Monosomic analysis of the acid extractable amino acids (free amino acid pool) in leaves

Monosomics generated by the r-X1 deficiency are being used to study the free amino acid pool in Zea mays leaves. In the previous paper in this Newsletter, we demonstrated that the acid extractable amino acids (free amino acid pool) remain remarkably stable throughout development from the seedling stage to anthesis. Therefore, the stage at which leaf samples are taken from the plant appears to make no difference in a study of the relative proportions of the different acid extractable amino acids in the maize leaves. This remarkable constancy of the acid soluble amino acid proportions is extremely helpful in a comparison of a specific monosomic type with its diploid (disomic) siblings because monosomics mature at a much slower rate than their diploid siblings (monosomics reach anthesis two to three weeks later than their diploid siblings). Thus, even though monosomics and disomics may be sampled at the same time they are at a different stage of maturity. If they are sampled at the same stage of maturity, they are of a different chronological age. However, since differences in the amino acid pool are not found as the plant matures, the above considerations are unimportant.

The acid extractable amino acids in specific monosomic types are being compared with those in their diploid sibling control in an attempt to learn more about the control of amino acids in the amino acid pool. If a gene expressing dosage effects, affecting the amino acid pool, is located on a specific chromosome, then the amino acid pool of a plant monosomic for that chromosome will be different from that found in its diploid sibling control. In this way we are comparing one vs. two copies of all genes on a given chromosome. In this paper, we describe the differences that have been found in monosomic 6 plants.

The experimental procedures are the same as those described in the previous paper. All plants used in this study were from field plantings. The diploid control plants used in this study were the same plants analyzed throughout development in the previous study. R/r-X1 plants of the W22 genetic background were crossed as females by a second inbred, Mangelsdorf's multiple chromosome tester. Monosomics are generated at a frequency in excess of 11% from this cross.

Monosomic 6 plants were detected by their distinctive plant morphology. They have darker foliage and leaves that point more upward (almost liguleless in phenotype) than their disomic siblings.

The results at the seedling stage are presented in Table 1. The amino acids analyzed in this study are the neutral and acidic amino acids. The basic amino acids could not be analyzed due to mechanical problems with the amino acid analyzer. Means and standard errors were computed for the percent of each amino acid in monosomic 6 plants and in disomic controls. The means and standard errors for the controls were based on four individual plants. The means and standard errors for the monosomic 6 class were derived from three plants. The data were tested for significant differences with a t test. The extremely high levels of hydroxyproline found in all samples is unexplained. It is unusual for plant tissue to contain high levels of hydroxyproline. Therefore, the compound absorbing in the same position as hydroxyproline is either hydroxyproline, or a similar 5 carbon imino ring compound resembling hydroxyproline. The most striking difference found in the monosomic 6 plants was the massive reduction in the aspartic acid levels in the acid-soluble amino acids. Monosomic 6 plants have only 29.1% of the aspartic acid found in their diploid controls. It is interesting to note that threonine levels drop significantly, and methionine levels are also slightly reduced, but not significantly, in the

Table 1. Relative percentages of neutral and acidic amino acids in the free amino acid pool in monosomic and diploid maize leaves.

	Diploid Controls Mean ± SE	Monosomic 6 Mean ± SE	Percent of diploid value
Phosphoserine	7.32 ± 0.44	5.68 ± 0.38	77.5
Aspartic Acid	10.75 ± 0.66	3.13 ± 0.05**	29.1
Threonine	2.63 ± 0.25	1.60 ± 0.02*	60.8
Serine	6.79 ± 0.45	5.78 ± 0.11	85.1
Asparagine	2.52 ± 0.37	1.90 ± 0.12	75.4
Glutamic Acid	11.26 ± 0.57	13.13 ± 0.50*	116.6
Glutamine	4.34 ± 0.56	2.25 ± 0.05*	51.8
Glycine	1.26 ± 0.26	1.28 ± 0.08	101.6
Alanine	28.83 ± 0.84	29.93 ± 0.46	103.8
Valine	3.65 ± 0.39	4.26 ± 0.26	116.7
Cystine	1.47 ± 0.05	3.23 ± 0.19**	219.7
Methionine	0.43 ± 0.02	0.37 ± 0.01	86.0
Isoleucine	0.66 ± 0.07	0.61 ± 0.02	92.4
Leucine	0.94 ± 0.11	1.22 ± 0.10	129.8
Tyrosine	0.93 ± 0.11	2.92 ± 0.20**	314.0
Phenylalanine	0.46 ± 0.05	0.60 ± 0.08	130.4
B-Alanine	2.29 ± 0.33	2.28 ± 0.11	99.6
Proline	1.79 ± 0.47	1.57 ± 0.03	87.7
Hydroxyproline	14.63 ± 1.00	18.21 ± 0.18*	124.5

*indicates number is significantly different at 0.05 significance level
**indicates number is significantly different at 0.01 level

monosomic 6 plants. Since these two amino acids are synthesized directly from aspartic acid in other plants (Dougall and Fulton, 1967, Plant Physiol. 42: 941), they presumably follow the same pathway in maize. We speculate, therefore, that a genetic factor controlling the amount of aspartic acid is located on chromosome 6. This gene could be a structural gene directly involved in the biosynthesis of aspartic acid or a regulatory gene. The reduction in the amount of threonine and possibly methionine are presumably due to the lack of aspartic acid as a substrate for further reactions.

Another interesting observation in the monosomic 6 plants is the significant decrease in glutamine (51.8% of the diploid level) accompanied by a significant increase in glutamic acid (116.6% of the diploid level). Glutamine is believed to be derived from glutamic acid in plants (Davies, 1964, Plant Biochemistry). We speculate, therefore, that a factor located on chromosome 6 is controlling the conversion of glutamic acid into glutamine. This factor could also be regulatory or structural in nature. The buildup of hydroxyproline levels, or a similar compound, in the monosomic 6 plants could also be related to the increased levels of glutamic acid, because hydroxyproline is also indirectly derived from glutamic acid.

The aromatic amino acid tyrosine is also sharply increased in monosomic 6 plants. The pathway for tyrosine and phenylalanine in animal and bacterial systems involves a branched pathway from a common precursor, prephenic acid (Horecker and Stadtman, 1971, Current Topics in Cellular Regulation). It is also known that tyrosine is synthesized by the direct hydroxylation of

phenylalanine in animals and bacteria. The same branched biosynthetic pathway from prephenic acid that occurs for bacteria and animals also occurs in plants (Miflin, 1973, in Milborrow, Biosynthesis and Its Control in Plants); little evidence is available on the plant's ability to hydroxylate phenylalanine directly to tyrosine. The fact that tyrosine is strikingly affected when phenylalanine remains essentially constant indicates an alteration has occurred in the biosynthetic pathway between prephenic acid and the formation of tyrosine. A possible explanation for this buildup of tyrosine could be the lack of genetic regulation within this segment of the biosynthetic pathway.

Another difference in the monosomic 6 plants is the significant increase in cystine (219.7%). This difference is not understood.

The same monosomic 6 plants were examined at the sporocyting stage and at anthesis. The data are presented in Table 2. The same changes observed at the seedling stage (Table 1) are also seen at these two subsequent developmental stages. Thus, those changes persist throughout development.

Table 2. Relative percentages of neutral and acidic amino acids in the free amino acid pool in monosomic 6 plants through development

	Seedling Stage Mean±SE	% of Diploid Value	Sporocyte Stage Mean±SE	% of Diploid Value	Anthesis Stage Mean±SE	% of Diploid Value
Phosphoserine	5.68±0.38	77.5	5.86±1.02	81.7	6.85±0.34	93.1
Aspartic Acid	3.13±0.05	29.1	3.62±0.24	33.9	3.39±0.08	31.8
Threonine	1.60±0.02	60.8	1.97±0.48	73.7	1.89±0.23	69.4
Serine	5.78±0.11	85.1	6.62±0.73	98.0	7.66±1.99	114.8
Asparagine	1.90±0.12	75.4	2.56±0.32	107.1	3.17±0.57	126.2
Glutamic Acid	13.13±0.50	116.6	13.04±0.35	115.1	12.06±0.47	107.7
Glutamine	2.25±0.05	51.8	2.49±0.49	57.7	2.47±0.27	61.4
Glycine	1.28±0.08	101.6	1.48±0.10	113.8	1.00±0.17	71.0
Alanine	29.93±0.46	103.8	29.60±1.18	111.9	29.10±1.61	110.7
Valine	4.26±0.26	116.7	3.85±0.57	114.5	4.67±0.40	130.4
Cystine	3.23±0.19	219.7	3.13±0.57	204.5	2.63±0.42	167.5
Methionine	0.37±0.01	86.0	0.37±0.02	80.4	0.40±0.03	95.2
Isoleucine	0.61±0.02	92.4	0.43±0.01	68.2	0.64±0.11	88.8
Leucine	1.22±0.10	129.8	1.05±0.19	109.3	1.18±0.06	120.4
Tyrosine	2.92±0.20	314.0	3.38±1.21	355.7	2.14±0.23	227.6
Phenylalanine	0.60±0.08	130.4	0.80±0.08	170.2	0.69±0.05	140.8
B-Alanine	2.28±0.11	99.6	0.87±0.24	64.2	0.82±0.08	37.4
Proline	1.57±0.03	87.7	1.42±0.17	81.1	1.20±0.24	71.8
Hydroxyproline	18.21±0.18	124.5	17.32±1.86	118.6	17.87±2.14	124.0

Although this study is in its early stages, this study might allow us to ascribe genetic control of certain amino acids to specific chromosomes. In addition, the amino acid biosynthetic pathways are poorly known in plants. If a coordinate increase or decrease is found for two or more amino acids in the same putative pathway (as for aspartic acid, threonine, and methionine) it would support that pathway. Also, if an increased concentration of a putative precursor and a concomitant decrease in its end product are found, this would also lend support for that pathway. In this case, a block would be present between the precursor and the end product.

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