cloned into the vector as well for homologous recombination.

Restriction sites XbaI and SbfI were added to the *rbcL* gene during cloning corresponding to unique 6+ cutter sites found in the pUC19 vector (Fig. 2). Root transformations were used because of the rapid regeneration time [7]. Plants produced aseptically were bombarded and grown under rapid liquid media selection (Fig. 3). Homologous recombination of the native TK *rbcL* gene, at the site of the mutation in the chloroplast genome, is required to introduce the A378V mutation [6]. There can be up to 10,000 copies of the circular genome in each chloroplast, and hundreds of chloroplasts per plant cell, so we must select for stable homoplastidic plants through gradually increasing selection pressure over time[2].

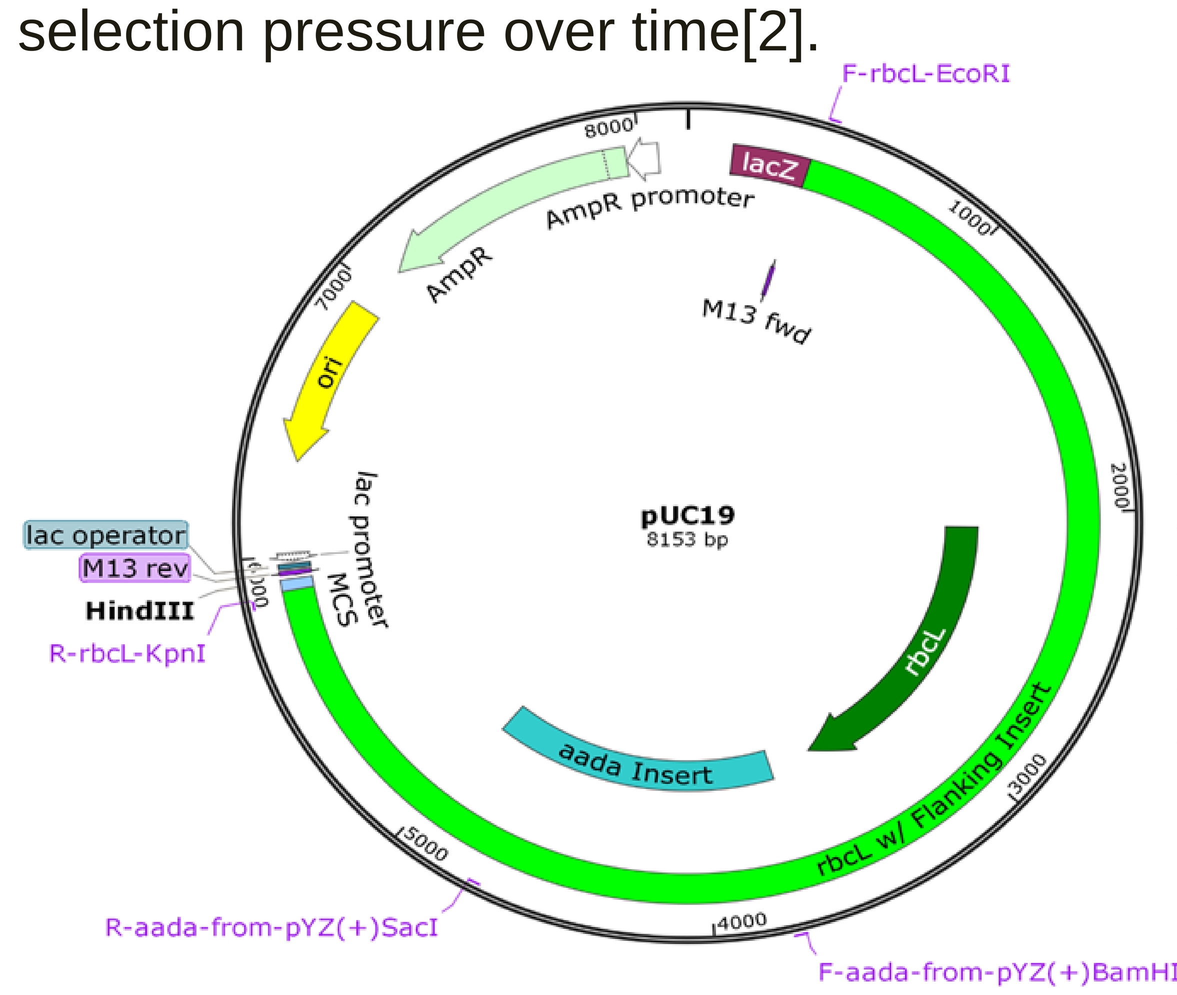

Figure 2. The full construct (~8 kb) to be used for native TK Rubisco modification is based on the pUC19 vector. The construct carries the modified rbcL gene along with the inserted downstream aadA.

METHODS (cont.)

Large quantities of TK roots for transformation targeting were produced using aseptic hydroponics (Fig. 3).

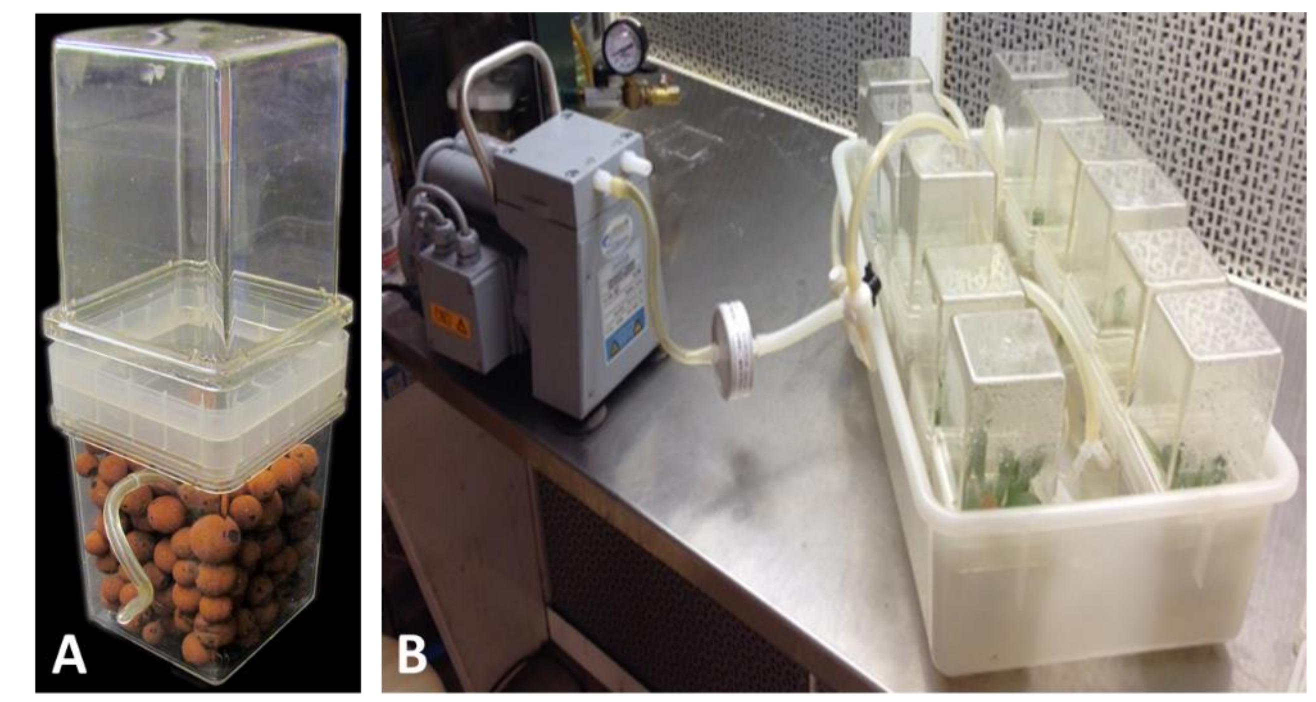

Figure 3. (A) Hydroponically grown TK roots being used as target tissue with spectinomycin as selection agent; (B) concentration is increased over time.

RESULTS AND DISCUSSION

Plants will be analyzed by extracting total DNA used for amplification of the *rbcL* gene and flanking regions for Sanger sequencing. Positive transformants will be grown to maturity in the greenhouse and growth chambers at normal and elevated O_2 levels, then characterized for size, inulin and rubber content.

Expected results will include:

1. First expression of A378V mutation in plants.
2. Plastidic expression using biolistic approach.
3. Reduced O_2 sensitivity in active organelles.

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