PROTEIN CONTENT AND AMINO ACID PROFILE OF POLLEN RESTORERS IN RAPESEED /B.NAPUS/ AND MUSTARD /B.JUNCEA/

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Anand et al. /1985/ isolated restorer genes for the CMS B.juncea from the putative monogenomic species through planned interspecific hybridisation. Selection for profuseness of pollen production and pollen viability have further led to development of restorer lines in each of restorer sources, RC and RN /restorer genes from B.campestris ssp. oleifera and B.nigra respectively, in B.juncea background/ where promising lines show as high fertility as 90-95 per cent in their crosses with CMS juncea. As differences in the levels of a number of substances, their accumulation or disappearance during development of androecium helps in understanding the sequence of events leading to male sterility and their control by the hereditary elements, the present study was conducted to analyse some of the biochemical constituents specially the protein content and amino acid profile of some of the improved restorers in each of RC, RN and their cross combination RC x RN and B.napus restorers /R Nap/ and to compare them with their respective CMS lines.

Materials and methods

Inflorescences of the three forms of CMS B.juncea viz. petaloid, stigmoid and rudimentary and their maintainer /i.e. normal fertile B.juncea/, highly promising restorers derived from B.nigra /RN/, B.campestris /RC/ and their cross RC x RN /all incorporated into B.juncea background/, MS x RC, MS x RN and MS x /RC x RN/, alongwith the inflorescence of male sterile, maintainer and restorer B.napus constituted the material for estimation of crude protein content. The modified Kjeldahl method of protein assay,

based on the estimation of reduced organic nitrogen was followed to determine the protein content.

For studying the amino acid profile, anthers were collected from the above mentioned sources using anthers collected from mature flower buds, a day prior to their opening. Amino acid composition both in protein bound and in free pool was analysed with the help of a Technicon Sequential Multi-sample /TSM/ amino acid auto analyzer.

Results and discussion

Cytoplasmic male sterility is fundamentally an aspect of the problem of gene action and nuclear cytoplasmic interaction. Cytoplasm is the seat of protein synthesis. Changed cytoplasm of male steriles might affect this vital process and lead to changes in amounts of protein synthesized, and also their constituents - the amino acids.

Among B.juncea types, RC x RN recorded maximum protein content /39.16%/. All the three forms of male steriles contained lower amount of protein /table-1/ than the normal male fertile /37.37%/. Among steriles, rudimentary type recorded the lowest protein /31.19%/. Both the restorers /RC and RN/ contained less protein than normal but their combination, RC x RN exhibited the highest amount of protein /39.16%/. Inflorescences of crosses involving restorers and MS showed higher protein content than normal fertile, the maximum /39.10%/ being that of MS x /RC x RN/. In B.napus, normal fertile and restorer /Rnap/ showed almost equal amounts of protein while the sterile form contained a reduced amount.

Since cytoplasm is the site for protein synthesizing organelles, reduced efficiency of these organelles in the sterile cytoplasm can very well be visualized. Deficiency of proteins could mean improper expression of vital genetic information. The possible role of defective enzyme system of the protein synthesizing pathway in the sterile cytoplasm is indicative of its close involvement with male sterility. The restorers RC and RN had protein content intermediate to MS forms and the normal fertile, while

RC x RN had two per cent higher protein content than normal fertile. This could be due to the interaction effect or complementation of restorer genes of the component monpgenomic species.

Protein content in the crosses between petaloid form of CMS and the restorers, showed an increase over male steriles and the normal fertile, possibly indicating that restoration mechanism operates through correction of deficient protein synthesis.

Amino acid analysis

It is expected that the bulk of the proteins in anthers would be constituted of enzymes, of which amino acids are the important constituents. Thus the differences in the amino acid composition of anthers of different forms would be suggestive of basic differences in protein quality and quantity. Inadequate synthesis of one or the other enzyme could cause metabolic imbalances in the anthers leading to sterility.

The amino acid profiles for the anthers of normal fertile, the three forms of male steriles, and the various restorers expressed as mg. amino acid per g anther for B.juncea and B.napus are presented in Tables 2 and 3 respectively.

Protein bound amino acids accounted for 80-90 per cent of the total amino acid content. Of the small amounts of amino acids present in the free pool, proline was the predominant one in all the lines, though in terms of absolute values it varied from line to line. This was followed by glutamic acid, alanine and threonine, in that order.

Protein bound aspartic and glutamic acids in all the three male sterile B.juncea forms were higher than normal except the protein bound aspartic acid of MS rudimentary which was less than normal. Among restorers and their crosses with the male sterile, RC x RN, MS x RC, MS x RN and restorer B.napus /Rnap/ recorded higher protein bound

aspartic and glutamic acid, while MS x /RC x RN/ showed higher amounts of aspartic acid alone. All the three male sterile B.juncea forms and MS stigmoid of B.napus had less than normal protein bound proline content. Proline content in free pool was lower than that of normal for B.juncea and B.napus male steriles.
Similarities among the three male sterile forms of B.juncea can be expected, as they have been reported to be interchangeable under extreme temperatures /Anand et al., 1985/. As in the case of male steriles, restorers also showed proline deficiency as compared to normal; for RN it was drastically low. Except for the protein bound proline in case of MS x /RC x RN/ and free and protein bound proline of Rnap which were higher than normal, all the restorers and their crosses with MS were deficient for proline.

Aberrant amino acid level found in male sterile anthers could be directly related to the male sterile condition. Nevertheless, the exact role played by either reduced proline content or increased aspartic acid level in the phenomenon of male sterility is not clearly understood.

Male sterile lines had lower methionine content than normal. Since, methionine is the chain initiator for protein biosynthesis, its deficiency can be linked to reduced protein synthesis of MS lines as revealed by the crude protein content of male sterile inflorescence.

Anthers of the three male sterile B.juncea forms and MS B.napus were further characterised by slightly lower contents of most of the neutral amino acids and slightly higher amounts of ammonia than the normal fertiles.

There were differences in the amino acid composition of restorers from B.nigra source /RN/ and B.campestris source /RC/. These could be due to differences in pollen sterility probably resulting due to chromosomal irregularities arising during the process of transfer of the restorer genes from the diploid progenitors. Higher amounts of all the protein bound amino acids, except histidine, proline and cystine in the RC x RN could probably be attributed

to interaction or complementation effect of restorer genes from the two component sources.

B.napus male sterile had higher protein bound lysine and ammonia, than normal. The restorer was found to have higher values than normal for all amino acids except for free pool cystine.

Percentage of amino acids incorporated /out of the total available/ for each of the sub-groups, in different types showed that in case of RC, percentages of basic and acidic groups incorporated into proteins were lower than normal by 12.45 and 9.35 per cent respectively. For RN, incorporation of acidic group was 11.49 per cent lower than that of normal. The crosses MS x restorers had higher incorporation percentages for neutral group of amino acid.

In the crosses of restorers with MS, acidic protein synthesis increased; and also proteins containing high amounts of neutral amino acids were synthesized. This probably indicates that neutral amino acids play some role in restoration mechanism.

Besides percentage incorporation of neutral group of amino acids was more than normal in all types of CMS and restorers in B.juncea but was less than normal in B.napus. These differences in combination with either increased or reduced incorporation of other groups seem to control the mechanisms of male sterility and restoration.

Marked difference for the amino acid profile of the normal, male steriles, restorers and the crosses of the male steriles and restorers indicated the relative changes occurring with respect to normal anthers and suggested that amino acids probably have a role in the mechanism of sterility and fertility restoration.

Summary

Protein content and amino acid profile of diffirent CMS B.juncea forms, their normal fertile maintainer lines and some of improved restorers in each of RC, RN and RC x RN /restorers from B.campestris and B.nigra respectively in

B.juncea background/ sources, and CMS B.napus, its normal fertile and restorer B.napus /RNap/ were compared to understand the sequence leading to male sterility. The study revealed that the three forms of male sterility viz. petaloid, stigmoid and rudimentary and RC and RN restorers showed lower protein content than the normal fertile, however, the restorers had higher amount than the CMS. RC x RN, CMS x RN and CMS x RC combinations exhibited higher protein content /more than 2 per cent/ than the normal fertile. Restorer /RNap/ and normal fertile B.napus had almost equal protein content /39.04 per cent/ but much higher than CMS napus /33.74 per cent/.

The free pool and protein bound amino acids in the various CMS forms of B.juncea and B.napus has shown that the proteins were deficient in methionine suggesting its effect in reducing the protein synthesis. Glutamic acid and proline in CMS forms were high and were indicative of synthesis of acidic proteins. Total protein bound amino acids were higher in RC and CMS x RC forms than CMS and were more or less equal to normal fertile. The overall study revealed that amino acids probably have a role in the mechanism of male sterility and fertility restoration.

References

Anand I.J., P.K.Misra, D.S.Rawat, 1985. Mechanism of male sterility in Brassica juncea I. Manifestation of sterility and fertility restoration. Cruciferae Newsletter 10: 44-46.

Table 1 : Protein content in the inflorescence of normal fertile, male sterile and restorer lines of *B.juncea* and *B.napus*.

Line	Protein content (%) on dry weight basis		
	Sample 1	Sample 2	Sample 3
B.juncea normal fertile	36.55	38.19	37.37
B. juncea male sterile			
i) Petaloid	32.75	33.99	33.37
ii) Stigmoid	30.98	33.23	32.10
iii) Rudimentary	30.08	32.30	31.19
Restorer *campestris* (RC)	36.68	36.54	36.61
Restorer *nigra* (RN)	35.25	35.56	35.40
RC x RN	38.99	39.33	39.16
MS x RC	37.89	39.24	38.56
MS x RN	38.55	38.82	38.63
MS x (RC x RN)	39.86	38.34	39.10
B.napus normal fertile	38.00	39.20	38.60
B.napus male sterile	34.32	33.17	33.74
B.napus restorer (Rnap)	39.09	38.99	39.04

Table 2: Amino acid content / mg/g anther/ in the anthers of male fertile, male sterile and fertility restorer lines of B.juncea.

	Normal/fertile/		Male sterile						RC		RN		RC x RN		MS x RC		MS x RN		MS x /RCxRN/	
			Petaloid		Stigmoid		Rudimentary													
	Fp[x]	Pb[xx]	Fp	Pb	Fp	Pb	Fp	Pb	Fp	Pb	Fp	Pb	Fp	Pb	Fp	Pb	Fp	Pb	Fp	Pb
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
tic																				
spartic acid	0.215	3.647	0.612	3.926	0.316	4.321	0.231	2.957	0.436	2.831	0.512	1.235	0.740	5.597	0.214	3.842	0.241	4.440	0.697	5.431
utamic acid	1.223	5.156	0.725	5.923	1.324	6.371	1.320	6.025	1.302	3.304	0.896	3.243	1.473	7.626	0.398	6.315	0.610	5.830	1.218	3.987
ub-total	1.438	8.803	1.337	9.849	1.640	10.692	1.551	8.982	1.738	6.135	1.408	4.478	2.213	13.223	0.612	10.157	0.851	10.270	1.915	9.418
ic																				
sine	0.322	5.401	0.132	3.624	0.323	4.963	0.318	5.467	0.386	2.419	0.085	0.973	0.370	7.177	0.179	3.971	0.259	4.435	0.346	3.926
istidine	0.051	2.063	0.098	1.963	0.049	2.121	0.064	1.985	0.118	0.867	0.073	1.925	0.086	2.003	0.415	2.198	0.045	2.205	0.125	1.987
rginine	0.427	3.413	0.796	3.241	0.392	4.208	0.538	3.512	0.626	1.708	0.273	2.976	0.556	4.083	0.312	3.789	0.245	2.788	0.486	3.128
ub-total	0.800	10.877	1.026	8.828	0.764	11.292	0.920	10.964	1.130	4.994	0.431	5.874	1.014	13.263	0.906	9.958	0.549	9.428	0.957	9.041
ral																				
erine	0.565	2.132	0.122	2.313	0.682	1.963	0.653	2.038	0.680	1.793	0.235	0.672	1.023	3.949	0.613	4.621	0.516	3.330	1.062	3.562
roline	5.868	3.893	3.326	4.923	4.153	4.735	3.987	4.986	2.222	1.757	0.735	0.679	3.872	3.114	0.976	3.418	1.582	2.760	2.978	4.213
lycine	0.162	2.660	0.098	2.342	0.159	2.581	0.189	2.573	0.220	1.860	0.135	1.926	0.228	3.832	0.078	4.362	0.125	3.190	0.213	2.875
lanine	0.883	2.227	0.318	2.142	0.763	2.231	0.867	2.327	0.598	1.359	0.720	0.972	0.853	4.025	0.373	3.192	0.495	2.990	0.783	3.135
aline	0.324	2.301	0.126	2.431	0.238	1.973	0.319	3.257	0.340	1.700	0.372	1.912	0.445	3.961	0.098	3.821	0.121	2.860	0.542	3.341
ystine	0.322	1.341	0.073	0.978	0.317	1.631	0.312	1.317	trace	1.181	0.124	0.736	0.288	1.189	0.486	2.721	0.512	1.170	0.231	2.173
ethionine	0.047	0.789	0.035	0.531	0.072	0.582	0.039	0.212	0.128	0.460	0.031	0.246	0.112	1.074	0.092	1.237	0.081	0.600	0.113	0.896
soleucine	0.174	2.075	0.152	2.312	0.157	2.063	0.187	2.068	0.128	1.838	0.183	1.972	0.259	3.773	0.187	4.216	0.138	3.050	0.395	2.986
eucine	0.227	3.887	0.080	2.968	0.232	3.851	0.219	2.352	0.226	2.833	0.053	0.873	0.337	6.558	0.135	3.897	0.190	4.980	0.238	4.312
hreonine	0.725	1.853	0.635	1.763	0.737	2.051	0.689	0.973	1.006	1.846	0.231	0.731	1.505	2.796	0.492	3.487	0.889	2.760	1.315	3.216
yrosine	0.147	2.641	0.173	2.562	0.158	2.638	0.143	2.651	trace	1.706	0.112	1.312	0.212	3.385	0.134	3.831	0.248	2.440	0.128	2.312
henylalanine	0.283	2.881	0.189	1.926	0.277	2.796	0.298	1.985	0.376	2.187	0.043	0.661	0.350	4.856	0.142	4.217	0.168	3.680	0.342	2.986
Sub-total	9.727	28.680	5.327	27.191	7.945	29.095	7.902	26.739	5.924	20.520	2.974	12.692	9.484	42.512	3.806	43.02	5.065	33.810	8.340	36.007
Amonia	0.114	0.328	0.321	0.673	0.123	0.826	0.147	0.731	0.254	0.307	0.042	0.098	0.159	0.528	0.312	0.618	0.134	0.540	0.182	0.581
Total	12.079	48.688	8.011	46.541	10.472	51.905	10.520	47.416	9.046	31.956	4.855	23.142	12.87	69.526	5.636	63.753	6.599	54.048	11.394	55.047

Fp=Free pool aminoacids Pb=Protein bound aminoacids

Table 3 : Amino acid content (mg/g anther) in the anthers of normal fertile, male sterile and fertility restorer (Rnap) lines of *B.napus*

Aminoacid	Normal fertile		Male sterile (stigmoid)		Rnap	
	Fp	Pb	Fp	Pb	Fp	Pb
Acidic						
Aspartic acid	0.081	3.313	0.109	2.130	0.142	4.846
Glutamic acid	0.371	3.498	0.371	2.669	0.441	7.232
Sub-total	0.452	6.811	0.480	4.799	0.583	12.078
Basic						
Lysine	0.086	0.303	0.149	2.179	0.281	7.087
Arginine	0.026	0.686	0.037	0.532	0.058	1.730
Histidine	0.061	2.102	0.445	1.845	0.173	4.757
Sub-total	0.173	3.091	0.631	4.556	0.512	13.574
Neutral						
1. Serine	0.206	2.317	0.497	1.352	0.451	3.718
2. Proline	1.676	1.505	1.148	1.078	4.203	3.324
3. Glycine	0.095	1.892	0.156	1.373	0.183	3.543
4. Alanine	0.322	1.687	0.459	1.409	0.855	3.827
5. Valine	0.097	1.659	0.129	1.561	0.355	3.561
6. Cystine	0.181	0.517	-	0.368	0.048	2.161
7. Methionine	0.018	0.516	0.101	0.240	0.073	1.293
8. Isoleucine	0.067	1.714	0.079	1.283	0.193	3.291
9. Leucine	0.09	3.679	0.115	2.105	0.282	5.554
10. Threonine	0.271	1.717	0.249	1.026	0.853	2.889
11. Tyrosine	0.088	1.959	0.079	1.196	0.263	3.548
12. Phenylalanine	0.123	2.229	0.093	1.279	0.146	4.540
Sub-total	3.237	21.391	3.105	14.270	7.925	41.249
Ammonia	0.055	0.232	0.108	0.531	0.805	0.432
Total	3.917	31.525	4.324	24.156	9.825	67.333

Fp = Free pool amino acid Pb = Protein bound amino acid