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BIOCHEMICAL ALTERATIONS IN MALE–STERILE BARLEY

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The biochemical alterations following male sterility were investigated by using the anthers and flag leaves of nine male fertile and male fertile and male sterile barley genotypes. The amount of proteins, starch and total sugars is significantly less in the mature anthers of male sterile plants as compared to their fertile counterparts. The decrease being 25–40% in protein content, 50–70% in starch and about 60–85% in the amount of total sugars. In contrast to anthers, the amount of these biochemical substances increases significanty in the flag leaves of male sterile plants at grain filling stage. The percentage increase in protein content ranges between 60–150% 65–100% in starch content and about 25 to 190% in total sugar content. The amount of total chlorophylls was also significantly high in the levels of male sterile plants. Whether these biochemical differences are the causes of consequences of male sterile gene action is not known.

Keywords : Male sterility; Biochemical alterations; Gene.

Introduction

Male sterility is a condition in which male sex is either absent or non-functional whereas female sex is functional. The sterility is rampant and useful in genetics and breeding (Kaul 1988). Barley, a self pollinated diploid feed and food grain plant and cytogenetically intensively explored, has over 50 male-sterile genes in its genome of which 33 are non-allelic. Suneson (1940) first documented the existence of male sterility in field grown barley in 1936. Austenson (1948) detected another *ms* gene (ms_2), Kasha and Walker (1960) detected ms_3 gene. In all genetic male sterile cases, the genes conditioning sterility are monogenic recessive. The features of male- sterility introduce alterations in anther biochemistry, physiology and function. In most of the cases, they appear as consequences rather than causes. In order to investigate the biochemical alterations following male sterility, alterations in sugars, starch and proteins in the anthers of barley are studied.

Materials and Methods

Nine non-allelic, single recessive genes controlled male sterile female fertile barley mutants were used.

Flag leaves were used for the estimation of total chlorophylls, proteins, starch and sugars at the grain filling stage. Mature anthers were taken for the estimation of protein, starch and sugars after the *ms* genes action. The chlorophylls were extracted by the method outlined by Arnon (1949). Protein was measured by using Folin reagent followed by Lowry *et al.* (1951) method. Starch and sugars were estimated by Dubois *et. al.* (1956) method.

Observations

The difference in starch, sugars and protein contents in the anthers of male fertile and male sterile segregants is shown in Table 1. In all the genotypes, these biochemical components show a gradual or drastic reduction in the anthers of sterile plants as compared to the corresponding fertile ones. The percentage decrease in proteins is nearly 40% in msk_6, msk_7, msk_8 and msk_9. In the remaining genotypes, the percentage decrease in protein content is about 25%. Like proteins, the starch content in the anthers of male sterile plants decreases significantly. This reduction is about 50% in msk_1, msk_3, msk_5 and msk_8. The maximal reduction by about 70% over its male fertile occurs in the msk_2. Likewise, ths decrease in sugar content is about 85% in msk_1 and msk_2 while the reduction is nearly 60% in msk_3, msk_6 and msk_8 over their fertile counterparts.

In contrast to anthers, the amount of biochemical substances increases significantly in the flag leaf of male sterile plants. The male sterile genes not only delay senescence but considerably enhance the chlorophyll content (Table 3). Maximum increase is about 75%. It occurs in the male mutant msk_4. The amount increases to about 25% in msk_3, msk_6 and msk_7. Similarly, a drastic increase in the protein content (150%) in msk_3 followed by msk_1 (125) occurs in male sterile plants. About 60% increase in leaf protein content occurs in msk_3, msk_8 and msk_9 male sterile mutants (Table 2). Similarly, the starch content increases to about 65–70% in msk_2 msk_7. This increase is more than 100% in msk_3. Like the starch content, the sugar content of the leaves in sterile plants increases significantly over their corresponding male fertiles. The increese is maximum in msk_3 where the increase is about 200%. This is followed by msk_1 where the increase is about 150% (Table 2).

Discussion

Male sterile plants are characterised by lack of viable pollen or mis-differentiation or mis-development of anthers or their contents. Such features induce alterations in anther biochemistry, cytochemistry, cyto-

Table 1. Biochemical alterations (mg/100 fresh weight) in fhe mature anthers of male fertile and male sterile barley genotypes

Geno–types	Starch mf	Starch ms	% reduc–tion	Sugars mf	Sugars ms	% reduc–tion	Proteins mf	Proteins ms	% reduc–tion
msk_1	0.402 a_1x_1 ±0.071	0.202 a_1x_2 ±0.023	49.7	0.574 a_1x_1 ±0.062	0 102 a_1x_2 ±0.015	82.2	0.263 a_1x_1 ±0.033	0.199 a_1x_2 ±0.022	24.3
msk_2	0.575 a_2x_1 ±0.083	0.179 a_2x_1 ±0.021	68.8	0.691 a_2x_1 ±0.075	0.104 a_1x_2 ±0.031	84.9	0.487 a_2x_1 ±0.061	3.384 a_2x_2 ±0.043	21.1
msk_3	0.601 a_2x^1 ±0.092	0.320 a_3x_2 ±0.040	46.7	0.475 a_3x_1 ±0.053	0.210 a_2x_2 ±0 022	55.7	2.396 a_3x_1 ±0.042	0.290 a_3x_2 ±0.031	26.3
msk_4	0.602 a_2x_1 ±0.081	0·387 a_4x_2 ±0.047	35.7	0.549 a_4x_1 ±0.071	0.386 a_3x_2 ±0.045	40.5	1.100 a_4x_1 ±0.078	0.830 a_4x_2 ±0.020	25.4
msk_5	0.645 a_3x_1 ±0.090	0.343 a_5x_2 ±0.036	46.8	0.569 a_1x_1 ±0.065	0.318 a_4x_2 ±0.039	44.1	0.670 a_5x_1 ±0.083	0.496 a_5x_2 ±0.055	25.9
msk_6	0.860 a_4x_1 ±0.113	0.668 a_6x_2 ±0.081	22.3	0.660 a_2x_1 ±0.072	0.230 a_5x_2 ±0.026	65.1	0 471 a_2x_1 ±0.061	0.286 a_3x_2 ±0.031	39.2
msk_7	0.834 a_4x_1 ±0.115	0.518 a_7x_2 ±0.056	37.8	0.430 a_3x_1 ±0.049	0.211 a_2x_2 ±0.023	51.0	0.487 a_2x_1 ±0.063	0.302 a_3x_2 ±0.034	38.0
msk_8	0.985 a_5x_1 ±0 123	0.508 a_7x_2 ±0.501	48.4	0.793 a_5x_1 ±0 082	0.300 a_4x_2 ±0.03	62.1	0.489 a_2x_1 ±0.061	0.284 a_3x_2 ±0.031	42.0
msk_9	0.825 a_4x_1 ±0.101	0.520 a_7x_2 ±0.047	37,0	0.440 a_3x_1 ±0.041	0.220 a_5x_2 ±0.025	50 0	0.495 a_2x_1 ±0.062	0 295 a_3x_2 ±0.032	40.4

N = 25; ± = Standard Diviation; mf = male fertile; ms = male sterile.

Table 2 Biochemical alterations (mg/100 mg fresh weight) in the leaves of male fertile and male sterile barley genotypes at grain filling stage

Geno-type	Starch mf	Starch ms	% increase	Sugars mf	Sugars ms	% increase	Proteins mf	Proteins ms	% increase
msk_1	$0.304a_1x_1$ $\pm$0·090	0 601a_1x_2 $\pm$0 050	97.7	0 212a_1x_1 $\pm$0.019	$0.555a_1x_2$ $\pm$0.049	161.7	0 296a_1x_1 $\pm$0.020	$0.670a_1x_2$ $\pm$0.039	126.3
msk_2	$0.240a_2x_1$ $\pm$0.070	$0.395a_2x_2$ $\pm$0.090	64.5	$0.190a_2x_1$ $\pm$0.023	$0.302a_2x_2$ $\pm$0.034	58.9	$0.274a_2x_1$ $\pm$3.037	$0.683a_2x_2$ $\pm$0.045	149.2
msk_3	$0.171a_3x_1$ $\pm$0.060	0 404a_2x_2 $\pm$0.080	136.2	$0.105a_3x_1$ $\pm$0.027	$0.308a_2x_2$ $\pm$0.037	193.3	$0.305a_3x_1$ $\pm$0.030	$0.483a_3x_2$ $\pm$0.056	58.3
msk_4	$0.500a_4x_1$ $\pm$0.090	0 689a_3x_2 $\pm$0.090	37.8	$0.540a_4x_1$ $\pm$0.024	$0.566a_1x_2$ $\pm$0.054	4 8	$0.205a_4x_1$ $\pm$0.030	$0.240a_4x_2$ $\pm$0.030	17.1
msk_5	$0.278a_2x_1$ $\pm$0.080	0 385a_2x_2 $\pm$0.070	38 4	$0.230a_5x_1$ $\pm$0.021	$0.445a_3x_2$ $\pm$0.039	93.4	$0.434a_5x_1$ $\pm$0.036	$0.709a_5x_2$ $\pm$0.069	63.3
msk_6	$0.555a_5x_1$ $\pm$0.090	0 660a_1x_2 $\pm$0.050	18.9	$0.502a_6x_1$ $\pm$0 027	$0.623a_4x_2$ $\pm$0.021	24.1	0 412a_6x_1 $\pm$0.025	$0.513a_6x_2$ $\pm$0.035	24.5
msk_7	$0.270a_2x_1$ $\pm$0.050	$0.457a_4x_2$ $\pm$0 060	69.2	$0.195a_2x_1$ $\pm$0.010	$0.288a_5x_2$ $\pm$0.017	47.6	$0.415a_6x_1$ $\pm$0.039	$0.604a_7x_2$ $\pm$0.027	45.5
msk_8	0 462a_4x_1 $\pm$0.080	0 575a_1x_2 $\pm$0.070	24.4	$0.430a_7x_1$ $\pm$0.026	$0.563a_1x_2$ $\pm$0.026	30.9	$0.477a_7x_1$ $\pm$0.018	$0.595a_8x_2$ $\pm$0.026	24.7
msk_9	$0.400a_6x_1$ $\pm$0.050	$0.505a_5x_2$ $\pm$0.090	26.2	$0.192a_2x_1$ $\pm$0.021	$0.290a_5x_2$ $\pm$0.017	51.0	$0.450a_7x_1$ $\pm$0.038	$0.570a_9x_2$ $\pm$0.034	26.6

N=25; $\pm$ = Standard Deviation; mf = male fertiie; ms = male sterile.

Table 3. Total chlorophyll content (mg/g fresh weight) in the leaves of male fertile and male sterile plants at grain filling stage

Genotype	Chlorophyll a+b mf	Chlorophyll a+b ms	% increase
msk_1	1.03 a_1x_1 ±0.11	1.22 a_1x_2 ±0.10	18.4
msk_2	1·47 a_2x_1 ±0.12	1.95 a_2x_2 ±0.16	32.6
msk_3	1.09 a_1x_1 ±0.14	1.37 a_3x_2 ±0.11	25.6
msk_4	1.05 a_1x_1 ±0.12	1.83 a_2x_2 ±0.12	74.2
msk_5	1.29 a_3x_1 ±0.11	1.57 a_4x_2 ±0.10	21.7
msk_6	1.01 a_1x_1 ±0.09	1.33 a_3x_2 ±0.13	31.6
msk_7	1.26 a_3x_1 ±0.12	1.54 a_4x_2 ±0.14	22.2
msk_8	1.14 a_4x_1 ±0.10	1.23 a_1x_2 ±0.12	7.9
msk_9	1.15a_4x_1 ±0.13	1.21 a_1x_2 ±0.10	5.2

N = 25; ± = Standard Deviation; mf = male fertile; ms = male sterile

The differences have been computed by DMRT after using Dancan's multiple range test.

Values of the two mean pairs (horizontal) followed by different symbols of alphabet (a) differ significantly from each other at 5P level.

Values of the various means (Linear array) followed by different symbols of alphabet (x) differ significantly from each other at 5P level.

chemistry and physiology in some plants (Kaul 1988). In many male-sterile mutants investigated biochemically, decreased or disturbed carbohydrate and protein metabolism in the anthers occurs in the male-sterile plants of beets (Chauhan and Kinoshita 1980), pepper (Markova and Daskaloff 1976), sunflower (Pirev 1966), wheat (Fuka sawa 1957, Savchenko *et al.* 1968), Indian mustard (Banga *et al.* 1984) and rice (Kaul 1988). Likewise in the anthers of the male sterile mutants of barley investigated presently, there is a strong reduction in carbohydrate and protein contents whereas, the protein content is decreased by 25-40%, the starch decreases by 50-70% and sugars by 60-85% of their corresponding male fertile line. The decrease is genotype specific. Likewise, Singh (1988) found reductions in starch, proteins, proline and nucleic acids in the anthers of some other male sterile mutants of barley. This reduction in certain biochemical components essential for growth, developmet, maintenance and functioning of anthers of male steriles appears a common feature. In fact, reduction or complete absence of various biochemical components like amino acids, proline, nucleic acids, cytokinin content, lower reducing activity of enzyme dehydrogenase phosphorylase has been reported in the male sterile anthers of cereal crops by Kaul (1988).

Many male sterile genotypes of maize, pea, rice, tomato and wheat have higher leaf chlorophyll content than their male fertile counterparts (Kaul 1988). In all the nine male sterile barley mutants, the chlorophyll content significantly increases in the leaves. This renders the male steriles dark greener in colour than their corresponding male fertiles. This colour difference assists in differentiating male sterile from the male fertiles in the field. In 12 other barley male sterile mutants, Singh (1988) detected increased chlorophyll content in the leaves.

Carbohydrates, the principal cell wall components and reserve food material of cereal seeds, are highly influenced by *ms* genes as there are either considerable deviations or significant increase in leaf carbohydrates of nine barley male sterile mutants investigated presently and 12 mutants investigated by Singh (1988). In all these barley male sterile mutants, the carbohydrates content is significantly increased in the leaves. Unfortunately, not much is known about the leaf carbohydrate and protent differences between male sterile and male fertile plants (Kaul 1988).

In the present studies, compared to their anthers, the amount of carbohydrates and proteins increases in the leaves of male sterile plants at grain filling stage. But whether such alterations of vital substances like carbohydrates and proteins are the causes or consequences of *ms* gene action is not known. But this is certain that since these biochemical components are essential to provide nutrition and energy, their depletion starves the developing microspores to death. Thus, a drop in the biochemical components in the anthers is a regular feature of male sterile plants.

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