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**Development of Peanut Cultivars with Enhanced Resistance to Fungal Disease
Project Report for January – June 2003**

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Project Goals:

To produce transgenic peanut containing the oxalate oxidase gene in Virginia-type cultivars, to evaluate gene expression, genetic transmission and disease resistance in multiple transformants, and to select lines with superior performance

Project Results:

1. *Recovery and growth of transgenic plants*

Transgenic plants have been obtained from three Virginia-type cultivars (Wilson, Perry and NC-7). Many plants have now grown sufficiently to begin flowering and of those, a few have progressed to produce pegs. We will plant seed recovered from these initial transformants to study the patterns of genetic transmission of the oxalate oxidase transgene and to increase seed for field-testing in 2004.

2. *Testing for gene expression and resistance to oxalic acid*

Transformed plants have been tested for activity of the oxalate oxidase transgene (see Figure 1). Plants with the transgene showed a two- to three-fold increase in activity of oxalate oxidase in comparison to non-transformed controls.

Transformants that produced high levels of oxalate oxidase were then tested to determine their ability to resist exposure to oxalic acid, a compound produced by *Sclerotinia minor* during infection. A leaf-lesion bioassay was used to measure the response of plants to different levels of oxalic acid (Table 1). Plants expressing oxalate oxidase developed smaller lesions in response to oxalic acid than plants that did not contain the transgene.

Figure 2 shows a graph of results for three different peanut lines: 1) non-transformed Perry as control is shown in red, 2) P9 transformed Perry line is shown in green, and 3) P30 transformed Perry is shown in blue. Lesions increased in size with increasing concentrations of oxalic acid for non-transformed Perry but remained small for two transformed lines, P9 and P30.

We will present these results at the annual meetings of the American Peanut Research and Education Society (APRES) in July.

Respectfully submitted, June 2003.

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Oxalate Oxidase Activity in Transgenic Peanut

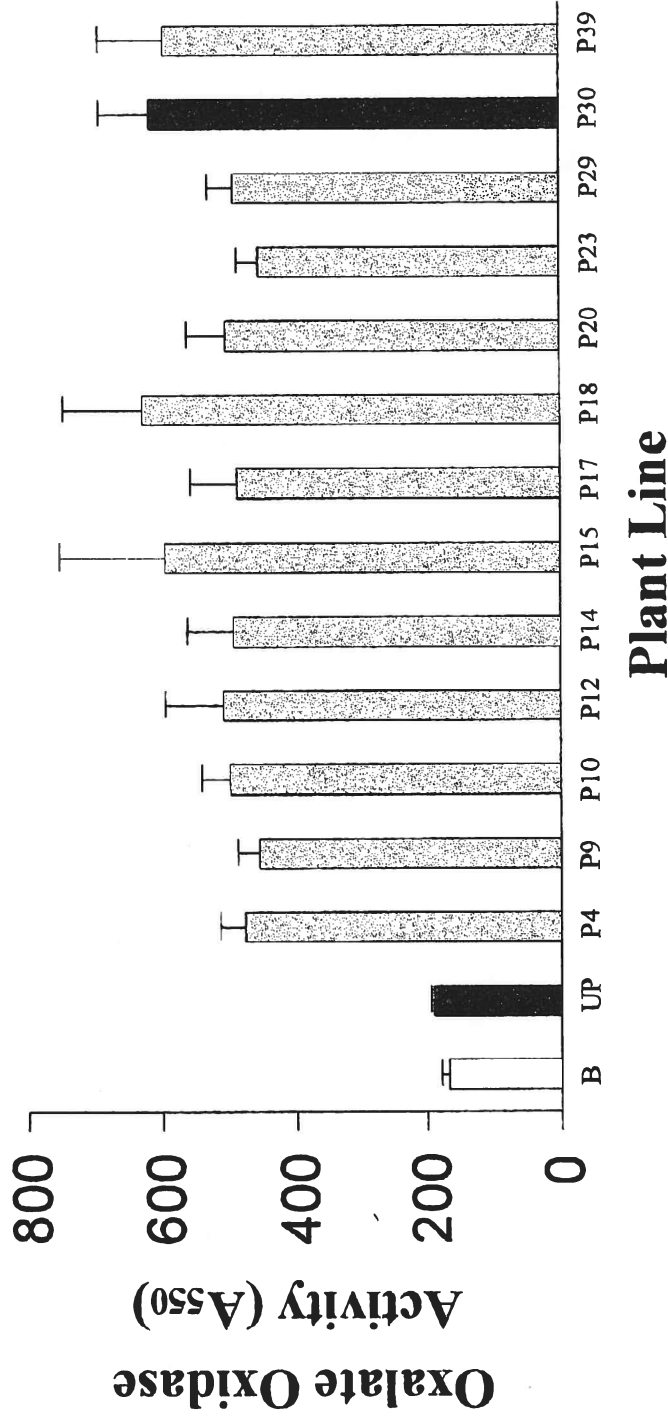


Fig.1: Oxalate oxidase activity in primary transgenic peanut lines (cv. Perry). Means are shown \pm SEM from 4 replicates. B = buffer only control. UP = untransformed Perry control.

Oxalic Acid Concentration (mM)	Lesion Size (mm ²)												
	UP	P4	P9	P10	P12	P14	P15	P17	P18	P23	P29	P30	P39
0	0.09 ± 0.04	0.01 ± 0.04	0.15 ± 0.11	0.13 ± 0.05	0.08 ± 0.06	0.12 ± 0.05	0.14 ± 0.10	0.14 ± 0.05	0.08 ± 0.04	0.06 ± 0.01	0.08 ± 0.04	0.16 ± 0.05	0.10 ± 0.05
5	0.59 ± 0.06	0.12 ± 0.03	0.27 ± 0.12	0.16 ± 0.04	0.05 ± 0.02	0.09 ± 0.01	0.18 ± 0.06	0.10 ± 0.05	0.20 ± 0.05	0.05 ± 0.01	0.16 ± 0.12	0.14 ± 0.05	0.10 ± 0.02
10	1.28 ± 0.10	0.21 ± 0.03	0.47 ± 0.16	0.24 ± 0.06	0.10 ± 0.04	0.21 ± 0.05	0.43 ± 0.08	0.29 ± 0.06	0.22 ± 0.13	0.15 ± 0.05	0.22 ± 0.10	0.16 ± 0.03	0.10 ± 0.02
20	2.35 ± 0.20	0.68 ± 0.13	0.88 ± 0.14	0.50 ± 0.10	0.35 ± 0.17	0.43 ± 0.11	0.58 ± 0.17	0.47 ± 0.26	0.92 ± 0.15	0.47 ± 0.26	0.40 ± 0.10	0.28 ± 0.05	0.19 ± 0.02
50	6.01 ± 0.34	1.16 ± 0.01	1.26 ± 0.21	0.84 ± 0.17	0.82 ± 0.25	1.30 ± 0.50	1.17 ± 0.19	0.87 ± 0.16	0.99 ± 0.16	0.91 ± 0.04	1.09 ± 0.16	0.40 ± 0.07	0.74 ± 0.10
100	9.68 ± 0.10	1.97 ± 0.31	2.19 ± 0.16	1.80 ± 0.12	1.21 ± 0.17	2.49 ± 0.39	5.27 ± 2.16	2.10 ± 0.08	2.67 ± 1.95	1.93 ± 0.50	1.62 ± 0.12	0.34 ± 0.12	1.83 ± 0.16
200	20.1 ± 0.35	3.46 ± 1.02	5.41 ± 0.61	3.54 ± 1.77	1.82 ± 0.26	7.10 ± 1.90	5.16 ± 2.16	3.25 ± 0.35	4.00 ± 1.02	2.87 ± 0.65	2.83 ± 0.55	1.60 ± 0.19	2.23 ± 0.44

Table 1: Effect of the exogenous application of oxalic acid on leaflets of primary transgenic peanut. UP = untransformed Perry, P4 - P39 = different transgenic lines. Means are shown ± standard deviation from 4 replicates. Leaflets were wounded with a 21 gauge needle before placing 15 µl of oxalic acid at 4 places on a leaflet. Lesions were assayed after 6 hours.

Effect of Exogenous Application of Oxalic Acid on Transformed Peanut

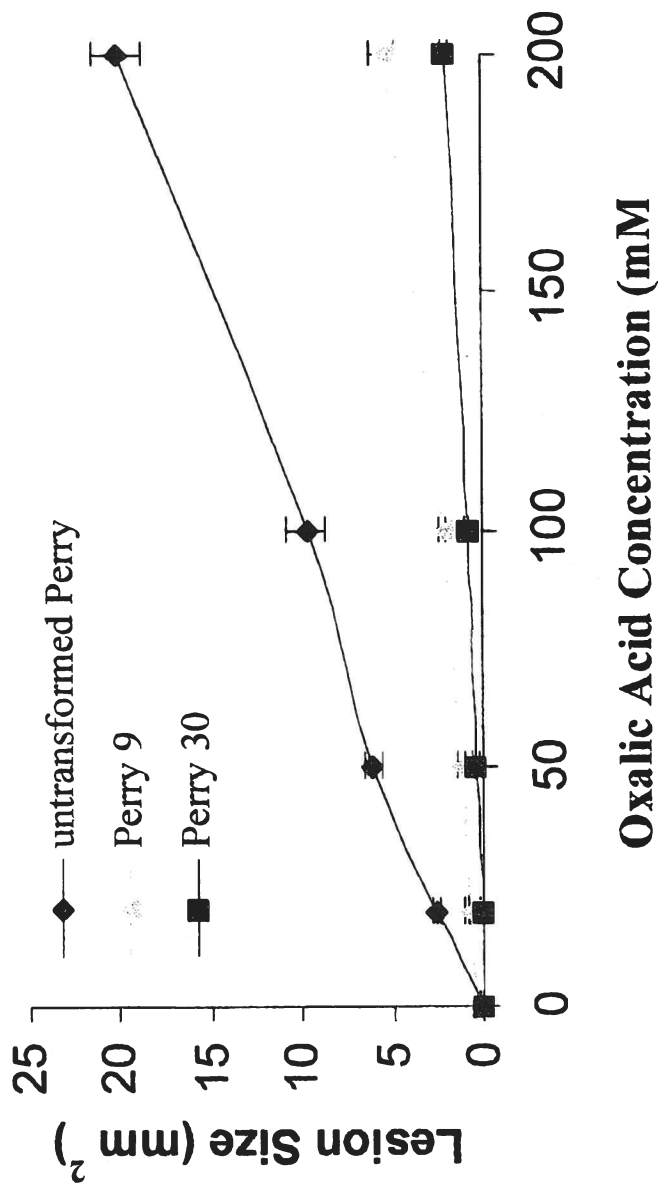


Fig.2: Effect on lesion size of applying oxalic acid to a wounded leaf surface. Natural physiological levels of oxalic acid during Sclerotinia minor infection is between 5 and 10 mM. Means are given ± standard deviation from 4 replicates

Peanuts engineered to contain an oxalate oxidase gene show a greatly improved ability to resist oxalic acid, a compound produced by *Sclerotinia minor* during infection

