Screening for Cell-Wall Mutants Deficient in Ferulate Ester Synthesis in Maize

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Introduction

Ferulic acid (FA) esters are involved in the cross-linking of lignin to polysaccharides in plant cell walls. Such cross-linking exerts a great impact on cell-wall accessibility, extensibility, and plasticity and also adversely affects cell-wall digestibility. Reduction of this cross-linking has markedly increased the rate and extent of cell-wall digestibility in a maize cell culture system. The goal of our research is to develop maize lines with increased cell-wall digestibility for use as silage for the feeding of dairy and beef cattle. We intend to identify and clone the gene responsible for FA ester synthesis and the gene will be reversely engineered back into plants to reduce the cross-linking. Our approach is to screen transposon tagged maize lines for mutants with reduced FA ester concentration in order to identify and isolate the gene of interest.

Materials and Methods

The transposon tagged maize lines were obtained by crossing the mum-9 transposon mutator into four inbred maize lines (A188, A619, A632, and W23). The selfed F_2 and F_3 progeny from these crosses were grown either in the greenhouse or in the field. The first true leaves were removed from 14 to 21 d old seedlings and dried at 50°C overnight. The samples were weighed and incubated in 1 ml of 2 N NaOH (20 h at 39°C) to release phenolic esters. The extracts were acidified with 6 N HCl to pH 1.5-2, placed on ice for 1 hr, and centrifuged for 10 min. The supernatant was transferred to sample vials for phenolic analysis using HPLC. Ferulic and *p*-coumaric (PCA) acid concentrations were estimated using commercial FA and PCA as external standards. Plants that had FA concentrations or FA/PCA ratios two standard deviations above or below the line mean were selected for further analysis and selfed. Mature tissues from selected plants were analyzed using the same procedure for their FA and PCA ester concentrations.

Results and Discussion

More than 12,000 maize seedlings have been analyzed for their FA and PCA ester concentrations and 397 plants were identified with unusually low or high FA concentrations and FA/PCA ratios. Of the selected plants, 140 produced viable seed. After analyzing mature tissues of the 140 selected seed-bearing plants, only 10 plants exhibited the desired traits of low FA concentrations or low FA/PCA ratios as both seedlings and at maturity. The progeny of these 10 plants have been evaluated in a greenhouse trial to verify the heritability of the desired traits. Progeny of three plants passed this evaluation. Lignin composition analysis by pyrolysis-GC-MS analysis indicated that one of the three selected plants exhibits unusually low syringyl unit abundance.

During the 1995 growing season we sowed 12,000 seeds, but only 8300 (72%) germinated and survived to the initial sampling. To our surprise, none of the plants analyzed was deficient in ferulate esters. In fact, FA concentrations in the cell wall were maintained at fairly constant levels within populations of uniform genetic background (Fig 1). Distribution of FA concentrations and FA/PCA ratios were truncated at the low ends of the curve (Fig. 2), suggesting that plants with low FA concentrations or low FA/PCA ratios died prior to screening.

Table 1 summarizes the viability of the selected 397 plants. One hundred sixteen (29%) died before pollination. Most of these dead plants had either low FA concentrations or low FA/PCA ratios. Mortality for the entire nursery population was only 12%. One hundred twenty (30%) of the selected plants exhibited abnormal developmental and growth patterns while only 15% of the overall population showed such abnormalities. These observations suggest that a threshold FA concentration in the cell wall is needed to maintain normal plant growth and development.

Conclusion

Massive screening has produced three putative mutants with reduced FA ester synthesis. Gene cloning using a polymerase-chain-reaction based screening technique is in progress. While our goal continues to be improvement of cell-wall digestibility through reduced cross-linking, the data from our screening experiment suggest that complete removal of FA ester synthesis may be catastrophic to plant growth and development.

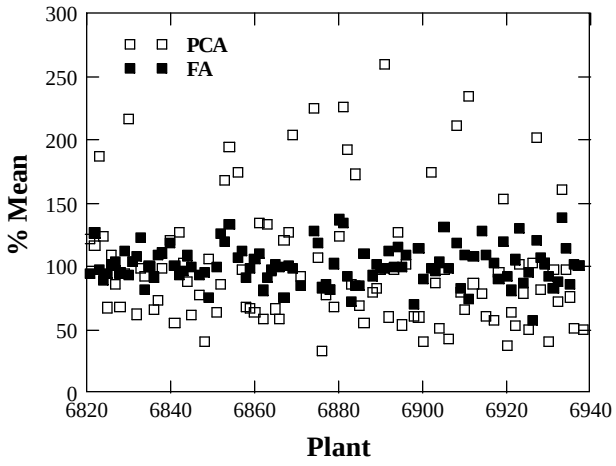

Figure 1. Distributions of FA and PCA concentrations. All 116 plants were from the same ear. Concentrations are expressed as a percent of the population mean.

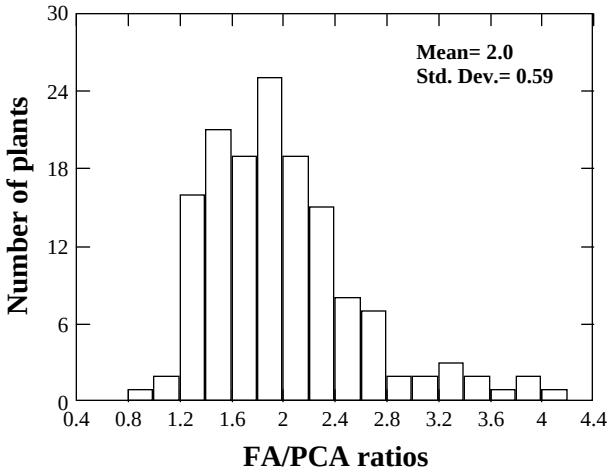

Figure 2. Distribution of FA/PCA ratios. All plants were from the same ear.

Table 1. Viability at maturity of maize plants selected based on seedling screening.

	High FA/PCA	Low FA/PCA	High FA	Low FA
Number of plants selected	130	162	19	87
Healthy plants	52	41	4	34
Dead plants	12	84	0	20
Abnormal	21	10	10	12
Male sterile	27	14	2	7
Female sterile	7	6	1	4
Infected Plants	1	7	2	10