The role of a grapevine-derived acetolactate synthase gene as a selectable marker for precision breeding of *Vitis*.

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Introduction

Precision breeding (PB) is a newly-enabled approach to plant genetic improvement that transfers only specific desirable traits among sexually-compatible relatives via the mitotic cell division pathway to avoid the genetic disruption imposed by meiosis. PB builds upon decades of both fundamental and applied research aimed at bypassing the disruption of sexual reproduction (meiosis) by allowing gene insertion to be accomplished via the significantly more stable and predictable mitotic cell division pathway. Recent advances in the development of cell culture protocols for efficient plant regeneration combined with crop genome sequencing have opened new avenues for the movement of specific functional traits among sexually compatible crop cultivars. A grapevine derived MybA1 transcription factor was recently studied and characterized for its use as a reporter gene in plant transformation. We are currently studying the grape-derived tolerant acetolactate synthase (ALS) gene, VvALS2, that might potentially confer herbicide resistance and can be used as a marker gene for selection of modified events in cell culture. In the current study, the effect of different herbicides including Monument® and Image, on inhibition of tobacco shoot cultures and grape embryogenic cultures will be studied to determine the optimum levels of herbicide that can be used for selection at the cell culture level. These studies will enable the use of the acetolactate synthase gene for the recovery of modified events in cell culture and regeneration of whole plants with traits of interest.

Materials and Methods

Kill Curve: Five concentrations of Monument® 75WG (Sygenta International AG; Basel, CH) herbicide were used to determine tobacco sensitivity to the ALS inhibitor herbicide. The recommended rate of use is 500mg for every 2 gallons of water. The concentration for the active ingredient tested were 0, 0.1, 0.5, 1.0, and 5.0 mg per L. Leaf disks were grown on DD2 medium with the different concentrations and observed for growth.

Explant preparation. 300 seeds of tobacco cultivar 'Samson' were surface-sterilized in 50% commercial bleach solution followed by three washes in sterile distilled water. Seeds were then transferred to MS medium and maintained at 25°C and 18 h photoperiod. Leaf discs were obtained from 21 day old seedlings and used in gene insertion studies.

Preparation of Agrobacterium. Agrobacterium harboring the VvALS2 gene were grown overnight on a rotary shaker at 28°C in MG/L medium. The overnight culture was then centrifuged at 6000 rpm for 8 min to obtain a bacterial pellet. The pellet was resuspended in X2 medium and grown for an additional 4 h prior to gene insertion experiments.

Agrobacterium-mediated transformation. Tobacco leaf discs were used as explants for gene insertion experiments. Explants were submerged in *Agrobacterium* containing a mutated grapevine ALS gene, for eight minutes and then blotted on sterile filter paper to remove excess bacterial culture. Explants were then transferred to regeneration medium for shoot proliferation. Modified shoots identified were excised from proliferating cultures and transferred to MS medium containing 200 mg l^{-1} each of carbenicillin and cefotaxime and 100 mg l^{-1} kanamycin for rooting.

Results and Discussion

The acetolactate synthase (ALS) genes code for the ALS enzyme involved in the production amino acid biosynthetic pathways (Sato et al., 2009). This enzyme functions to catalyze reactions for amino acid synthesis in the chloroplast. Sulfonylurea herbicides bind to the ALS enzymes and disrupt their catalytic functions. This causes the cell to effectively halt the synthesis of leucine, isoleucine, and valine amino acids (LaRossa and Schloss 1984). This causes stagnation in plant growth due the lack of essential building blocks. Plants can have an altered gene coding for ALS yielding ALS enzymes resistant to ALS inhibitors (Shimzu et al., 2011). This allele has a nucleotide difference that creates a missense mutation. The result is the production of an enzyme that still has the ability to perform its normal functions but has an altered herbicide binding site. Plants that contain the altered ALS gene are not affected by ALS herbicides (Shimzu et al., 2011) since these herbicides cannot bind and cannot interrupt plant growth. We are currently evaluating the effect of different levels of herbicide on tobacco shoot cultures and obtaining transgenic plants expressing the VvALS2 gene.

Conclusions

This study is ongoing and we are currently amassing datum from experimental repetitions to support conclusions made which pertain to the feasibility of VvALS2 use as a selectable marker in transgenic cultures. Transgenic tobacco plants expressing the VvALS 2 gene will be sprayed with herbicide to study plant resistance. Additionally grapevines expressing ALS2 will also be generated to study its potential as a selectable marker for precision breeding of grapevine.

References

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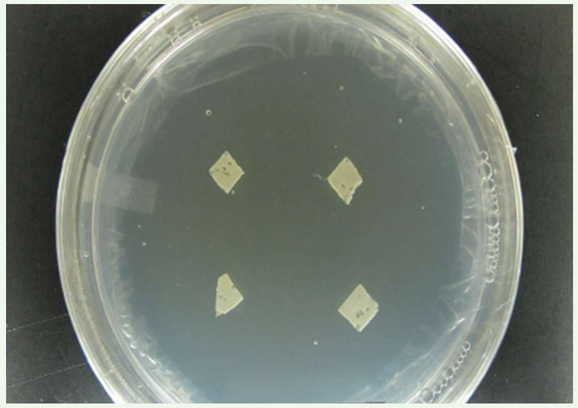
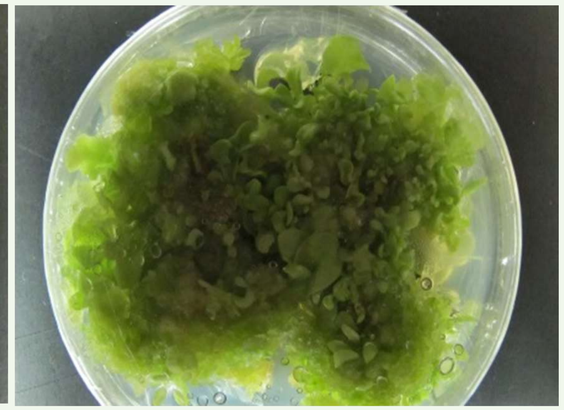
Table 1: Serial concentrations of Monument® 75WG (ALS inhibitor herbicide) integrated into DD2 medium.

Treatment Level	Monument® 75WG mg	Stock of 10 mg/10 ml	Medium DD2
M-1	0	0	100
M-2	0.1	0.1 ml	100
M-3	0.5	0.5 ml	100
M-4	1	1 ml	99
M-5	5	5 ml	95



Monument @ 1.0 mg per l



Control – no herbicide

Fig 1. Transgenic tobacco expressing plant pigmentation genes. a) Tobacco response to a 1mg/l of herbicide and control; b) Control tobacco plate; c) Cocultivation of tobacco with agrobacterium ; d) Transformed tobacco cultures in laminar air flow hood.

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