

Production of some wheat lines resistant to some unfavorable conditions

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This study aimed to introduce a stress-related gene in order to improve tolerance of one Egyptian wheat cultivar (Giza 164) to oxidative stress, i.e., drought and salinity. This goal was achieved by introducing plant expression vector pLe-GST Thar harboring one of oxidative stress tolerance-related genes namely Le-GST, *Lycopersicon esculentum* glutathione s-transferase, that generates a signal for scavenging and detoxification of the highly reactive oxygenated compounds (ROS) into the genome of the cultivar (Giza 164) using immature embryo-derived calli as a target tissue and microprojectile bombardment as a transformation device. However, the Le-GST gene was driving by CaMV 35S promoter. Plant expression vector pLe-GST/bar also contained bar gene as a selectable marker for the herbicide bialaphos resistance. The main results could be summarized as follows:

1. The plasmid pLe-GST/bar was constructed by ligating the 2.09 kb HindIII fragment containing bar gene cassette, which has been taken from pAB6 (9.45 kb), to the 4.696 kb pAM3 5 S/Le-GST-T3.
2. Calli were obtained via immature embryo culture on TW callus induction medium for transformation experiments.
3. A number of transformation experiments were conducted in which bialaphos, at 0 or 2 mg/L concentrations was used as a selective agent during the callus induction period.
4. For plant regeneration, a concentration of 0 or 2 mg/L bialaphos was used and regenerated calli were transferred to the rooting medium.
5. For rooting, one-half strength medium with 0 mg/L bialaphos was used as a selective agent and all rooted plantlets were transferred to the soil for acclimatization.
6. Putative transgenic plants were tested for bar gene expression by painting a segment of a well developed green leaf with one-half the recommended dose of basta (1g/L), the commercial name of the herbicide, on both sides.
7. Two transgenic plants were shown to resist the herbicide with no symptoms of necrosis. These two transgenic plants were chosen for subsequent molecular analysis.
8. Presence of both Le-GST' and bar genes was confirmed using PCR and PCR/Southern in which they resulted in the production of the expected DNA sizes of both genes, i.e. 840 and 400 bp, respectively.
9. PCR was done for the nine T1 transgenic progeny for both Le-UST and bar genes which gave the expected band sizes.
10. On the expression level, RT-PCR for Le-GS7' gene showed the expected band with the approximate size confirming its expression in three T1 plants.
11. It is recommended that this transgenic event will be evaluated under drought and/or salt stress using a higher number of individuals and replicates in subsequent generations.