Table 2. Recombination frequency in the sh-wx region of chromosome 9 where heterozygous trisomic 4 and diploid sibling plants were testcrossed as female parents.

| Plant | Plant type | No. kernels scored | % recombination in sh-wx region | $\bar{X} \pm SE$ |
|---|---|---|---|---|
| 75-603-1 | Trisomic 4 | 141 | 21.3 | |
| 75-603-11 | Trisomic 4 | 207 | 22.2 | |
| 75-603-12 | Trisomic 4 | 288 | 19.1 | 20.9 ± .9 |
| 75-602-6 | Diploid | 478 | 19.5 | |
| 75-602-13 | Diploid | 456 | 19.5 | |
| 75-603-9 | Diploid | 426 | 17.6 | 18.9 ± .6 |

A highly significant ($p<.005$) increase in recombination in the sh-wx region occurred in males (pooled trisomics and diploids) over that which took place in females (pooled trisomics and diploids). Numerous other investigators have also found that recombination in male maize flowers is higher than in female flowers (reviewed by Phillips 1969, Genetics 61:117, and Ghidoni 1975, Genetics 81:253).

When all diploid crosses (both as male and female parents) are compared with all trisomic crosses (both as male and female parents), the data are nearly significantly different ($p=.06$). Therefore, recombination appears to be higher in trisomic 4 plants than in diploid plants. Although the recombinational frequency in male flowers of trisomic 4 plants (25.6 ± 1.6%) is higher than that in their sibling diploids (22.4 ± .6%), the increase was not statistically significant ($p=.12$). The increase in recombination in the female flowers induced by the addition of an extra chromosome 4 (from 18.9 ± .6% to 20.9 ± 9%) was smaller ($p=.37$). Ghidoni (1975, Genetics 81:253) found that trisomy of chromosome 6 increased recombination in the sh-wx region in both sexes of maize by approximately the same amount.

As noted before, I testcrossed heterozygous monosomic 4 and control diploid maize plants as males to determine if monosomy could alter recombination in the sh-wx region. Monosomic 4 plants had only 11.2 ± .8% whereas diploid control plants had 23.0 ± 0.8%. Therefore, monosomic 4 plants had only approximately half the amount of recombination found in their diploid controls. If the amount of recombination in the sh-wx region in chromosome 9 was proportional to the number of chromosomes 4, then a recombinational frequency in the trisomic 4 plants male flowers would be expected to be 1-1/2 times that found in the diploid siblings (or 1.5 x 22.24% = 33.36%). This obviously was not the case because the recombination frequency increased to only 25.61%. It therefore appears that a plateau in recombinational alteration is being reached. These data indicate that monosomic-diploid comparisons are a far more sensitive system for measuring this continuously variable quantitative trait, and presumably other quantitative traits, than disomic-trisomic comparisons.

David F. Weber

## The homeostatic nature of the acid extractable amino acids (free amino acid pool) in leaves

Since the acid extractable amino acid pool (free amino acid pool) is an intermediate, and not an end-product of a series of reactions involved in protein

synthesis, it would seem reasonable to believe that it should be a highly sensitive parameter to examine, one that would reflect in a very efficient way any changes in amino acid synthetic activity or utilization in the plant. In the present study, we determined the relative proportions of the different acidic and neutral acid extractable amino acids at three different developmental stages in the same maize plants to determine what, if any, changes would occur during development in the leaves. The basic amino acids could not be analyzed because of technical problems with the amino acid analyzer.

All plants analyzed were from a cross between W22 inbred line and inbred Mangelsdorf's Multiple chromosome tester. Both lines were obtained from K. Satyanarayana. Each of two sets of two diploid sibling plants were sampled separately at each of three different developmental stages: seedling stage, sporocyte stage, and anthesis. The seedling stage was approximately two weeks after germination when the plants had 5-6 leaves. The sporocyte stage was approximately six weeks after germination when the plants had 10-12 leaves. This is when microsporocyte samples would be taken from the plant for meiotic analysis. Anthesis is the time of pollen shed. Three-gram leaf samples were excised from the uppermost leaves of the same plants at each of the three developmental stages. All leaf samples were from plants grown under field conditions. The samples were taken at the same time of day (7-8 A.M.) to reduce environmental variations. All samples were placed on ice and transported back to the laboratory for immediate preparation and analysis.

Amino acid extracts were prepared from leaf samples as follows. Three g of leaf tissue was placed in a Virtis homogenizing cup with 9 ml of water. Each sample was homogenized in a Virtis "45" homogenizer at a setting of 60 for 10 min and placed over a boiling water bath for 1 min. One ml of 30% sulfosalicylic acid was then added as a deproteinizing agent and the homogenate was then reheated for 1 additional min and rehomogenized for 10 min. The homogenate was centrifuged in a clinical centrifuge (International Model CL) for two ten min periods at 2900 rpm to remove all unwanted plant material. The supernatant (the amino acid extract) was then adjusted to a pH of 2.0-2.3. No glutathion was present in the samples so the standard oxidation procedure involving 2M $NaSO_3$ solution was unnecessary. The samples were maintained at -22 C until used. A JEOL model JLC-6 AH fully automatic Amino Acid Analyzer was used to analyze the samples.

The data obtained from this approach are summarized in Table 1. The amount of each amino acid is expressed as the percent of that amino acid in the total of all neutral and acidic amino acids from that sample. This was calculated by adding the μm/ml values of all the amino acids sampled to obtain a grand total. The proportion of each amino acid was then computed as a percentage of this total. The mean and standard errors were calculated for each amino acid fraction at each of the three developmental stages. By examining the means and standard errors for each amino acid during the three developmental stages, it appears that no major differences took place during development. To determine if this is true, the data were tested using a two way analysis of variance with replication over a developmental time period, and between full sibling families. The significance levels (0.05 and 0.01) for each amino acid are listed in Table 2. The F scores indicate that the percent of each amino acid does not change through development. Since none of the F values are significant at the 1% significance level, and all but one of the values are not significant at the 5% significance level, this indicates that the acid extractable acidic and neutral amino acids are an extremely stable component in the plant throughout development. Since the acidic and neutral amino acids are stable through development, it is not unreasonable to assume the remaining basic amino acids are just as stable. The F values between full family siblings are significant in many cases. These data show that

Table 1. RELATIVE PERCENTAGES OF ACIDIC AND NEUTRAL AMINO ACIDS IN THE FREE AMINO ACID POOL IN LEAVES OF FOUR *Zea mays* PLANTS AT THREE DEVELOPMENTAL STAGES.

| Amino Acid | Seedling Stage | | | | | Sporocyte Stage | | | | | Anthesis | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Family A Plant # | | Family B Plant # | | $\bar{X} \pm SE$ | Family A Plant # | | Family B Plant # | | $\bar{X} \pm SE$ | Family A Plant # | | Family B Plant # | | $\bar{X} \pm SE$ |
| | 1* | 2* | 1 | 2 | | 1 | 2 | 1 | 2 | | 1 | 2 | 1 | 2 | |
| Phosphoserine | 8.05 | 8.10 | 6.32 | 6.81 | 7.32± 0.44 | 7.92 | 8.00 | 6.20 | 6.57 | 7.17± 0.46 | 8.11 | 8.07 | 6.44 | 6.78 | 7.35± 0.43 |
| Aspartic Acid | 9.91 | 12.53 | 9.57 | 11.01 | 10.75± 0.66 | 10.03 | 11.93 | 9.76 | 10.88 | 10.65± 0.48 | 10.13 | 11.69 | 9.60 | 11.18 | 10.65± 0.47 |
| Threonine | 3.04 | 3.11 | 2.07 | 2.32 | 2.63± 0.25 | 3.27 | 3.07 | 2.01 | 2.34 | 2.67± 0.29 | 3.21 | 3.15 | 2.12 | 2.41 | 2.72± 0.27 |
| Serine | 7.88 | 7.17 | 6.20 | 5.91 | 6.79± 0.45 | 7.56 | 7.25 | 6.32 | 5.89 | 6.75± 0.39 | 7.82 | 7.00 | 6.11 | 5.77 | 6.67± 0.46 |
| Asparagine | 1.93 | 2.40 | 3.63 | 2.15 | 2.52± 0.37 | 1.85 | 2.13 | 3.51 | 2.07 | 2.39± 0.37 | 2.04 | 2.32 | 3.59 | 2.11 | 2.51± 0.36 |
| Glutamic Acid | 9.79 | 11.76 | 11.03 | 12.47 | 11.26± 0.57 | 10.11 | 11.55 | 11.33 | 12.32 | 11.32± 0.45 | 9.96 | 11.51 | 10.93 | 12.37 | 11.19± 0.50 |
| Glutamine | 4.17 | 5.98 | 3.40 | 3.84 | 4.34± 0.56 | 4.22 | 5.28 | 3.78 | 3.96 | 4.31± 0.33 | 4.37 | 4.69 | 3.33 | 3.72 | 4.02± 0.30 |
| Glycine | 0.98 | 0.79 | 1.29 | 1.98 | 1.26± 0.26 | 1.10 | 0.96 | 1.38 | 1.76 | 1.30± 0.17 | 1.16 | 1.03 | 1.39 | 1.99 | 1.39± 0.21 |
| Alanine | 27.60 | 29.38 | 27.37 | 30.97 | 28.83± 0.84 | 27.00 | 22.69 | 26.56 | 29.55 | 26.45± 1.41 | 26.70 | 22.13 | 27.52 | 28.74 | 26.27± 1.44 |
| Valine | 4.00 | 4.57 | 2.77 | 3.29 | 3.65± 0.39 | 3.93 | 3.23 | 2.89 | 3.49 | 3.36± 0.19 | 4.12 | 4.02 | 2.85 | 3.36 | 3.58± 0.29 |
| Cystine | 1.48 | 1.60 | 1.34 | 1.48 | 1.47± 0.05 | 1.58 | 1.56 | 1.46 | 1.55 | 1.53± 0.02 | 1.67 | 1.68 | 1.41 | 1.52 | 1.57± 0.06 |
| Methionine | 0.45 | 0.41 | 0.50 | 0.38 | 0.43± 0.02 | 0.57 | 0.39 | 0.48 | 0.40 | 0.46± 0.04 | 0.49 | 0.39 | 0.47 | 0.36 | 0.42± 0.03 |
| Isoleucine | 0.76 | 0.77 | 0.67 | 0.46 | 0.66± 0.07 | 0.77 | 0.72 | 0.65 | 0.41 | 0.63± 0.07 | 0.85 | 0.78 | 0.75 | 0.52 | 0.72± 0.06 |
| Leucine | 1.18 | 1.07 | 0.83 | 0.69 | 0.94± 0.11 | 1.09 | 1.26 | 0.78 | 0.73 | 0.96± 0.12 | 1.23 | 1.14 | 0.85 | 0.71 | 0.98± 0.12 |
| Tyrosine | 0.63 | 0.89 | 1.06 | 1.17 | 0.93± 0.11 | 0.61 | 0.94 | 1.01 | 1.26 | 0.95± 0.13 | 0.66 | 0.91 | 0.99 | 1.20 | 0.94± 0.11 |
| Phenylalanine | 0.33 | 0.57 | 0.47 | 0.50 | 0.46± 0.05 | 0.47 | 0.55 | 0.42 | 0.47 | 0.47± 0.02 | 0.44 | 0.52 | 0.50 | 0.53 | 0.49± 0.02 |
| B-Alanine | 2.16 | 2.83 | 2.79 | 1.39 | 2.29± 0.33 | 2.23 | 2.49 | 2.69 | 1.44 | 2.21± 0.27 | 2.39 | 2.38 | 2.84 | 1.94 | 2.38± 0.18 |
| Proline | 1.59 | 3.11 | 1.67 | 0.82 | 1.79± 0.47 | 1.48 | 2.87 | 1.50 | 1.17 | 1.75± 0.37 | 1.38 | 2.56 | 1.54 | 1.20 | 1.67± 0.30 |
| Hydroxyproline | 13.96 | 15.39 | 16.93 | 12.26 | 14.63± 1.00 | 14.21 | 13.11 | 17.37 | 13.74 | 14.60± 0.94 | 13.27 | 14.03 | 16.77 | 13.59 | 14.41± 0.80 |

*Represents sibling plants 1 and 2 under representative family.

Table 2. F values as determined by variance analysis.

| AMINO ACID | Significance of F values at 5% and 1% error | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TIME | 5% | 1% | FAMILY | 5% | 1% | TIME X FAMILY | 5% | 1% |
| Phosphoserine | 15.69 | ns. | ns. | 3020.17 | * | * | 0.05 | ns. | ns. |
| Aspartic Acid | 00.34 | ns. | ns. | 34.19 | * | ns. | 0.03 | ns. | ns. |
| Threonine | 2.20 | ns. | ns. | 741.34 | * | * | 0.14 | ns. | ns. |
| Serine | 1.00 | ns. | ns. | 599.00 | * | * | 0.01 | ns. | ns. |
| Asparagine | 5.50 | ns. | ns. | 382.38 | * | * | 0.00 | ns. | ns. |
| Glutamic Acid | 10.70 | ns. | ns. | 1632.00 | * | * | 0.00 | ns | ns. |
| Glutamine | 1.33 | ns. | ns. | 40.64 | * | ns. | 0.22 | ns. | ns. |
| Glycine | 1.57 | ns. | ns. | 100.37 | * | * | 0.13 | ns. | ns. |
| Alanine | 3.08 | ns. | ns. | 7.29 | ns. | ns. | 0.48 | ns. | ns. |
| Valine | 0.41 | ns. | ns. | 11.72 | ns. | ns. | 1.36 | ns. | ns. |
| Cystine | 1.79 | ns. | ns. | 10.52 | ns. | ns. | 1.15 | ns. | ns. |
| Methionine | 1.83 | ns. | ns. | 1.66 | ns. | ns. | 0.09 | ns. | ns. |
| Isoleucine | 26.66 | * | ns. | 393.33 | * | * | 0.02 | ns. | ns. |
| Leucine | 0.19 | ns. | ns. | 55.53 | * | ns. | 1.13 | ns. | ns. |
| Tyrosine | 0.43 | ns. | ns. | 500.28 | * | * | 0.02 | ns. | ns. |
| Phenylalanine | 0.27 | ns. | ns. | 0.00 | ns. | ns. | 0.54 | ns. | ns. |
| B-Alanine | 0.68 | ns. | ns. | 3.57 | ns. | ns. | 0.11 | ns. | ns. |
| Proline | 0.26 | ns. | ns. | 33.84 | * | ns. | 0.12 | ns. | ns. |
| Hydroxyproline | 0.05 | ns. | ns. | 3.38 | ns. | ns. | 0.27 | ns. | ns. |

*means number is significantly different statistically; ns. means number is not significantly different.

phosphoserine, threonine, serine, asparagine, glutamic acid, glycine, isoleucine and tyrosine proportions differed significantly between these two families. The remaining amino acids did not exhibit family differences. This conclusion is difficult to explain because all progeny were F1 hybrids between the same two inbred lines, thus genetic differences should be small. The most reasonable explanation is that environmental factors experienced by each different family during its growth and development in the field were responsible for the differences. Possible influencing factors could be: temperature, moisture, light levels, leaching, insect damage, disease, or levels of nitrogen in the soil. No significant differences were found between families over a period of time. Similar comparisons are being made for specific monosomic types through their development.

These data establish that the relative amounts of the different neutral and acidic amino acids in the amino acid pool in maize leaves are remarkably stable during the development of the plant. Thus, the stage of maturity at which a sample would be taken from a plant is relatively unimportant. The free amino acid pool must be under a very stringent genetic regulatory mechanism, one that deserves further analysis.

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