

Genetic mapping indicates that *orobanche1* does not appear to be associated with the XanL subunit of Mg-protoporphyrin IX monomethyl ester cyclase.

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The yellow green/tan necrotic seedling mutant *orobanche1*, isolated by Dale and Anderson (MNL 24:13), was originally placed to chromosome 6. This placement was made due to the segregation of *y1* in *oro1* stocks (Ed Coe, personal communication). However, during propagation of *oro1* stocks at the Stock Center, it became clear that *oro1* is either not linked to *y1*, or is very loosely linked at best.

By studying the accumulation of chlorophyll biosynthetic precursors in *oro1* seedlings, Peter Mascia (1978. Mol Gen Genet 161:237-244) found a block in the conversion of Mg-protoporphyrin monomethyl ester to protochlorophyllide. This step is catalyzed by the enzyme Mg-protoporphyrin IX monomethyl ester cyclase (MPEC). In barley, this enzyme is multimeric, comprised of several subunits encoded by different genes (Rzeznicka et al. 2005. PNAS 102:5886-5891). One of these subunits, XanL, has been cloned, and its sequence was used in a BLAST search of the maize genome. Two hits were obtained: GRMZM2G081462 on chromosome 8 and GRMZM2G043109 on chromosome 3. A second barley gene, *ycf54*, may also be associated with MPEC (Bollivar et al., 2014. FEBS J. 281:2377-2386). The maize gene was cloned by Belcher et al. (2015. Biochimica et Biophysica Acta 1847:1004-1016) and is associated with gene model GRMZM2G010196 on chromosome 1.

The *oro1* mutant phenotype is partially reversed by a dominant allele at a second locus, *orom1* (Mascia, 1978), suggesting the possibility that *oro1* is part of a duplicate factor pair. Since XanL has two homologues in maize, on chromosomes 3 and 8, we decided to use waxy marked translocations to determine whether *oro1* maps to either of these two chromosomes. We decided to use the translocations *wx1 T3-9(8447)* and *wx1 T8-9d* because they have breakpoints closest to the physical location of these two genes. The mapping crosses and results are presented in Tables 1 and 2. Neither translocation showed linkage of *wx1* with *oro1*. These results suggest that *oro1* does not encode XanL in maize. The possibility remains that *oro1* is associated with one of the other subunits of MPEC, or perhaps it is related to some other aspect of the conversion of Mg-protoporphyrin monomethyl ester to protochlorophyllide.

Table 1. Linkage data for *oro1-6577* and *wx1 T3-9(8447)*. Cross: *wx1 N + X [wx1 T3-9(8447) + X Wx1 N oro1-6577]*

Class	Phenotype	No.
Parentals	Wx N oro	18
	Wx T oro	3
	wx T +	25
	wx N +	2
Recombinants	Wx N +	39
	Wx T +	0
	wx T oro	25
	wx N oro	1

% crossing over *wx1 - oro1* = 57.5 +/- 4.7

% crossing over *T3-9(8447) - wx1* = 5.3 +/- 2.1

Table 2. Linkage data for *oro1-6577* and *wx1 T8-9d*. Cross: *wx1 N + X [wx1 T8-9d + X Wx1 N oro1-6577]*

Class	Phenotype	No.
Parentals	Wx N oro	26
	Wx T oro	0
	wx T +	25
	wx N +	1
Recombinants	Wx N +	31
	Wx T +	0
	wx T oro	31
	wx N oro	0

% crossing over *wx1 - oro1* = 54.4 +/- 4.7

% crossing over *T8-9d - wx1* = 0.9 +/- 0.9