



University of Zabol

Department of Plant breeding and Biotechnology

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Agricultural Biotechnology

Title

Seed oil content increasing in safflower (*Carthamus tinctorius* L.) by transferring of
gene combinations *DGAT1*, *CYP78A* & *WR11*

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Abstract:

In this study, in order to increase the amount of safflower seed oil, in first stage *in silico* study of SBP sequence as a possible specific promoter in seeds was performed and seed specific *cis-elements* identified. Then, molecular analysis and histochemical assay were performed on tobacco model plant with SBP promoter and the results showed that this sequence probably has a specific function as a precursor in seed. In the second stage, three genes *DGATI*, *CYP78A* and *WRI1* were choose as effective genes in seed oil content increasing in oilseeds, and use in five gene constructs under the control of specific seed promoters Napin and SBP. Then, the function of these genes in tobacco model plant with stable and transient gene transferring was investigated and confirmed. Expression of genes by Real time PCR method showed the proper function of the promoters and gene constructs. Transgenic tobacco plants were successfully transferred to the second and third generations. Analysis of oil and fatty acid content in third generation transgenic tobacco plants showed an increase in seed oil content between 1.9 to 2.2 times in transgenic plants with different gene constructs. After obtaining acceptable results in tobacco model plant, callus formation and callus regeneration were optimized in safflower cultivars. According to the results, the use of TDZ and NAA were identified as suitable hormones for callus formation. Also, for the first time, the *in vitro* micro grafting method for adaptation of regenerated Iranian cultivars safflower to the soil environment was performed by more than 70% success. Then, gene transfer optimization to Iranian Safflower cultivars was successfully performed by GUS gene transfer, which showed that Padideh and strain EHA105 showed the best response to transgenics. Finally, transgenic safflower plants in the first, second and third generations were confirmed by molecular analysis. Seeds of safflower plants from transgenic pDGAT-WRI1 genes showed a 15.8% increase in oil content.

Key words: Fatty acid, Transgenic, Recombinant gene structure, Seed specific promoter, Genetic engineering