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The use of pollen irradiation in barley breeding

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Summary. Second generation progenies (M_2) derived from crosses using barley pollen irradiated at 500, 1,000 1,500 and 2,000 rads increasingly resembled the maternal parent. 'Golden Promise', but contained some characteristics derived from the paternal parent, 'Magnum'.

Key words: Barley – *Hordeum vulgare* – Irradiated pollen – Plant breeding

Introduction

It has recently been demonstrated that by irradiating pollen of *Nicotiana rustica* immediately prior to fertilisation only a portion of the paternal genome is expressed in the derived hybrid material (Jinks et al. 1981; Caligari et al. 1981). The phenomenon had previously been observed using pollen irradiation before making inter-specific crosses, also in the genus *Nicotiana* (Pandey 1975, 1978), where the same principles appeared to apply. Davies (1981) has pointed out that the plant breeder may wish to use pollen irradiation to transfer only a single, or a small number of characteristics from one parent to another, either by intra- or inter-specific crossing. With conventional plant breeding techniques this could only be achieved by a time-consuming series of three to six back-crosses. It would therefore be of considerable interest if the same phenomenon was demonstrated in a self-pollinating, commercially important crop species, for example a small-grained cereal such as barley, *Hordeum vulgare,* L.

Materials and methods

Experiments were conducted with the pure-breeding barley cultivars 'Golden Promise' as female parent and 'Magnum' as male parent. 'Golden Promise' is mildew susceptible, which is a recessive trait, and has an erect juvenile growth habit determined by a single recessive gene. This gene, in conjunction with other modifier genes, reduces mature plant height so that the two traits are highly, but not completely, correlated. 'Magnum' has mildew resistance, conferred by two dominant genes, Ml_v plus an unknown factor X. Under glasshouse conditions 'Magnum' has a tall phenotype, although it does carry a recessive dwarfing gene. This gene confers a semi-prostrate juvenile growth habit and is non-allelic with that present in 'Golden Promise'. 'Golden Promise' was emasculated by the egg-topping method (Pope 1944). Whole ears of 'Magnum' were irradiated immediately before pollination. using gamma radiation from a Cobalt 60 source at the Western General Hospital, Edinburgh. A series of pilot experiments established that the maximum radiation dose which resulted in viable embryos in barley was 2,000 rads. To ensure that as many embryos as possible survived, gibberellic acid (GA_3) was applied at 75 ppm one day after pollination to encourage embryo development. Approximately 14 to 18 days after pollination the developing caryopses were removed, surface sterilised and the embryos transferred to tubes containing B5 medium (Gamborg et al. 1968) and incubated at 20 °C in a 16 h per day light regime. Green plantlets were transferred to peat blocks (Jiffy 7) and later were potted on into 12 cm plastic pots containing John Innes No. 2 compost. Four radiation doses, 500, 1,000, 1,500 and 2,000 rads, were used to produce hybrid progenies for study, together with the F_1 derived from the use of unirradiated pollen. The first generation material, designated M_1, was grown together with the parents and F_1, to maturity over the winter in a heated glasshouse (12° to 20 °C) again in a 16 h per day light regime, produced by using sodium lighting. Individual plants were grown in a randomised block design and observations were made on both juvenile and mature plants.

The second generation material, produced by self-pollinating the M_1 plants and designated M_2, was grown together with the parental genotypes and the F_2 produced by self-pollinating the F_1. A replicated randomised complete block experimental design was used and the material was grown in the field at The Murrays Farm, Pathhead, East Lothian (Grid Reference NI 413633) in 1982. Each family was represented in each of four randomised complete blocks as a row of up to 20 plants. Plants were spaced 5 cm apart within rows and rows were separated by 20 cm. A spring wheat plant was grown at each end of every row as a guard plant. There were limitations in seed quantity in some families due to reduced fertility in some M_1 plants, and therefore some rows contained less than 20 plants. The data presented in this paper were recorded on single plants at the juvenile stage for mildew resistance and the expression of the dwarfing gene, and on mature plants for height.

Results

A summary of observations made on the F_1 and M_1 generations is given in Table 1. The F_1 data confirm the dominance relationships described. The M_1 population means for the four radiation doses show the same trends as reported by Jinks and his co-workers. With increasing radiation dose the means become progressively more like those of the recessive, maternal parent and less like the F_1. Recessive genotypes, susceptible to mildew and of erect growth habit occur with increasing frequencies. There is a similar trend in population variances but this is masked in the standard errors of the means by the unequal population sizes. The numbers of plants at the four doses, derived by approximately similar amounts of effort, demonstrate the increased difficulty of obtaining viable embryos at the higher radiation doses. However, this is offset by the increasingly maternal phenotypes of these derivatives, which would make the effort worthwhile. In addition, some M_1 plants at each of the radiation doses were partially or, in some cases, fully sterile and data from the sterile plants were not used in the calculation of the M_1 population means. One haploid plant ($n=7$ confirmed by root-tip chromosome count) was treated with colchicine to double the chromosome number and restore fertility. The diploid sectors of this plant resembled 'Golden Promise' morphologically but produced only one grain on self-pollination. This grain was tested electrophoretically and resembled 'Golden Promise' for hordein protein pattern (Type 1A of Shewry et al. 1979).

Table 2 presents a summary of the observations on the F_2 and M_2 generations. The segregation ratios are presented as numbers of single plants while, for height, the family means are given. It should be noted that not all of the M_1 plants produced were scored in the randomised experiment, but that these plants did contribute to the M_2.

Mildew resistance behaves as a trait determined by a single dominant gene. The use of mobile seedling nurseries (M. J. C. Asher, unpublished) established that the resistance derived from Ml_v would be overcome, in the field, by the virulent pathotype v_{Mlv}, which was of widespread occurrence in the pathogen population. Therefore, in effect only factor X was being scored. Juvenile growth habit was recorded as the four phenotypic categories tall, semi-prostrate, erect or double-dwarf. This latter phenotype exhibits some features of each of the dwarfing genes but the effect on final plant height is largely additive, thus giving a phenotype which is extremely short. Chi-squared tests for goodness of fit to the appropriate segregation ratio were also made, where appropriate. The F_2 conforms with the segregation expected from a single mildew resistance gene but juvenile growth habit did not fit the expected 9:3:3:1 ratio. This may have been due to difficulties in distinguishing the four phenotypes with absolute

Table 1. Summary of data from the M_1 generation

Genotype	No. of plants	Mildew reaction (resist: suscep)	Juvenile growth habit (normal: erect)	Height (cm) ± SEM
'G.P'	4	0:4	0:4	72.5±1.94
'Magnum'	5	5:0	5:0	96.0±3.83
F_1	9	9:0	9:0	102.8±2.03
M_1 500	39	37:2	34:5	89.2±2.03
M_1 1000	19	18:1	14:5	87.0±2.23
M_1 1500	13	9:4	4:9	70.6±2.43
M_1 2000	4	3:1	2:2	79.0±3.81

Table 2. Summary of data from the M_2 generation

Genotype	No. of families	Mildew reaction (resist: suscep)	Juvenile growth habit (tall: prost: erect: double-dwarf)	Height (cm) ± SEM
'G.P.'	5	0: 373	0: 0: 385: 0	60.5±0.46
'Magnum'	5	375: 0	0: 390: 0: 0	67.9±1.25
F_2	17	860: 291	436: 376: 277: 48	72.7±0.55
M_2 500	79	3052:1640	1515:1369:1548:151	69.3±0.61
M_2 1000	20	472: 651	219: 210: 673: 38	66.0±1.17
M_2 1500	15	75: 812	34: 33: 893: 2	61.5±0.92
M_2 2000	4	4: 223	0: 0: 233: 0	59.4±1.26

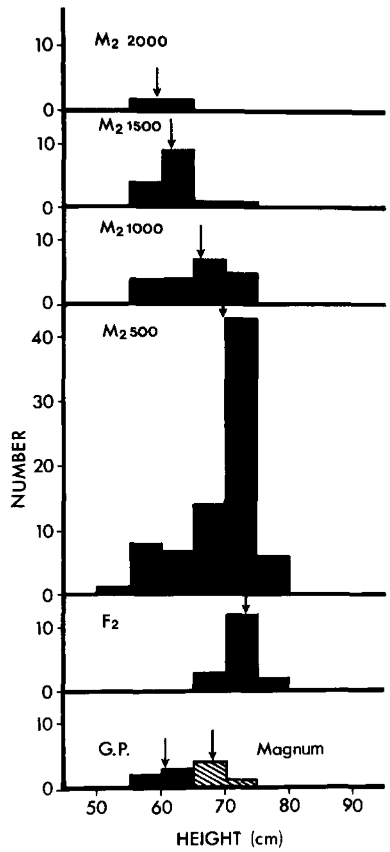

Fig. 1. Histograms of second generation family heights. Each datum is the mean of up to 80 plants per family, grown in four replicate plots of up to 20 plants each

certainty at a single scoring data or to differential viability. In each case, the appropriate comparison for the M_2 populations is with the observed F_2 segregation ratio. Chi-squared tests for heterogeneity indicated that for all of the populations where a test was appropriate ($n > 4$) there was a significant difference from the F_2. Inspection of the data reveals an increasing proportion of maternal phenotypes with increasing radiation dose. For plant height the trends present in the M_1 are confirmed in the M_2, although, not surprisingly, there is a pronounced difference in population means between the glasshouse and the field-grown plants. The data for height are also presented in histogram form in Fig. 1, and the differences between the various M_2 populations can be clearly seen, with the mean of the population derived from pollen receiving 2,000 rads not differing from that of the female parent, 'Golden Promise'. A note of caution should be added here, since it is not possible to deduce from the present data the extent to which mutations, which are likely to be in the direction of reduced height, induced in the paternal 'Magnum' genome also contributed to the observed effects. Crosses to test these contributions have been made but not yet assessed at M_2. However, for the major gene characters the only phenotypes observed were due to parental alleles or combinations of them, and so unless mutation has produced these specific alleles it would not seem to be a major component for such characters.

The M_1 phenotype was not a reliable indicator of M_2 phenotype, with correlations generally in the range 0.3 to 0.7 for juvenile growth habit (values were assigned such that 0 = erect and 1 = tall in M_1 or segregating in M_2 respectively) and for mature plant height. A dramatic example of this is afforded by inspection of growth habit scores in the plants produced from the 2,000 rads treatment, of the four M_1 plants produced, two were normal and two were erect (Table 1) whereas in their M_2 progenies (Table 2) all 233 plants were erect. The correlations for mildew were not calculated since glasshouse data for winter-grown material were considered atypical of field performance.

Conclusions and discussion

It is apparent from the data presented that similar effects were observed in *Hordeum* as were reported in *Nicotiana* (Jinks et al. 1981; Caligari et al. 1981), and in *Triticum* (Snape et al. 1983) and therefore it is likely that similar mechanisms are operating. Parthenocarpy can be eliminated as a possible mechanism because emasculated ears left bagged, but unfertilised, did not set any seed. Using the technique as a plant breeder might, it was possible to isolate M_2 families which were homozygous erect but segregating for the mildew resistance factor *X* of 'Magnum'. 'Golden Promise' and 'Magnum' also have distinct hordein electrophoretic patterns (Shewry et al. 1979) and, although only a few families have so far been examined, it was possible in at least one family, which was both erect and mildew susceptible, to demonstrate the *Hor-1* and *Hor-2* hordein banding patterns of 'Magnum'. This enabled us to eliminate accidental self-pollination of the original female parent as a source of at least one of the apparently maternal types. Electrophoresis would also allow mildew resistances such as Ml_a which are closely linked to the *Hor-1* and *Hor-2* loci on chromosome 5 to be manipulated very precisely, although in this cross Ml_a was not present in either parent. There are other major gene differences, for example bib and collar lodicules in 'Golden Promise' and 'Magnum' respectively, which will be scored on the harvested grain and will extend the scope of the analysis. However, in this preliminary report, it is our intention only to demonstrate that the technique would appear feasible in barley and to commend it to plant breeders. Many of the considerations which apply to back-crossing as a technique apply also to pollen irradiation, except that by using pollen irradiation the breeder can probably produce, in two generations and one cycle of selection, changes which would require between three and six generations of back-crossing to achieve. However, in the case of pollen irradiation, the recessive parent should be used as female and the dominant parent as male, whereas with back-crossing the direction in which the cross is made is not important. Since the M_1 is not a good guide to M_2 phenotype, little, if any, selection can be applied to the M_1 generation, so pollen irradiation would be equally effective when using dominant or recessive alleles, in contrast to back-crossing where the manipulation of dominant alleles is considerably easier. Certainly, it would now seem possible to contemplate the more systematic transfer of a large number of different sources of mildew resistance, even the production of complex multilines or mixtures of near-isogenic lines, using pollen irradiation. Using conventional back-crossing these would require prohibitively large resources. It is also possible now to investigate how this technique affects the potential for inter-specific and inter-generic transfer of genetic information to *Hordeum,* which has often not been successful using conventional wide-crossing techniques.

As with the *Nicotiana* work (Jinks et al. 1981), the under-lying mechanisms are unknown so some of the material was examined cytologically in an attempt to gain some understanding of the processes involved. Combinations of 'Golden Promise' × irradiated 'Golden Promise' and 'Magnum' × irradiated 'Magnum' were made to examine the extent to which mutation was influencing the results. Several M_1 plants of 'GP' × 'GP' (500 rads) were examined at first metaphase of pollen meiosis. Multivalent chromosome associations were apparent in one plant, particularly trivalents plus univalents or quadrivalents with occasionally the association of higher numbers of chromosomes, at a frequency of at least one per cell. The remaining plants were all normal, although in some preparations the chromosomes appeared to be sticky. The maternal parent, 'Golden Promise', was also examined and consistently formed seven bivalents, mostly of the ring type. It is probable therefore, that chromosomal re-arrangements are occurring and that some of the M_1 sterility stemmed from the formation of translocation heterozygotes.

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