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MORPHOLOGICAL STUDIES ON MALE-STERILE BARLEY

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Nine non-allelic single recessive *ms* genes induced complete male sterility when present singly in double recessive state in barley genome. These genes also influence tillering capacity of the barley plant. But unlike other male sterile genes, none of the nine genes influences shoot height, ear length, flag leaf area, spikelets/spike and days to heading. However, the male sterility caused the spikes to become lax after flowering. Anthers of all the male sterile plants were shrivelled, shrunken and non-dehiscent. Their size and weight was significantly lower than that of their fertile counterparts. All the male steriles are pollenless and their anthers lodge degenerated microspores which are clumped or stranded.

Keywords : Male sterile; Gene; Barley; Genome.

Introduction

Inability to produce functional male gametes is known as male sterility. It includes male sex suppression or transformation of male sex organs into non-male organs. The sterility is controlled by genes which adversely affect the male sex differentiation and development, or its function. These genes seem to exhibit pleiotropic action as they affect some other plant traits as well. This is evidenced by the action of nine non-allelic single recessive *ms* genes in barley. Results of this study comprise the text of this paper.

Material and Methods

Screening of male steriles : The male sterile plants were screened through pollen fertility test using 2% acetocarmine Whereasthe fertile pollen grains stained, the sterile grains remained unstained. On the basis of this staining difference, the male sterile plants wereidentified They were tagged and later studied for agronomic performance and anther and pollen characteristics.

Data recording : Data for various morphological traits of randomly selected 15 plants, 5 plants per replication avoiding the border plants, of each genotype were recorded for male fertile and male sterile plants. Significance of mean difference was evaluated by Duncan's multiple range test as given by Gomez and Gomez (1984).

Morphological studies: The male fertiles and steriles were studied for the following traits :

(i) *Shoot height* : The height of the main shoot was measured in cm from the ground level to the tip of the ear, excluding awns just prior to harvest.

(ii) *Days to heading* : The date on which 50% of the ears emerged from the boot leaf was noted and the number of days taken from date of sowing to heading were calculated.

(iii) *Number of tillers/plant* : The total number of first crop tillers (including primary and secondary tillers) in case of fertile plants and total number of first crop + late or second crop tillers in case of steriles were counted at the time of harvesting.

(iv) *Ear length* : The length of the main spike was measured in cm from the ring at the base of the rachis to the tip of the uppermost spikelet excluding awns.

(v) *Number of spikelets/ear* : Total number of spikelets of the main spike was taken as the number of spikelets per ear.

(vi) *Flag leaf area* : This was determined at the grain filling stage by multiplying flag leaf length by its maximal width and a coefficient whose value for barley is 0.6438 (Starling 1980).

Pollen and anther charecteristics : Five spikes from each male sterile genotype were selected randomly. At least two florets were sampled from each of the five spikes just prior to filament elongation and pollen dehiscence. Fertile spikes were also taken for comparison. Observations on pollen appearance ratings were also made while studying pollen stainability. The pollen appearance ratings were (1) normal, (2) mostly shrivelled, (3) shrivelled and very much reduced and clumped. Pollen stainability was determined by staining the pollen in 20% acetocarmine and by counting the number of non-stained and stained pollens present in five microscope fields per preparation from each floret. Thus, in all 50 observations were made for each male sterile genotype. Percentage fertility of each genotype was calculated by the following formula :

$$\% \text{ Fertility} = \frac{\text{Number of sterile pollen}}{\text{Total number of pollen}} \times 100$$

Anther size : For determination of the anther size, five spikes were selected at random form male sterile plants and their male fertile sibs of each of the nine male sterile mutants. Five florets were selected from each

of the spikes at filament elongation stage and placed in Langlet's modification II of Navashin's solution (CARF fixative) for 24 hours. They were then washed in distilled water and stored in 70% ethyl alcohol. Five anthers, one each from five random florets, from the total of twenty-five florets collected were placed on a microscope slide in 70% ethyl alcohol. Measurements were made with an ocular micrometer.

Anther weight : Five spikes were selected at random separately from each of the male sterile and male fertile genotypes and six florets were selected from each of the spikes at filament elongation stage. 30 anthers were dissected out from the total of 30 florets. Fresh weight of the 10 anthers was recorded in a single pan electric balance. Two more similar observations were also made. Mean values of the observations were recorded.

Observations

1. Morphological studies : Mean values of various metric traits are given in Table 1 and the traits are described in the following :

There was no significant difference in between the male steriles and their male fertile counterparts in shoot height, spike length, spikelets/spike, Flag leaf area and Days to heading.

However unlike others, the differences in the tiller number of male sterile and male fertile plants are significant in all the genotypes. In all the male steriles, the tiller number is significantly increased. The increase is about 90% in msk1 and msk2. In six genotypes i.e., in msk3, msk4, msk5, msk6, msk7 and msk9, the increase in tillering capacity is about 50–60%. In msk8, the sterile plants have about 40% more tillers than their corresponding fertile ones.

2. Phenological Studies :

General Vigour : Due to the absence of any phenotypic marker in the early stages of growth and development, the growth stages seedling growth, leafing and flowering and general vigour of male fertile and male sterile plants cannot be assessed separately. All the genotypes exhibit normal growth and vigour at this stage.

Spike characteristics : The sterile spikes can easily be distinguished after flowering stage. The fertile barley flower opens slightly at pollination time because of lodicule action and then closes when the ovary is fertilized. The sterile flower opens similarly on the first day of flowering but the ovary remains unfertilized due to absence of selfing. As a result, the florets reopen due to swelling of ovary and lodicules on the 2nd and 3rd days after flowering (Fig. 1).

3. Ovary characteristics : Some interesting changes were observed in the ovary of the sterile florets at the time of pollination. The opening of the florets exposes the stigma at the time of pollination but due to the absence of pollination for the first few days of flowering in the male steriles, the ovary swells (Fig. 2) and keeps the flower open until 7 days. But by 9th or 10th day, the ovary shrinks and florets close.

4. Stomium and filament elongation : The anthers of fertile plants have distinct and well developed stomium and exhibit filament elongation. On the other hand, none of the male steriles had stomium in their anthers. The latter remain non-dehiscent and did not exhibit filament elongation.

5. Anther and pollen characteristics :

(i) *Pollen stainability* : The pollen grains of fertile anthers are engorged with reserves and exhibit nearly cent per cent stainability (Fig. 3). In contrast to male fertiles, the microspores/pollen of all the male steriles exhibit almost nil staining with aceto-carmine as all these genotypes produce shrivelled, reduced, clumped and aborted microspores (Fig. 4). Pollen stainability test reflects complete male sterility in all the male sterile mutants studied presently.

(ii) *Pollen appearance* : Pollen grains of fertile plants are rounded, unshrivelled, turgid, free and engorged with reserve food materials while the pollen grains produced by the majority of male steriles are shrivelled reduced and clumped (Fig. 4) The pollen stainability and appearance in various genotypes is indicated in Table 2.

(iii) *Anther size* : Anthers of all male sterile plants are shrivelled, shrunken, rudimentary and underdeveloped which accordingly are significantly shorter in size than their fertile counterparts. The anther length of male fertiles ranges between 3.70 mm in msk 6 to 3.04 mm in msk9. On the other hand, the anther length of male steriles ranges from 1.90 mm in msk2 to 3.11 mm in msk7. Likewise, the width of male sterile anthers is also less than their fertile counterparts. The anther size of male fertile and steriles are given in table 3.

(iv) *Anther weight* : Since the anthers of male steriles are not fully developed and they do not produce pollen, they exhibit less weight than the fertile ones. This is evidenced from the Table 3.

6. Phenotypic alterations at maturity : Phenotypically, the male steriles do not differ from male fertiles during vegetative phase of growth, but after flowering and during anthesis, the male steriles can be easily identified from the male fertile plants.

Fig. 1. Male fertile and male sterile spikes of barley showing compact and closed (Fertile, A) and loose and open spikelets (Sterile, B).

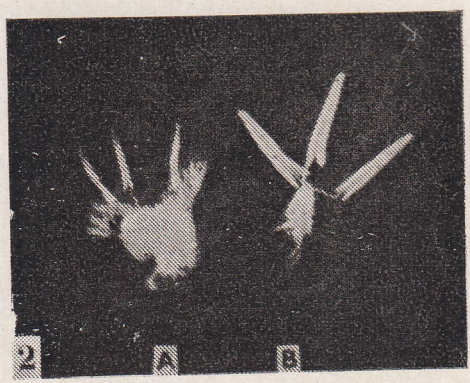

Fig. 2. Unfertilized highly swollen ovary with undeveloped anthers of male steriles (A) and fertilized ovary with well developed anthers of male fertiles (B).

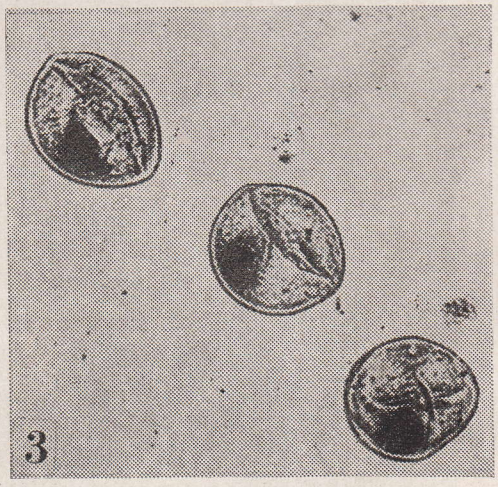

Fig. 3 Fertile pollen exhibiting stained nucleus, high starch content well developed exine and intine.

Fig. 4 Sterile microspores shrivelled, clumped, lacking nuclei and starch.

Table 1 : Comparative performance of male-fertile (MF) and male sterile (MS) barley genotypes.

Genotypes	Shoot height (cm)		Tiller Number		Flag Leaf Area (cm^2)		Spike length (cm)		Spikelets/Spike		Days to Heading	
	MF	MS	MF	MS	MF	MS	MF	MS	MF	MS	MF	MS
msk[1]	89.80a ±2 12	89.40a ±1.50	5 70a ±0.60	10.70a ±0.47	6.00a ±0.03	6.10a ±0.13	8.09a ±0.26	8.84a ±0.19	27.30a ±1.28	28.0a ±0.80	98	98
msk[2]	115.0b ±1.87	116 80b ±1.71	14.10b ±0.55	27.40b ±0.66	7 0b ±0 01	7.11b ±0 03	12.40b ±0.52	12.90b ±0.51	27 70a ±2.37	29 0a ±0.76	95	96
msk[3]	89.4a ±1.96	85 20a ±1 52	14.0b ±1.14	20.70c ±1.41	5 40c ±0.02	5 35c ±0.12	7.80a ±0 28	7.30d ±0.18	23 80b ±0.88	22.7b ±0.74	100	100
msk[4]	104.6c ±1 46	102.60c ±1 44	8 50c ±0 43	12.90d ±0 63	6 25a ±0.03	6.20a ±0.02	7.77a ±0.09	7.90d ±0 20	26 60a ±0.68	27.4a ±0 80	99	99
msk[5]	113 6b ±1.55	112.0b ±2.75	8.60c ±0 65	12.0d ±0.66	7.10b ±0.01	7.15b ±0.12	10 3c ±0.14	10 03e ±0.08	29.0c ±0.70	28.3a ±0.36	102	102
msk[6]	72.9d ±0.42	72.30d ±1.28	7.10d ±0 41	11.30d ±0.60	5.35c ±0 01	5 30c ±0.03	6 5d ±0.31	6.52f ±0 30	17 8d ±0.71	18 1c ±1.05	100	100
msk[7]	102.9c ±0 74	101.9c ±1.22	7.80d ±0 52	12.20d ±0.70	5 30c ±0.02	5.32c ±0.01	13 0b ±0.15	12.70c ±0 10	30 2c ±1.66	28 4a ±1.55	97	98
msk[8]	93.0a ±1.02	92 7a ±0 67	9.20e ±0 36	14.40e ±0.36	7.15b ±0 01	7.16b ±0 03	7.18a ±0.11	6.97f ±0.18	22 6b ±1.0	21 6b ±0 55	102	102
msk[9]	93 3a ±1.14	93.3a ±1.0	8 0c ±0 37	12.10d ±0.50	7.25b ±0 01	7.28b ±0 01	6.8d ±0.09	7.09d ±0.11	23 8b ±1.05	24 1b ±0.76	98	97

* Mean value of 15 observations. ± Standard deviation.

Mean value of the trait in a vertical column not followed by the same alphabet differs from each other at 5P level, as revealed by Duncan's multiple range test.

Table 2 : Pollen appearance and stainability of male fertile and male sterile barley genotypes.

Genotype	Pollen appearance and stainability
Male fertile	Pollen normal in appearance. Rounded non-shrivelled and turgid with reserve food material. 98.5–99% stainability.
Male steriles	
msk^{1}	Microspores reduced and form a mass of degenerated tissue in the later stages. Stainability nil.
msk^{2}	Microspores very much reduced and clumped forming an amorphous mass of degenerated microspores. Stainability nil.
msk^{3}	Shrivelled microspores, free and/or clumped. Stainability nil.
msk^{4}	Reduced and clumped forming amorphous mass. Stainability nil.
msk^{5}	Shrivelled and clumped microspores, 0 5–1% stainability.
msk^{6}	Reduced and clumped microspores, Stainability nil.
msk^{7}	Reduced, shrivelled microspores forming amorphous mass. Stainability nil.
msk^{8}	Very much reduced microspores which are clumped and form an amorphous strand of degenerated microspores in later stages. Stainability nil.
msk^{9}	Microspores reduced and clumped to form an amorphous tissue. Stainability nil.

Table 3 : Anther dimensions and fresh weight of male-fertile (MF) and male sterile (MS) barley genotypes.

Genotypes	Anther length (mm)		Anther width (mm)		Anther weight (mg)	
	MF	MS	MF	MS	MF	MS
msk^1	3.07 a_1x_1 ±0.02	2.5 a_2x_1 ±0.04	0.406 a_1x_1 ±0.003	0.375 a_2x_1 ±0.007	0.420 a_1x_1 ±0.02	0.130 a_2x_1 ±0.012
msk^2	3.40 a_1x_2 ±0.004	1.90 a_2x_2 ±0.03	0.448 a_1x_2 ±0.001	0.340 a_2x_2 ±0.004	0.430 a_1x_1 ±0.05	0 029 a_2x_2 ±0.005
msk^3	3.50 a_1x_2 ±0 003	2.90 a_2x_3 ±0.04	0.507 a_1x_3 ±0.002	0.465 a_2x_3 ±0 007	0.540 a_1x_2 ±0 02	0.048 a_2x_3 ±0.002
msk^4	3.50 a_1x_2 ±0.04	2.50 a_2x_1 ±0.03	0.509 a_1x_3 ±0.003	0.255 a_2x_4 ±0 014	0.570 a_1x_2 ±0.02	0.075 a_2x_4 ±0.005
msk^5	3 30 a_1x_3 ±0.003	2.00 a_2x_2 ±0.04	0 450 a_1x_2 ±0.004	0.425 a_2x_5 ±0.008	0.370 a_1x_3 ±0.025	0.133 a_2x_1 ±0.02
msk^6	3.70 a_1x_4 ±0.004	3.00 a_2x_3 ±0 04	0 485 a_1x_4 ±0.002	0.430 a_2x_5 ±0 06	0.530 a_1x_2 ±0.02	0.046 a_2x_3 ±0.004
msk^7	3.50 a_1x_2 ±0.003	3.11 a_2x_3 ±0.04	0.453 a_1x_2 ±0.003	0.428 a_2x_5 ±0.01	0.560 a_1x_2 ±0.014	0.056 a_2x_5 ±0.002
msk^8	3.60 a_1x_5 ±0.003	1.95 a_2x_2 ±0.014	0 507 a_1x_3 ±0.002	0.325 a_2x_6 ±0.01	0.370 a_1x_3 ±0.017	0.160 a_2x_6 ±0.014
msk^9	3.04 a_1x_1 ±0.03	2.51 a_2x_1 ±0.23	0.405 a_1x_1 ±0.002	0.205 a_2x_7 ±0.01	0.380 a_1x_3 ±0.008	0.180 a_2x_6 ±0.02

— Mean value of a trait of male fertile or male sterile in a horizontal row not followed by the same subscript (a_1, a_2,———) differ from each other at 5P level.

— Mean values of a trait of male fertile or male sterile in a vertical column not followed by same subscript (x_1, x_2,———) differ from each other at 5P level, as revealed by Duncan's multiple range test.

Whereas the male fertile plants lodge at maturity due to seed formation and their foliage turn yellow, the male steriles remain erect, as they produce none or a few outcrossed seeds and their foliage remains green and fresh. The male sterile plants in addition to first crop tillers (primary+ secondary) produce second crop tillers at later stages of growth and development. Second crop tillers are shorter than primary tillers and remain green while primary and secondary tillers mature and dry up.

Discussion

In the male sterile mutants studied presently, all the nine non-allelic recessive male sterile (*ms*) genes cause not only complete male sterility, but also influence the phenotypic traits like tillering capacity, anther, pollen and spike characteristics. But these male sterile genes have no influence on shoot height, days to heading, ear length, number of spikelets/spike and flag leaf area. Whereas, a reduction in shoot height of male steriles was observed by Kasha and Walker (1960) and Singh (1988) in barley, in the nine male sterile mutants of barley studied presently, such reduction was statistically not significant. In fact, reduction in shoot height is not a constant feature of male steriles but an occasional one as the reports of reduction in other plants are occasional, infrequent and inconsistent (Kaul 1988).

shpande *et al.* (1981) reported increases in ear length and flag leaf area in a male sterile mutants of barley, but these alterations were not detected in the mutants studied presently. Singh (1988) also did not observe any increase in ear length in twelve monogenic male sterile mutants of barley. Therefore, increases in the ear length and flag leaf area are not caused by all male sterile genes in barley, but probably occasionally by a few.

The open flowered male sterile mutants found in present investigation are morphologically distinct from the fertile counterparts. Singh (1988) also reported such male sterile plants in barley that appeared lax after flowering. Whether such open flowered mutants are agronomically useful or not is unknown. There usefulness will depend upon their natural outcrossing rate. If the rate is appreciable, their agronomic and breeding values are high as they will form females in a barley hybrid seed production on commercial scale.

Underdeveloped anthers having degenerated pollen grains as found presently is a characteristic feature of the majority of male sterile plants (Kaul 1988). But three male sterile mutants, ms^6, ms^8 and ms^{16} of barley develop normal anthers and normal-looking pollen. However, the pollen produced by these three male steriles is non-functional (Roath and Hockett

1971). Thus, these three mutants are exceptional male sterile mutants of barley, as almost all the barley male sterile mutants are pollenless (Kaul 1988) as are the nine mutants studied presently and the twelve ones studied by Singh (1988). However, due to such variable and diverse results obtained early and presently, it is desirable to investigate more male sterile mutants of barley in particular and cereals in general in order tocharacterise male steriles in cereals.

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