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Amino Acid Comparisons in Male Sterile Wheat Derived from *Triticum timopheevi* Zhuk. Cytoplasm and its Fertile Counterpart

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Summary. Anthers, seeds, seedlings and flag leaves of fertile and cytoplasmic male sterile (*Triticum timopheevi*) lines of bread wheat were examined for free and acid-hydrolyzable (bound) amino acids. Free asparagine was found to be high in anthers of the male sterile line and free proline high in anthers of the fertile line.

Among the bound amino acids, proline was lower, and aspartic and glutamic acid were higher, in male sterile than in fertile anthers. Retardation of glutamic and aspartic acid conversion to proline was believed to be a cause of proline deficiency in the male sterile line.

The total amount of bound amino acids was higher in anthers, seed and flag leaves of the male sterile line compared with the fertile analogue.

Introduction

Male sterility in various plants is known to affect amino acid content in comparison with their fertile counter-parts (Fukasawa, 1954; Alam and Sandal, 1967; Silvolap, 1968). Most reports, however, are devoted to studies of free amino acids and little is known about bound amino acids in male steriles. In wheat (*Triticum aestivum* L. em. Thell.) having sterility from *Triticum timopheevi* Zhuk. cytoplasm, the latter was reported to influence the protein content of seeds borne on male sterile plants (Wilson, 1968; Rai *et al.* 1970). It would be valuable, therefore, to examine for differences in individual bound amino acids between male sterile and fertile wheat.

Amino acids are known to be influenced by the cytoplasmic source and other factors (Khoo and Stinson, 1957). A paper chromatographic survey of free amino acids (Fukasawa, 1954) revealed an accumulation of asparagine and a deficiency of proline in the anthers and mature leaves of male sterile durum wheat derived from *Aegilops caudata* cytoplasm and its fertile analogue. While similar amino acid differences were found between male sterile corn and its fertile counterpart (Fukasawa, 1954), Khoo and Stinson (1957) also noticed an accumulation of alanine. This was confined to the Texas cytoplasmic male sterile type and did not appear in the A, B and S male sterile types. In sorghum, anthers with genetic cytoplasmic male sterility were found by Brooks (1962) to have a higher amount of glycine, in contrast to higher amounts of aspartic acid, serine and alanine in the fertile counterpart. Brooks concluded that in male sterile plants the glycine pathway to purine synthesis was probably blocked. In sudan grass (*Sorghum vulgare* var. Sudanese (Piper)), Alam and Sandal (1967) found that fertile anthers at the pre-pollen stage were higher in alanine, proline, glutamic acid and tyrosine while threonine was higher in sterile anthers. In flower buds of male sterile lines with functional (tomato), cytoplasmic (sorghum) and nuclear (sunflower) sterility, a higher content of asparagine and a lower amount of proline were found compared with their fertile counterparts (Silvolap, 1968). Flower buds from these male sterile types also contained higher levels of free amino acids.

On the other hand, Sarvella *et al.* (1967) did not find any consistent differences among the male sterile, fertile and restored versions of corn in individual or total acid hydrolyzable (bound) amino acids. They concluded that the observed differences had no causal relationship with male sterility, and so did Sarvella and Stojanovic (1968), when they analyzed leaves and flower buds of several genetic and cytoplasmic male sterile lines of cotton for free and protein bound amino acids.

The objectives of the present investigation were to examine free and bound amino acid differences in anthers at different developmental stages in seed, seedlings and flag leaves from normal fertile and male sterile (*Triticum timopheevi* cytoplasm) bread wheat, and to determine the relationship of any amino acid differences to male sterility.

Materials and Methods

Genesee and a male sterile line, Ms 48 from *Triticum timopheevi*, derived from male sterile Bison with six backcrosses to Genesee, were studied from plants grown in the field at the Elora Research Station, Ontario, Canada. Anthers were collected at four developmental stages, namely, pre-tetrad (stage I), young microspore (stage II), immature pollen grain (stage III) and mature pollen grain (stage IV). Collection of anthers of stages I and II were based on microscopic examination. Flag leaves of stage I plants were sampled from the field and seven-day-old seedlings were grown indoors at room temperature. All samples were stored in stoppered vials at −30 °C until used.

Qualitative analyses of free amino acids in fresh anthers were performed by one dimensional thin-layer chromatography. Samples were extracted in 90% ethyl alcohol. Carrier plates, 20×20 cm, were coated with silica gel G and activated plates were developed using solvent isopropanol, distilled water, benzene and n-butanol (70:25:25:10). Amino acids were visualized by spraying 0.5% ninhydrin solution (BDH). Only the amino acids that showed differences between the male sterile and fertile line were identified by comparing them with standard amino acid solutions.

Quantitative analyses of bound amino acids were done on the "Technicon" ion exchange, automatic amino acid analyser. Freeze-dried, 50 mg samples were hydrolyzed for 16 hours in test tubes in an atmosphere of nitrogen. Hydrolysates were filtered, two aliquots of distilled water were added and evaporated to dryness on a "Buchler" evapomix at 35 °C. Finally, the residue was dissolved for analysis in a sodium citrate buffer with a pH of 2.5. Duplicate analyses of each sample were performed. Tryptophan was destroyed during hydrolysis. The values reported for methionine and cysteine were low because of oxidation during hydrolysis.

Since the total bound amino acid content was significantly higher in the samples from the male sterile line than in the fertile counterpart, the comparisons of individual amino acids were made on the basis of equal amounts of total amino acids from the male sterile and fertile line. Amino acid differences between the male sterile and fertile line were tested statistically.

Results and Discussion

A deficiency of proline in the anthers of the male sterile line as compared with the fertile line was found in both the analysis of bound amino acids (Table 1) and in the analysis of free amino acids. Proline deficiency was pronounced in stages III and IV, the later stages of anther development. During these two stages the proline content of fertile anthers averaged 14.71 and of male sterile anthers 11.03 μ moles/100 mg of dried sample, a 25% difference. A 10% difference in proline between the two lines was observed at stage II. Proline deficiency during the advanced stages of anther development in male sterile versions of various crop plants appears to be a common occurrence (Fukasawa, 1954; Khoo and Stinson, 1957; Sarvella *et al.*, 1967) but proline's role as a causative agent is not known. Fowden (1963) considered that proline was important in determining the structure of protein. Nutrient requirements during microsporogenesis and pollen development are believed to be critical (Vasil, 1967) and a deficiency of proline in male sterile anthers may have a direct bearing on pollen abortion. Proline decline in this study began in stage II and became more pronounced in stages III and IV when pollen degeneration had already occurred (Rai, 1970). Therefore, to have a primary causative effect on pollen sterility, proline deficiency would have to occur in stage I, which suggests that proline deficiency may not be the primary cause of pollen abortion in wheat and hence sterility. This agrees with the statement of Britikov *et al.* (1964), who reported that proline deficiency in non-viable pollen grains was probably a consequence of certain defects of meiosis or of preceding stages of microsporogenesis.

Glutamic acid content showed its highest value, 30.18 μ moles /100 mg dried sample, in the fertile line at stage I, but decreased steadily to 18.42 μ moles at stage IV, a 39% decline. Proline content in the fertile line during the same period increased 23% from 12.11 to 15.01 μ moles (Table 1). In the male sterile line, glutamic acid declined 25% from 26.67 to 19.86 μ moles from stage I to IV and, in contrast to the fertile line, proline declined nearly 12% from 12.11 to 11.40 μ moles. This suggests a retardation in the conversion of glutamic acid to proline in male sterile anthers, as glutamic acid is considered to be a precursor of proline in plants (Coleman and Hegarty, 1957; Vogel and Bonner, 1954). This conclusion supports the hypothesis of Fukasawa (1962) in that the slow conversion of glutamic acid to proline may be partially responsible for the deficiency of proline in male sterile plants. More evidence based on male sterile wheat lines with different genetic backgrounds is needed.

Higher levels of free asparagine, based on thin layer chromatography, or of aspartic acid, analysed quantitatively, were found in male sterile anthers compared with the fertile counterpart (Table 1). As the relationship between aspartic acid and proline in plants is not clear, it remains to be seen whether the observed deficiency of proline in male sterile anthers was related to the accumulation of aspartic acid. Panalaks *et al.* (1963) showed that asparagine was accumulated in etiolated soybean seedlings by either the breakdown of protein or by amidation of aspartic acid released from reserve protein. The consistently higher amount of asparagine in male sterile anthers during the pre-tetrad stage I in this study may be taken as evidence of the metabolic inertness of male sterile anthers. A cytohistological study of the male sterile line also indicated abnormal development of the pollen mother cells during the pre-tetrad stage (Rai, 1970).

With the exception of methionine and arginine, which had slightly higher levels in the flag leaves and seeds, respectively, of male sterile plants, none of the bound individual amino acids were found to differ among the vegetative tissues analysed from male sterile and fertile plants (Table 2). This may not be unexpected, since the only visually observed change associated with *T. timopheevi* cytoplasm was shrunken seed (Rai, 1970). In all other respects, male sterile and fertile plants appeared phenotypically similar.

The total amounts of unadjusted amino acids in the seed, the seedlings and the flag leaves were higher in male sterile plants than in their fertile counterparts by 27, 4 and 18% respectively (Table 2). Likewise, in male sterile anthers at stages I and II, total unadjusted amino acid content was 8 and 4%

Table 1. *Bound amino acid contents (μmoles/100 mg dried sample) in male sterile (Ms) and fertile (F) anthers at different stages of its development*

Amino acids	Stages of Anther								LSD* (.05)
	I		II		III		IV		
	F	Ms	F	Ms	F	Ms	F	Ms	
Aspartic acid	21.68	23.48	16.60	19.97	16.89	18.82	15.04	17.55	1.00
Threonine	11.42	11.62	9.27	9.04	7.88	8.54	7.19	8.14	0.94
Serine	15.39	15.89	11.72	11.94	9.92	9.98	9.90	9.58	0.74
Glutamic acid	30.18	26.67	24.62	23.04	20.28	22.50	18.42	19.86	0.86
Proline	12.11	12.11	12.54	11.33	14.41	10.65	15.01	11.40	0.63
Glycine	25.11	25.19	17.96	18.50	16.19	16.86	15.07	15.63	1.62
Alanine	27.30	28.20	23.90	24.90	23.67	23.45	19.39	19.69	1.51
Cysteine	0.94	1.04	1.17	1.03	0.82	0.93	0.70	0.76	0.19
Valine	16.88	17.34	12.66	12.31	12.77	12.89	13.24	13.13	1.20
Methionine	3.69	3.96	3.39	3.51	3.16	1.16	3.39	1.64	0.67
Isoleucine	10.27	10.90	7.08	7.68	6.48	5.89	5.88	5.49	0.89
Leucine	19.10	18.99	16.11	16.66	12.79	12.69	12.50	12.20	1.07
Tyrosine	5.66	5.18	4.80	4.71	4.68	4.76	4.86	5.75	0.77
Phenyl Alanine	9.01	9.67	6.94	6.41	6.46	6.31	6.45	5.95	0.57
Histidine	5.59	5.08	3.98	3.26	2.88	2.98	2.88	3.15	0.71
Lysine	22.24	22.64	15.99	14.73	12.95	11.21	11.68	10.21	0.85
Arginine	13.87	12.56	9.57	9.28	8.40	10.87	6.44	7.91	1.38
Total amino acids μmol/100 mg anther (adjusted)	250.48	250.48	198.30	198.30	180.63	180.63	168.04	168.04	
Total amino acids μmol/100 mg anther (unadjusted)	250.48	271.88	198.30	206.19	180.63	183.79	168.04	154.90	7.78
Total amino acids μmol/1000 anthers	99.70	106.10	141.60	140.50	195.20	147.70	280.00	138.30	

* Least significant difference $P \leqq .05$

Table 2. *Bound amino acid contents (μmoles/100 mg dried sample) leaves of male sterile (Ms) and its counter fertile (F) line*

Amino acids	Seed		Seedling		Leaves		LSD* (0.05)
	F	Ms	F	Ms	F	Ms	
Aspartic acid	4.16	3.53	18.50	18.60	12.08	11.52	0.66
Threonine	1.96	2.10	4.00	4.02	7.46	7.22	0.39
Serine	3.50	3.30	4.61	4.93	8.87	9.11	0.44
Glutamic acid	18.60	18.57	10.14	9.75	15.53	15.14	0.86
Proline	7.12	6.80	3.10	2.80	7.66	7.07	0.63
Glycine	5.52	5.16	8.09	7.79	14.00	14.71	0.84
Alanine	3.70	3.77	8.40	8.65	14.00	14.00	0.47
Cysteine	0.51	0.39	0.42	0.50	0.50	0.45	0.25
Valine	4.10	4.56	7.00	7.06	9.00	9.41	0.53
Methionine	1.00	1.21	1.00	0.96	0.90	1.55	0.39
Isoleucine	1.70	1.94	3.40	3.13	5.70	5.33	0.35
Leucine	4.75	4.52	4.58	4.82	11.50	11.57	0.74
Tyrosine	1.21	1.36	1.51	1.67	3.39	3.31	0.30
Phenyl Alanine	2.30	2.51	2.48	2.42	5.51	5.26	0.46
Histidine	1.31	1.26	1.46	1.48	2.30	2.41	0.26
Lysine	2.20	2.10	5.18	5.36	9.65	9.25	0.71
Arginine	2.20	2.76	3.75	3.68	5.50	5.53	0.33
Total amino acids μmol/100 mg sample (adjusted)	65.84	65.84	87.62	87.62	132.83	132.83	
Total amino acids μmol/100 mg sample (unadjusted)	65.80	83.72	87.62	91.09	132.83	154.76	4.70

* Least significant difference $P \leqq .05$

higher respectively (Table 1). It is possible, therefore, that *T. timopheevi* cytoplasm has an effect on amino acid synthesis in male sterile plants. Higher total crude protein content of seeds from *T. timopheevi* derived male sterile lines as compared with their fertile analogues was reported by Wilson (1968) and Rai *et al.* (1970). The evidence that mitochondria from male sterile (*T. timopheevi* cytoplasm) wheats have higher metabolic activity than their fertile lines (Srivastava *et al.*, 1969) may be extended to explain the basis for higher amino acids present in the male sterile wheats. Flower buds of male sterile lines of sorghum, sunflower and tomato are also known to contain higher amounts of amino acids when compared with their fertile counterparts (Silvolap, 1968).

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