Theor Appl Genet (1987) 74:402–408



# Investigation of partial sterility in advanced generation, sodium azide-induced lines of spring barley*

**M. L. Crispi [1], S. E. Ullrich [1] and R. A. Nilan [2]**

[1] Department of Agronomy and Soils, [2] Department of Agronomy and Soils and Program in Genetics; Washington State University, Pullman, WA 99164-6420, USA

Received January 26, 1987; Accepted February 23, 1987
Communicated by J. Mac Key

**Summary.** The partial sterility found in several advanced generation, sodium azide-induced lines of spring barley (*Hordeum vulgare* L.) was investigated. Plants of mutant lines were reciprocally crossed with plants of their untreated mother lines. Spike sterility was measured in the selfed offspring of the plants crossed and in $F_1$ and $F_2$ progeny. Pollen sterility and endosperm development were analyzed in the selfed offspring of the plants crossed. Results indicated that the sterility was inherited in the mutant lines and was not caused by translocations, inversions, endosperm lethals, embryo-endosperm lethals, or major gene mutations. Furthermore, the sterility was not cytoplasmically inherited, and was essentially eliminated in the $F_1$ and $F_2$ of crosses between partially sterile lines and their fertile parents. Results suggest that the sterility may be caused by an environmental interaction with deleterious, homozygous recessive, minor gene mutations that were in the heterozygous condition when the mutant lines were originally selected.

**Key words:** Sodium azide – Partial sterility – Minor genes – *Hordeum vulgare* L. – Mutations

## Introduction

The use and importance of chemical and physical mutagens in plant genetics and breeding is well known (Konzak et al. 1984). An effective and efficient mutagen must induce a high number of mutations and a low number of deleterious effects. One deleterious effect caused by some chemical and physical mutagens is partial sterility. Partial sterility, unlike complete male sterility, is of little value in a breeding program and is usually considered an impediment in cultivar development.

The possible causes of partial sterility in barley (*Hordeum vulgare* L.) include: chromosomal structure changes (i.e. heterozygosity for segmental interchanges, inversions, duplications and deficiencies), changes in the number of chromosomes (i.e. euploidy or aneuploidy), nuclear factors, cytoplasmic influence, physiological factors, and environmental influence (see review, Yu and Hockett 1979). Of these, Ekberg (1969) has defined five genetic mechanisms that would result in a barley plant having 50% to 75% seed set on each spike. The five types are: translocations, inversions, endosperm and embryo-endosperm lethals, and gene mutations. The differentiation of these types is well documented (Ekberg 1969; Yu and Hockett 1979). Sodium azide, considered an effective and efficient mutagen that causes few, if any, chromosomal aberrations in barley (Kleinhofs et al. 1978), has not been characterized in terms of the type(s) of partial sterility it can induce in barley.

Azide has been used in the Washington State University Barley Improvement Program to produce several types of useful mutants. Unfortunately, many of these mutants exhibit varying degrees of partial sterility several generations after their initial selection. The sterility has been a major problem, limiting the use of these mutants in genetic and breeding studies. This study was conducted to characterize the type(s) of partial sterility in some selected mutant lines in order to recognize, understand, manipulate, and, hopefully, avoid partial sterility problems of this nature in the future.

* Scientific paper No. 7441, College of Agriculture Research Center, Washington State University, Pullman, Wash., USA, Project No. 1006

**Table 1.** List of mother lines and their sodium azide induced mutants used in the crossing program

| Mother line and mutants[a] | Row type | M-generation at year of crossing | Spike sterility the year before crossing[b,c] (%) |
|---|---|---|---|
| Advance | 6 | – | 12.0 |
| Ant 503 | 6 | 8 | 22.7 |
| Ant 525 | 6 | 7 | 28.7 |
| SD 11081-81 | 6 | 4 | NA |
| Morex | 6 | – | 1.6 |
| Ant 531 | 6 | 7 | 3.1 |
| SD 11094-81 | 6 | 4 | NA |
| Karla | 6 | – | 1.9 |
| Ant 504 | 6 | 8 | 16.2 |
| WA 9037-75 | 2 | – | 2.5 |
| Ant 514 | 2 | 8 | 14.8 |
| Ant 517 | 2 | 8 | 4.9 |
| SD 11005-81 | 2 | 4 | NA |
| WA 9044-75 | 2 | – | 2.0 |
| Ant 520 | 2 | 7 | 0.6 |
| 72 Ab3484 | 2 | – | NA |
| Ant 513 | 2 | 8 | 5.2 |

[a] Ant: proanthocyanidin-free mutant; SD: semi-dwarf mutant
[b] Percent empty floret values based on the mean of 25 randomly selected heads from each line
[c] NA: Data not available at time of crossing

## Materials and methods

*Parental material*

Six- and two-row spring barley (*Hordeum vulgare* L.) lines and their sodium azide-treated mutants used in the crossing program are described in Table 1. "Ant" designates proanthocyanidin-free (and anthocyanin-free) mutants, and "SD" designates semi-dwarf mutants. The Ant lines used had higher levels of sterility (percent blank florets) than their mother line. SD lines, the sterility levels of which were unknown, were included as mutant checks. Line 72 Ab3484 was generously provided by Dr. D. M. Wesenberg, USDA/ARS, Aberdeen, Idaho, USA. All other lines were obtained from seed stocks maintained by the Washington State University Barley Improvement Program.

*Reciprocal crosses and $F_1$, $F_2$ segregation*

Five reciprocal crosses were made between plants of each sodium azide-induced ANT or SD mutant and its untreated mother line, as well as between plants of each such ANT and SD mutant with the same mother. All crosses were made in Pullman, USA during the summer of 1984. Selfed seed was harvested from each plant crossed. Two (or three) of the five reciprocal crosses made in all of the above-mentioned combinations were increased on the basis of the level of sterility of each plant involved in the cross and the number of $F_1$ seed obtained. Twelve $F_1$ seeds from one of the two (or three) selected crosses were greenhouse planted in Pullman, USA and twelve $F_1$ seeds from the other selected cross(es) were field planted in Yuma, USA during the fall of 1984–85. Due to few $F_1$ seeds, only one reciprocal cross was chosen for each semi-dwarf/mother line cross and planted in the greenhouse in Pullman. $F_2$ seed were harvested according to pedigree. $F_2$ seed was not obtained from all two-row $F_1$ plants grown in the greenhouse, due to inadequate light conditions.

Selfed (20 seeds), $F_1$ (10–30 seeds), and $F_2$ (150–225 seeds) seeds from each of the two (or three) selected crosses were planted in spring, 1985, at Pullman, USA. Selfed plant and $F_1$ seeds were space-planted approximately 15 cm apart, and $F_2$ seeds were space-planted approximately 5 cm apart. Plants were field rated for presence/absence of anthocyanin pigmentation and for spike sterility.

*Spike sterility*

In summer, 1984, sterility data for each plant used in a cross was determined by calculating the percentage of blank florets per spike on two spikes of that plant. The top and bottom spikelets of each spike were not counted as these are the last to develop and are frequently sterile. In summer, 1985, sterility data for each plant was calculated by averaging the percentage of blank florets of two (for $F_2$ plants) or three (for selfed and $F_1$ plants) of the most mature spikes per plant. One of the two or three spikes was bagged before anthesis to determine any cross pollination effect. As before, the top and bottom of each spike were not counted.

*Endosperm development*

Five immature spikes were harvested at random from each group of selfed plant offspring, 10 to 14 days after anthesis, in summer, 1985. Endosperm development was analyzed using the classification system as described by Yu and Hockett (1979).

*Pollen sterility*

Immature spikes, just emerging from the boot leaf, were harvested, one per plant, from six randomly selected plants from each group of selfed plant offspring, in summer, 1985, and stored in 95% ethanol. Three anthers, from centrally located florets, were removed from each spike. Anthers were squashed and stained with an iodine-potassium-iodide (IKI) solution (2,000 ml distilled $H_2O$, 10 g IKI, 1.8 g $I_2$). Approximately 125 pollen grains per anther were microscopically analyzed. Pollen grains that were abnormally shaped (shrunken cytoplasm, or devoid of cytoplasm) were considered sterile (Yu and Hockett 1979). Percent pollen sterility per plant was calculated by summing the number of sterile pollen grains for the three anthers and dividing by the total number of pollen grains analyzed from the three anthers. Percent pollen sterility for a selfed plant offspring group was calculated by averaging the percent pollen sterility values of the six plants.

*Statistical analysis*

Summary statistics were calculated and nonparametric statistical analyses performed using the computer program MSU-STAT (MS-DOS version 2.20), Montana State University (Lund 1983). Unless otherwise noted, mean comparisons were by Wilcoxon's signed-rank test or Spearman's rank correlation coefficient (Steel and Torrie 1980).

## Results

*Spike sterility, 1985*

Partial sterility data for 1985 are summarized in Tables 2 and 3. The results of Mann-Whitney tests (Steel and Torrie 1980) showed that in 15 of the 22 crosses, one

**Table 2.** Summary of partial spike sterility and pollen sterility data for all plants crossed and selfed offspring of plants crossed in 1984. NS: not significant

| Selfed line of offspring | Cross number | *n* | Mean pollen sterility (1985) (%) | Spike sterility (%) in | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 1985 | | | | 1984 |
| | | | | Mean of selfed offspring | $s^2$ | F value | Mann-Whitney test | Mean of plants crossed |
| Morex | 1 | 22 | 10.2 | 3.1 | 15.92 | 3.255** | ** | 0.0 |
| Ant 531 | | 26 | 5.0 | 9.3 | 51.84 | | | 0.0 |
| Morex | 2 | 39 | 10.7 | 1.9 | 4.65 | 11.48** | ** | 0.0 |
| Ant 531 | | 30 | 6.3 | 5.8 | 53.46 | | | 0.0 |
| SD 11094-81 | 3 | 33 | 3.7 | 2.8 | 5.42 | 3.830** | NS | 10.8 |
| Ant 531 | | 35 | 3.4 | 3.5 | 20.77 | | | 14.5 |
| SD 11081-81 | 4 | 11 | 6.7 | 11.9 | 128.36 | 8.779** | NS | 10.8 |
| Ant 525 | | 9 | 7.1 | 12.0 | 14.63 | | | 25.6 |
| SD 11081-81 | 5 | 14 | 7.6 | 15.1 | 571.68 | 59.95** | NS | 14.3 |
| Ant 525 | | 18 | 4.9 | 4.0 | 9.54 | | | 18.9 |
| SD 11081-81 | 6 | 25 | 9.2 | 5.1 | 57.86 | 2.001 | NS | 0.0 |
| Ant 525 | | 14 | 4.6 | 3.9 | 28.92 | | | 45.9 |
| Advance | 7 | 28 | 6.2 | 1.9 | 5.81 | 117.2** | ** | 2.8 |
| Ant 525 | | 9 | 5.4 | 30.4 | 678.61 | | | 54.8 |
| Advance | 8 | 31 | 9.0 | 1.2 | 1.40 | 11.14** | * | 2.5 |
| Ant 525 | | 25 | 3.7 | 3.5 | 15.62 | | | 15.4 |
| SD 11081-81 | 9 | 18 | 8.7 | 3.4 | 28.51 | 1.970 | ** | 24.2 |
| Ant 503 | | 30 | 4.1 | 12.2 | 56.19 | | | 40.0 |
| SD 11081-81 | 10 | 13 | 6.8 | 6.9 | 35.01 | 1.469 | NS | 15.4 |
| Ant 503 | | 21 | 6.3 | 5.3 | 23.80 | | | 44.4 |
| SD 11081-81 | 11 | 15 | 9.2 | 7.5 | 187.96 | 1.426 | ** | 2.3 |
| Ant 503 | | 13 | 5.3 | 15.3 | 131.79 | | | 44.2 |
| Advance | 12 | 27 | 12.0 | 1.3 | 2.30 | 43.50** | ** | 8.3 |
| Ant 503 | | 20 | 12.4 | 10.4 | 100.20 | | | 23.5 |
| Advance | 13 | 29 | 4.6 | 1.1 | 1.13 | 58.99** | ** | 2.7 |
| Ant 503 | | 22 | 7.3 | 12.8 | 77.12 | | | 16.6 |
| Advance | 14 | 21 | 7.5 | 1.4 | 3.84 | 1.417 | NS | 10.5 |
| SD 11081-81 | | 23 | 12.7 | 1.5 | 2.70 | | | 9.7 |
| Karla | 15 | 30 | 6.5 | 1.1 | 3.31 | 1.622 | NS | 0.0 |
| Ant 504 | | 30 | 5.6 | 1.0 | 2.04 | | | 0.0 |
| Karla | 16 | 31 | 4.0 | 1.4 | 3.32 | 13.43** | * | 12.2 |
| Ant 504 | | 27 | 7.6 | 4.1 | 44.66 | | | 5.5 |
| 72 Ab3484 | 17 | 19 | 4.7 | 1.0 | 1.77 | 9.347** | ** | 0.0 |
| Ant 513 | | 20 | 6.3 | 5.2 | 16.52 | | | 0.0 |
| WA 9044-75 | 18 | 20 | 2.3 | 0.5 | 0.78 | 30.96** | ** | 0.0 |
| Ant 520 | | 18 | 5.9 | 3.8 | 24.23 | | | 0.0 |
| WA 9037-75 | 19 | 19 | 5.2 | 1.1 | 3.95 | 162.9** | ** | 5.3 |
| Ant 517 | | 8 | 10.7 | 22.2 | 562.16 | | | 0.0 |
| WA 9037-75 | 20 | 15 | 4.6 | 1.4 | 5.65 | 2.132 | ** | 4.5 |
| Ant 514 | | 17 | 3.6 | 6.3 | 12.05 | | | 0.0 |
| SD 11005-81 | 21 | 15 | 7.1 | 1.5 | 3.28 | 14.17** | ** | 0.0 |
| Ant 517 | | 8 | 10.2 | 23.5 | 46.45 | | | 47.8 |
| SD 11005-81 | 22 | 13 | 7.5 | 1.5 | 2.13 | 22.69** | ** | 0.0 |
| Ant 517 | | 15 | 8.7 | 10.8 | 48.36 | | | 0.0 |

*, ** Significant at the 0.05 and 0.01 probability levels, respectively

parent (a mother line, or SD mutant) had less sterility than the other parent (always an Ant mutant) (Table 2). In 12 of these 15 pairs, the variance of the more sterile parent was statistically greater than that of the more fertile parent (Table 2).

Reciprocal cross sterility data were combined for each of the 15 crosses when it was determined that there were no reciprocal cross differences (data not shown). When data from the 15 crosses were compared by population ($P_1$, $P_2$, $F_1$, $F_2$), the percent sterility of the fertile parent ($P_1$) (either a mother line or SD mutant) and $F_1$, and the percent sterility of the $F_1$ and $F_2$ were statistically equal (Table 4). The $F_2$ differed slightly from the $P_1$. The $P_1$, $F_1$, and $F_2$ data were highly

**Table 3.** Summary of 1985 partial spike sterility data from $F_1$ and $F_2$ generations of all crosses

| Cross | Cross no. | Generation | $n$[a] | Mean sterility (%) | $s^2$ |
|---|---|---|---|---|---|
| Morex/Ant 531 | 1 | F1 | 8 | 2.1 | 7.25 |
| | | F2 | 145 | 2.1 | 11.92 |
| | 2 | F1 | 10 | 0.6 | 0.09 |
| | | F2 | 169 | 1.7 | 3.60 |
| Ant 531/SD 11094-81 | 3 | F1 | 2 | 1.5 | 0.25 |
| | | F2 | 83 | 1.4 | 4.02 |
| Ant 525/SD 11081-81 | 4 | F1 | 5 | 2.1 | 4.83 |
| | | F2 | 158 | 2.6 | 25.64 |
| | 5 | F1 | 3 | 0.9 | 0.03 |
| | | F2 | 162 | 2.7 | 28.14 |
| | 6 | F1 | 11 | 0.9 | 2.28 |
| | | F2 | 207 | 2.8 | 32.49 |
| Advance/Ant 525 | 7 | F1 | 11 | 2.6 | 6.25 |
| | | F2 | 194 | 1.7 | 9.06 |
| | 8 | F1 | 7 | 0.9 | 8.64 |
| | | F2 | 141 | 1.2 | 9.35 |
| SD 11081-81/Ant 503 | 9 | F1 | 8 | 1.0 | 0.87 |
| | | F2 | 194 | 2.7 | 16.17 |
| | 10 | F1 | 4 | 0.8 | 0.85 |
| | | F2 | 183 | 1.5 | 5.83 |
| | 11 | F1 | 4 | 2.8 | 10.64 |
| | | F2 | 168 | 4.2 | 78.80 |
| Advance/Ant 503 | 12 | F1 | 10 | 1.4 | 2.28 |
| | | F2 | 122 | 1.2 | 1.81 |
| | 13 | F1 | 10 | 2.0 | 2.13 |
| | | F2 | 199 | 1.3 | 3.49 |
| Advance/SD 11081-81 | 14 | F1 | 11 | 0.9 | 1.21 |
| | | F2 | 173 | 1.2 | 11.72 |
| Karla/Ant 504 | 15 | F1 | 19 | 0.4 | 0.42 |
| | | F2 | 155 | 0.6 | 1.26 |
| | 16 | F1 | 28 | 0.3 | 0.21 |
| | | F2 | 192 | 0.7 | 0.88 |
| 72 Ab3484/Ant 513 | 17 | F1 | 19 | 0.3 | 0.53 |
| | | F2 | 177 | 0.7 | 3.06 |
| WA 9044-75/Ant 520 | 18 | F1 | 20 | 1.0 | 1.40 |
| | | F2 | 157 | 0.6 | 1.13 |
| WA 9037-75/Ant 517 | 19 | F1 | 17 | 0.9 | 1.27 |
| | | F2 | 171 | 1.0 | 2.48 |
| WA 9037-75/Ant 514 | 20 | F1 | 17 | 1.4 | 2.75 |
| | | F2 | 177 | 1.1 | 2.97 |
| SD 11005-81/Ant 517 | 21 | F1 | 8 | 2.1 | 1.81 |
| | | F2 | 164 | 2.0 | 7.50 |
| | 22 | F1 | 4 | 1.9 | 2.69 |
| | | F2 | 183 | 1.3 | 3.83 |

[a] *n:* number of plants in indicated generation

significantly different from the sterility data of the more sterile parent ($P_2$; an Ant mutant). Furthermore, the following data pairs were significantly correlated: $P_1$ and $F_2$, $P_2$ and $F_1$, $P_2$ and $F_2$, and, $F_1$ and $F_2$. Identifiable segregation ratios for partial sterility were not evident in any of the populations.

A comparison of the variances for the 15 crosses, when combined by population, showed similar results (data not shown). A notable difference is that the $F_2$ variance (mean variance = 10.48) was greater than the $F_1$ variance (mean variance = 2.87). Furthermore, only the $P_1$ and $F_2$ variance data were significantly correlated with a Spearman's rank correlation coefficient ($r_s$) of 0.65. Population frequency distribution graphs were generated that illustrated these relationships (Fig. 1).

**Table 4.** Comparison of selfed plant offspring, $F_1$, and $F_2$ partial spike sterility data for crosses in which one parent is sterile ($P_1$) (mother line or SD mutant) and the other was partially sterile ($P_2$ Ant mutant). Results from Wilcoxon's signed-rank test are above the diagonal, those from the Spearman's rank correlation coefficients are below. NS: not significant

| | $P_1$ | $P_2$ | $F_1$ | $F_2$ | Mean sterility (%) | No. of crosses |
|---|---|---|---|---|---|---|
| $P_1$ | – | ** | NS | * | 2.0 | 15 |
| $P_2$ | 0.36 | – | ** | ** | 12.3 | 15 |
| $F_1$ | 0.51 | 0.66** | – | NS | 1.5 | 15 |
| $F_2$ | 0.90** | 0.60* | 0.71** | – | 1.6 | 15 |

*,** Significant at the 0.05 and 0.01 probability levels, respectively

The sterility of the plants crossed in 1984 was compared to the mean sterility of their 1985 selfed offspring. The 1984 percent sterility (overall mean = 12.1%) was significantly greater than the 1985 percent sterility (overall mean = 6.4%), and the means were significantly correlated ($r_s = 0.42$).

The effect of bagging spikes in 1985 was determined. For each of six groups of selfed plant offspring tested, the sterility of the bagged spikes (overall mean = 8.5%) was statistically equal (using the Mann-Whitney test) to the sterility of the unbagged spikes (overall mean = 8.0%; data not shown).

The sterility of field selected, anthocyanin-free $F_2$ plants was compared to that of pigmented $F_2$ plants from proanthocyanidin-free by normal crosses. The sterility means of the anthocyanin-free plants (overall mean = 2.2%) were statistically equal to the means of the pigmented plants (overall mean = 1.7%), and the means were highly significantly correlated ($r_s = 0.61$).

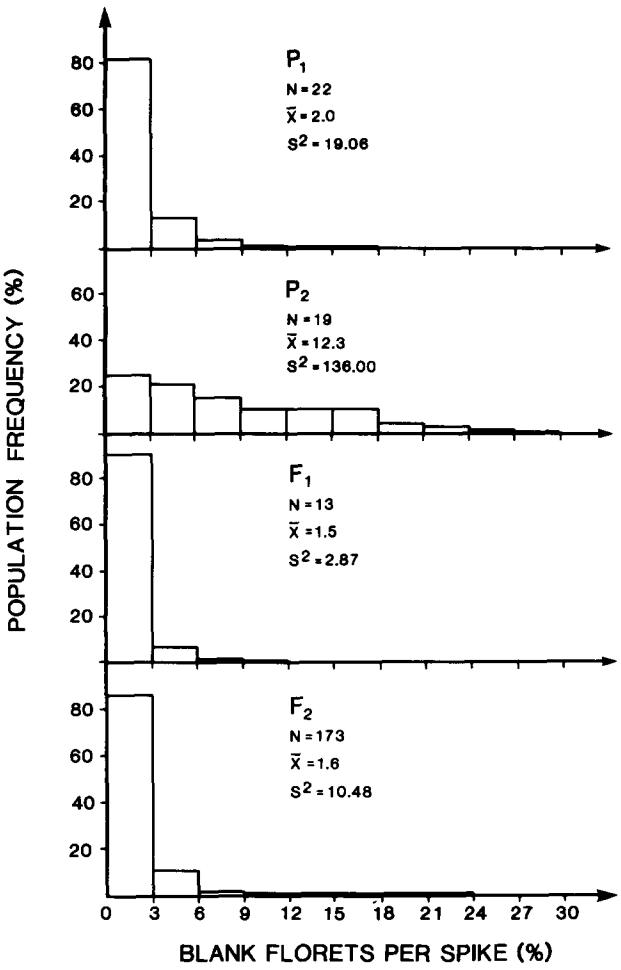


**Fig. 1.** Population frequency distributions for the partial sterility (percent blank florets per spike) of 15 crosses where $P_2$ (Ant mutant) was significantly more sterile than $P_1$ (mother line or SD mutant). Values shown represent the mean of the values from the 15 crosses for the respective classes

*Endosperm development*

For all selfed offspring of the plants crossed in 1984, the only type of defective endosperm development observed was the no-development (ND) type as described by Yu and Hockett (1979). Examples of this type of endosperm development in six-row and two-row lines are presented in Figs. 2 and 3, respectively.

*Pollen sterility, 1985*

Mean pollen sterility values for each group of selfed offspring of the plants crossed in 1984 are presented in Table 2. Pollen sterility values were compared for the 15 crosses where one parent was fertile (either mother line or SD mutant) and the other more sterile (Ant mutant). The pollen sterility means of progeny of fertile parents (7.1%) were statistically equal to the pollen sterility means of the progeny of more sterile parents (6.8%). The means of percent blank florets or spike sterility (6.4%) and percent pollen sterility (6.8%) of all parents ($n = 44$) were statistically equal and not correlated ($r_s = 0.21$). For the 15 parents considered sterile, the mean percent blank florets (11.5%) was statistically greater ($P = 0.05$) than the mean percent pollen sterility (7.0%) and they were not correlated ($r_s = 0.29$).

## Discussion

The results obtained in this experiment suggest that the sterility problem observed in selected azide-induced mutants is not one of the five types of partial sterility described by Ekberg (1969) and Yu and Hockett (1979). The absence of a ratio of one fertile to one partially sterile plant in any of the selfed offspring of

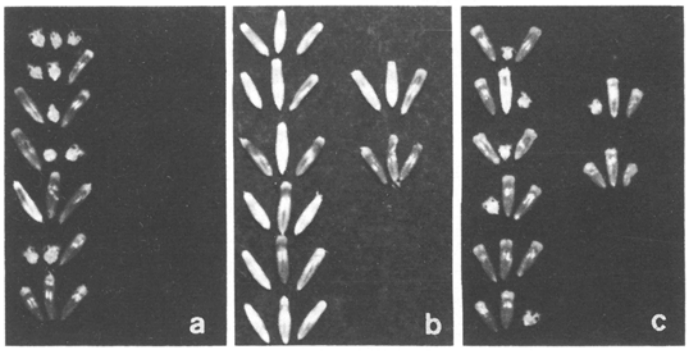


**Fig. 2a–c.** Endosperm development, 10–14 days after anthesis, of the six-row barley lines **a** Ant 525, **b** Advance, and **c** Ant 503

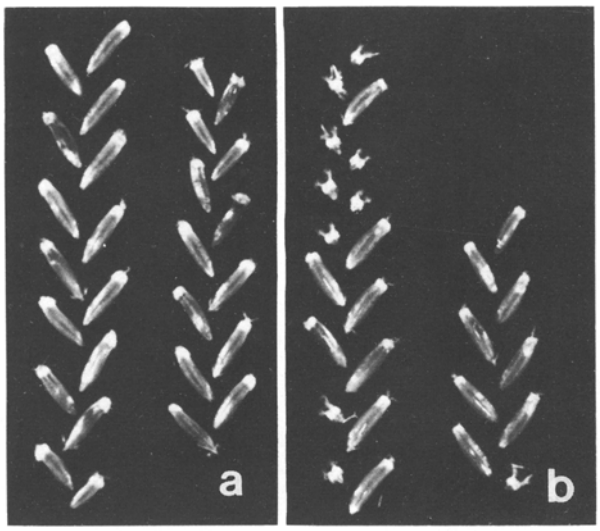


**Fig. 3.** Endosperm development, 10–14 days after anthesis of the two-row barley lines **a** WA 9037-75 and **b** Ant 514

the plants crossed in 1984 or the $F_1$, and the higher than theorized seed set and pollen fertility, suggest that partial sterility is not caused by either a translocation or an inversion. This is further supported by recent work in barley suggesting that chromosomal aberrations are not correlated with azide-induced partial sterility (W. Nilan, pers. commun.). Defective endosperm development of only the no-development (ND) type, in addition to the above observations, suggests that the partial sterility is not caused by endosperm or embryo-endosperm lethals, this would result in unequal caryopsis sizes (Ekberg 1969; Yu and Hockett 1979). The absence of segregation for sterility in the $F_2$ of any of the crosses suggests that the partial sterility is never caused by a major gene mutation.

The significant, positive correlation between the 1984 and 1985 sterility data suggest that the sterility is heritable. The sterility is not cytoplasmically inherited because there were no reciprocal cross differences. Furthermore, it is essentially eliminated by crossing an azide-derived, partially sterile mutant to its untreated mother line. Higher variances in the $F_2$ of these crosses, relative to the $F_1$, suggest a possible polygenic mode of inheritance. However, firm conclusions of this nature cannot be drawn due to the relatively low population sizes in this study.

The sterility did not appear to be caused by severe environmental stress, such as low moisture or high temperature. This usually produces sterility patterns in cereals where blank florets are predominantly located near the upper and lower ends of a spike (Morgan 1971; Wright 1972; Oosterhuis and Cartwright 1983; Morgan and King 1984). The more severe the stress at the time of anthesis, the larger the area of blank florets (Oosterhuis and Cartwright 1983). Blank florets were random and sterility patterns not evident in this experiment.

Environmental stress prevents normal dehiscence of anthers and decreases pollen fertility (Scoles and Evans 1979; Saini and Aspinall 1981). When rating endosperm development in this experiment, it was noted that blank florets usually contained indehiscent or undeveloped anthers. When pollen sterility was assessed, some florets contained only undeveloped anthers.

The above observations, as well as an observed lack of a correlation between pollen sterility and blank florets in this study, seem to suggest that the sterility could be caused by an environmental interaction with several deleterious, homozygous recessive, minor gene mutations that were in the heterozygous condition when the mutant genotype was originally selected. A similar mechanism of partial sterility was suggested by Sato and Gaul (1967), when investigating ethyl methanesulphonate-induced partial sterility in barley. Instead of minor gene mutations, the authors felt that many small, "invisible" deficiencies were the main cause of the sterility. Further speculation suggests that greater sensitivity to environmental fluctuations would result in sterility levels of mutants that vary greatly from location to location relative to the sterility levels of their untreated progenitors. Such variation has been

observed in yield trials of the mutants used in this study (data not shown). Another possible cause of the sterility is the alteration of a reproduction-related enzyme so that the activity of the enzyme becomes environmentally sensitive. Alterations of this type could be caused by one or a few, mutagen-induced, base substitutions in the nucleotide sequence that codes for the enzyme. Mutagen treatment may have modified the "translation" phase of protein synthesis so that the accuracy of mRNA "reading" is influenced environmentally. Misreading of mRNA that codes for a reproduction-related enzyme could result in the production of an altered enzyme, or no enzyme at all.

Sodium azide-induced meiotic abnormalities (synapsis deficiencies, abnormal chromatin condensation, and abnormal meiotic anaphases) may also be a cause of the partial sterility. Recent work suggests some association between sterility and these abnormalities, but no firm correlations have been found (Prina et al. 1983; W. Nilan, pers. commun.).

Elimination of the sterility by crossing the partially sterile line to its fertile mother line, as in this experiment, may have been caused by the remasking of deleterious mutations. Alternately, the replacement of recessive mutations with normal alleles (or nucleotide bases in the case of mutagen-induced base substitutions or deletions), through a process of gene conversion, would also result in highly fertile $F_2$ populations. A third possibility is that crossing over and recombination, in the meiotic cells of $F_1$ plants, may have broken apart linkages of mutant alleles that were essential for expression of the sterility. Evaluation of the sterility in the $F_3$, $F_4$, and $F_5$, of the crosses made in this experiment would be helpful in determining which, if any, of the three explanations is correct.

Three aspects of this study must be considered when evaluating its results. Firstly, relatively low population sizes may have produced some degree of genetic drift. Furthermore, drift would also be more likely in crosses where the mutant line was the pollen donor. Genetic drift was not evident though, when examining the segregation of anthocyanin pigmentation. Three normal to one anthocyanin-free ratios were observed in the $F_2$ of most of the normal/anthocyanin-free crosses (data not shown). Secondly, the use of IKI to determine pollen sterility may have resulted in lower estimates of sterility, as IKI stains cytoplasmic starch deposits. The presence of these deposits may not be indicative of pollen viability (Roath and Hockett 1971). Thirdly, the genes controlling the partial sterility would, most likely, be different for each mutant line. Hence, using data from several crosses (with different mutant and/or mother lines) to make statistical comparisons may be misleading and the results obtained may not be valid for each line.

In this study, it was evident that the partial sterility in azide-induced barley lines was not caused by translocations, inversions, endosperm lethals, embryo-endosperm lethals, or major gene mutations. The sterility was inherited in the selfed progeny of partially sterile plants and was not cytoplasmically inherited. It was essentially eliminated by crossing an azide-derived, partially sterile mutant to its untreated mother line. Although it was not conclusively shown, it appears that the sterility may be caused by an environmental interaction with deleterious, homozygous recessive, minor gene mutations that were heterozygous when the mutant line was initially selected. Future studies of this problem should be designed to determine if the sterility is controlled as suggested here, and the extent to which it is affected by environmental conditions.

*Acknowledgements.* The authors wish to thank A. Hodgdon, A. Kleinhofs, C. F. Konzak, W. Nilan, J. Cochran, W. Aprill, and A. Aydin, for their advice and assistance during the course of this study.

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