Overcoming the limitations to alkaloid biosynthesis in 
*Catharanthus roseus* hairy root cultures

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The *Catharanthus roseus* (Madagascar periwinkle) plant produces over 130 different alkaloids (nitrogen-containing organic compounds) including vincristine (VCR) and vinblastine (VLB), two powerful anticancer drugs. *C. roseus* produces extremely low levels of these alkaloids; to isolate 1 g of VLB, it takes approximately 500 kg of dried periwinkle leaves. Consequently, isolation of VCR and VLB from the plant is laborious and costly, ranging from $4 – 60 million/kg. Despite these barriers, these alkaloids have been used for the last 40 years for cancer treatment due to their unique mode of action and their effectiveness. For these reasons, VCR and VLB are excellent targets for enhanced production through biotechnology in order to meet their need and decrease their cost.

We are addressing these production barriers by using hairy root cultures derived from the *C. roseus* plant. Using hairy root cultures is advantageous because they eliminate the dependency on the availability of the plant, and they can be cultivated under optimum conditions that maximize growth and product formation. The culture period for optimizing growth was determined by performing a growth curve where cultures were harvested on days 5, 10, 15, 20, 25, and 30. The optimum subculture period was determined to be 21 days while the exponential phase (the optimum period for treatment) was determined to be between days 10 and 23.

The biosynthesis of VCR and VLB is complex, involving at least 35 chemical intermediates and 30 enzymes. One approach to enhance the production has been to apply methyl jasmonate (MeJA), a plant hormone produced as a stress response (such as infection by pathogens). MeJA increases alkaloid production by enhancing expression of multiple genes. This mechanism led to the discovery of a transcriptional regulator, ORCA (octadecanoid-responsive *Catharanthus* AP2-domain protein). ORCAs are induced by MeJA and are responsible for increasing the expression of multiple genes in the pathway and ultimately increasing the production of alkaloids in cell cultures. Additional transcriptional regulators from 5 different classes have been discovered in *Catharanthus*, including the class known as zinc finger proteins (ZCT).

We confirmed the above literature findings in our *C. roseus* hairy root cultures by monitoring Orca and Zct gene levels using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Hairy root cultures were elicited with 100µM MeJA during exponential growth phase (day 20). Significant increases in Orca and Zct gene levels occurred compared to non-treated cultures (30 fold and 4 fold, respectively).

Future work will investigate how ORCA and ZCT collaborate to control alkaloid gene expression and alkaloid production. A transgenic hairy root culture line will be generated to test our model for how alkaloid production is regulated.