

Soybean yield trials use as a control a line genetically incapable of being infected by nitrogen-fixing bacteria to measure soil nitrogen availability (pale green plants). Other plants are nodulated and use both soil and atmospheric nitrogen.



organisms are being studied.

One concrete example of enhancing nitrogen fixation and agronomic yield through genetic alteration of *Rhizobium* has been demonstrated in soybeans. A mutant of the commercial *R. japonicum* strain USDA 110 was selected for high nitrogen-fixation capacity in pure culture in R. C. Valentine's laboratory. Under microaerophilic conditions, similar to those encountered in the plant root nodule, the mutant strain C33 had 94 percent more activity than the parent strain 110. In subsequent tests with C33 on soybean plants grown under controlled environments, we measured approximately a 40 percent increase in dry matter and nitrogen content relative to plants infected with strain 110.

Those results stimulated field trials run at Davis in 1979 and 1980. Seed yield and total seed nitrogen (protein) content were increased significantly in 'Clark' soybean plants treated with mutant strain C33, which yielded 39.2 bushels per acre in 1979 and 44.6 in 1980, compared with 36 and 38.3 bushels, respectively, with USDA 110. Seed nitrogen content with C33 was 146 pounds per acre in 1979 and 186 pounds in 1980, compared with 136 and 159 pounds, respectively, with USDA 110. During both field seasons, more than 80 percent of the rhizobia reisolated from root nodules of field-grown plants were identified as the applied 110 or C33 strain on the basis of genetic markers.

A genetically similar, but nonnodulating, line of 'Clark' soybean yielded 27.1 and 17.7 bushels per acre containing 73.6 and 44.8 pounds of nitrogen per acre in 1979 and 1980, respectively. That decline in available soil nitrogen associated with different field sites was responsible for the more obvious promotive effect of strain C33 in 1980 when compared with 1979. Thus the genetically altered strain C33 was as good as strain 110 under the moderate soil nitrogen conditions used in 1979 and clearly superior under the greater nitrogen stress experienced in 1980.

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Herbicide tolerance

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Isolation of mutants tolerant or resistant to herbicides may become a valuable application of cell culture techniques. Every herbicide is restricted in use by the number of crops it damages or kills. Tolerant mutants of various plant species could broaden the usefulness of currently available herbicides. The advantages of searching for this kind of mutant using cell cultures are (1) accuracy and uniformity of herbicide exposure in culture, (2) the ease with which billions of cultured cells may be screened for ability to grow in the presence of the herbicide, and (3) the potential (as yet unrealized for most crop species) for easy isolation of recessive mutants using haploid cell cultures.

Paraquat-tolerant mutants of tomato were isolated using an interspecific hybrid strain called L2. This plant had 25 percent cultivated and 75 percent wild tomato ancestry and was selected for the wild tomato traits of rapid callus growth and efficient plant regeneration. When callus cells of this hybrid were placed on agar medium containing a lethal concentration of paraquat, spontaneously occurring presumptive mutant callus clones that could grow under these conditions appeared at a frequency of one out of four billion cells tested.

Diploid plants were regenerated from 10 of the 22 clones isolated. Although some of these plants were normal in appearance, others were abnormal (leaflets smaller and thicker than normal) with reduced vigor and

fertility. New callus cultures initiated from the regenerated plants typically had at least a 30-fold increase in paraquat tolerance relative to normal callus of strain L2. Paraquat tolerance was transmitted to sexual progeny in each of the three clones tested, confirming the hypothesis that the paraquat tolerance resulted from a mutation.

Limited tolerance was expressed at the plant level in regenerated plants from two out of seven clones tested. Although some were killed and the rest were damaged by paraquat, plants deriving from paraquat-tolerant clones PQT¹³ and PQT²² were significantly less damaged than plants of parent strain L2.

Each of these mutant plants is probably heterozygous for one or more alleles that confer the paraquat tolerance. The tolerance of different mutants may arise through more than one pathway. Thus, highly resistant plants might be achieved through inbreeding to produce homozygous tolerant genotypes, through hybridization to combine different tolerance alleles in a single strain, or through isolation of new and better mutants.

Eventually, herbicide-tolerant tomato mutants are likely to have agronomic applications. The greatest weed problems of tomato in California are the closely related nightshades, which are sensitive to the same herbicides as are tomato cultivars. Paraquat-tolerant tomato mutants, however, may be useful only as a model system, because the high human toxicity of this herbicide will restrict the times at which it can be safely applied, even to a tolerant crop of a species grown for edible fruits or foliage. Further experiments may produce tomato plants that tolerate other herbicides, perhaps achieving effective weed control together with the flexibility to choose compounds that pose the least risk to the environment.

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