DEVELOPMENT OF FRONTENAC BLANC, A COLD-HARDY DISEASE-RESISTANT WHITE WINE GRAPE

PROGRESS REPORT TO VITICULTURE CONSORTIUM EAST AND THE NEW YORK WINE AND GRAPE FOUNDATION

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Objectives:
To develop Frontenac blanc, a white-fruited version of the cold-hardy and disease-resistant wine grape variety Frontenac.

Background and Procedures:
The proposal is to use tissue culture to develop Frontenac blanc, a white-fruited version of the cold-hardy and disease-resistant wine grape variety Frontenac. Through tissue culture an individual cell collected from the interior cell layer of Frontenac gris will be grown into an entire vine. This vine will bear white fruit, but will be horticulturally identical to Frontenac. This white-fruited grape variety, called Frontenac blanc, will be useful to grape growers and wine makers across the colder regions of eastern North America.

Frontenac is a black fruited grape variety used for red wine. Frontenac is very hardy (to at least -30 ºF) and is cultivated across eastern North America, including New York, Vermont, Minnesota, Kansas, Nebraska, Illinois, Iowa, Indiana, Wisconsin, and Canada. Introduced in 1995, it has been quickly and enthusiastically adopted by grape growers, wine makers, and wine drinkers for its high quality red wines, often distinguished by cherry aromas.

A natural bud sport of Frontenac with gray skinned fruit was found in the University of Minnesota experimental vineyard. The gray skinned sport is called Frontenac gris. In all aspects of the vine, its horticulture, disease resistance, cold hardiness and other attributes it is identical to Frontenac. Only in the wine and fruit are differences realized. While the black fruited Frontenac produces red wines with cherry aromas, Frontenac gris is used for rosé or blush wines remarkable for their apricot and peach aromas (Frontenac gris also is used in white wine making).

The natural bud sport that resulted in Frontenac gris likely comes from differences in the pigmentation of the cell layers of this vine. All grapevines have two cell layers, respectively constituting the inner and outer portions of the plant. The skin of the berry is composed of cells from both the inner and outer portions (other plant parts may be of one or both portions). In a gray fruited variety, the outer cell layer is black (with anthocyanin pigment) and the inner cell layer is white (without anthocyanin pigment). The berry skin appears gray because of the layering of black cells over white cells. In a black fruited variety, the berry skin cells are black (pigmented) throughout the thickness of the skin.

Through tissue culture, it is possible to produce plants from either of the two cell layers. This technique has been used successfully on grapes in our lab. By generating somatic embryos from anther tissue one can separate the black and white cell layers of a gray fruited variety. New plants derived from individual cells of either the black or white layer will produce black or white fruit accordingly. In the case of Frontenac gris, a white-fruited variety "Frontenac blanc" will be recovered. The original Frontenac (with black fruit) also will be recovered.

To create Frontenac blanc, immature Frontenac gris flowers will be collected and shipped to The Ralph M. Parsons Foundation Plant Transformation Facility at the University of California, Davis in the spring of 2006 . Flowers will be surface sterilized and using a dissecting microscope the anthers will be excised and plated on a range of media known to promote embryo development from the anther filament cells. Once formed, embryos will be encouraged to germinate into whole plants. The entire procedure from collection of anthers to production of small plants will take between twelve and eighteen months.

Following the generation of the plants, genetic sequence testing will reveal which of the young plants will bear white fruit. Christopher Owens perfected the technique that allows "color testing" of small grapevines from breeding programs, predicting the skin color of the grapes they will eventually bear. This method will be applied (during 2007) to determine which of the young plants will bear white grapes—the new Frontenac blanc vines—so that these vines can be selected for vineyard planting.

Progress to Date:

Plant Material

Three shipments of Frontenac gris flower buds were sent to the Ralph M. Parson Plant Transformation Facility in Davis, CA during the spring of 2006. One shipment from Vincennes Indiana was received on 5/9/2006. These buds were more developed than normally used for the development of embryogenic callus cultures and only a small number of anthers were collected from this shipment and cultured *in vitro*. An additional shipment was received on 5/16/2006 from Lafayette Indiana and on 5/25/2006, from Minnesota and these were the appropriate developmental stage.

Results

Embryogenic callus was produced from Frontenac gris anthers from flowers from both the Indiana and Minnesota locations. Although nine different media were employed, two, (AIM and MSE) produced the majority of embryogenic callus to date (Table 1). Upon transfer to GERM media, embryos quickly germinated (Figure 1) and form vigorous plants upon transfer to rooting media (Figure 2). Plants are readily acclimated to soil and thirteen have been transferred to C. Owens in Geneva, NY for analysis.

Since multiple anthers were plated per flower, the developing callus may be of single cell decent or may be from independent anther filaments. If shoots arise from callus of single cell decent they should all express the same fruit color. However, if plants arise from independent cells on a single anther filament or from cell on independent filaments, they could have different fruit color from one another depending upon which cell layer generated the callus. For the purposes of this project, we are developing multiple plants per callus piece but maintaining them as clonal events knowing that they may be in fact be independent events. Known independent event are being delivered for analysis and multiple "clones" will be delivered later.

Beginning January, 2007, plants received from Davis, CA will be tested for the presence of the white alleles at the grapevine color locus by extracting DNA from leaf tissue of these plants and conducting a PCR-based assay to determine the genetic makeup of the color locus. Plants showing only white alleles at these locus will be saved to reach a sufficient growth stage to develop clusters.

Table 1. Summary of embryogenic callus formation from anthers plated on various embryogenic callus induction media

Location flower buds	Medium	# of Anthers plated	Date and Number of anthers developing embryogenic calli							
			08/09	08/22	09/06	10/02	10/31	11/14	12/05	12/21
Lafayette	LeGall et al., 1994 (NB)	182								
	Perl et al., 2004 (AIM)	128				2				2
	Franks et al., 1998 (GSICA)	116			1	3				2
	Hanson et al., 1999 (PT)	158								
	Mauro et al., 1995	97								
	Ling et al., 1999 (MSE)	133	6			2				
	HT	110								
	Franks et al., 1998 (PIV)	121								
	Torregrosa et al., 1998 (CIP)	50								
Location flower buds	Medium	# of Anthers plated	Date and Number of anthers developing embryogenic calli							
			08/09	08/22	09/06	10/02	10/31	11/14	12/05	12/21
Minnesota	LeGall et al., 1994 (NB)	140	1							
	Perl et al., 2004 (AIM)	150		4		2	1	1	2	1
	Franks et al., 1998 (GSICA)	130								
	Hanson et al., 1999 (PT)	160								
	Mauro et al., 1995	130								
	Xue et al., 1999 (MSE)	106	5	3		1				
	Iocco, et al., 2001 (HT)	109								
	Franks et al., 1998 (PIV)	221				1		1		2
	Torregrosa et al., 1998 (CIP)	131			1	1				

Figure 1. Frontenac gris somatic embryos developing from callus from excised anthers.

Figure 2. Regenerated plant produced from a somatic embryo of Frontenac gris, and acclimated to soil.