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# Matromorphy in *Pisum sativum* L.

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**Summary.** The possibility of obtaining instant pure breeding lines by matromorph seed development in *Pisum sativum* L. has been investigated. Two types of maternal parents, namely, homozygous for the recessive marker genes and heterozygous for the dominant marker genes were pollinated with *Lathyrus odoratus* and the 'P174' variety of *Pisum sativum* L. carrying dominant markers. For both pollinators, induction of matromorphy by prickle pollination, irradiated pollen and IAA treatment was examined. Promising matromorphs were identified in the $M_1$ generation which were studied in the $M_2$ generation for assessing their genetic status with respect to homozygosis. The success of pod set varied from zero to 28% with a varying number of matromorphic seeds following different treatments. The possible mechanisms for matromorphic origin have been discussed. The evidence presented herein favours induction of matromorphy in peas for the production of homozygous stocks. In addition, the recovery of double recessive seed markers of the maternal parents along with plant markers from the paternals has prospective implications in plant breeding as an alternative tool to recurrent back crossing.

**Key words:** Matromorphy – Maternals – *Pisum sativum* L. – Parthenogenesis – Prickle pollination

## Introduction

Matromorphs or maternals are non-hybrid diploid offspring which originate entirely from the maternal parent (Mackey 1972). Matromorphic induction of the seeds occurs without egg fertilization though pollination, which is often false or prickle, is essential in producing the necessary stimulus for the maternal seed to develop. Maternals produced by the doubling of the maternal haploid gamete during an early stage of cleavage are completely homozygous (Röbbelen 1966) and provide a means of producing instant inbred lines which are generally obtained by continuous selfing and selection over several generations. In the present investigation the occurrence of homozygous maternals for use as inbred lines has been assessed in peas.

## Materials and methods

For induction of matromorphic seed development in *Pisum sativum* L. we used two systems of pollination – (a) with an alien species and (b) with another variety of *P. sativum* L. In order to establish the homozygous or heterozygous status of the maternal progeny and to rule out any contribution from the pollen parent following pollination with (a) or (b), major gene markers were used. Two types of maternal parents carrying major gene markers were essential for covering all possibilities: (i) homozygous for recessive gene markers and (ii) heterozygous for marker genes. Any contribution from the pollen parent in the progeny of (i) pollinated with (a) or (b) can be detected by screening for the dominant gene marker. On the other hand, the homozygosity of the maternal progeny following pollination of type (ii) maternal parent can be established by examining the segregation of the marker in the $M_2$ generation. The maternal parent of type (i) was the 'Bonnevile' (BV) variety which has dwarf stature, white flowers, green pods and green and wrinkled seeds. The heterozygous maternal parents were two $F_1$ hybrids, BV×'P23-2' and 'T163'×BV. The genotype 'P23-2' is dwarf with round and yellow seeds. Therefore, the $F_1$ (BV×'P23-2') will have yellow and round seeds. The parent 'T163' is a very tall plant type and produces yellow and round seeds. The $F_1$ of 'T163' and BV, therefore, will have tall plants with round and yellow seeds.

The pollinator of type (a) was a strain of *Lathyrus odoratus* ('Moonlight') obtained from the nursery of the

Punjab Agricultural University, Ludhiana. It has flattened stems, purple flowers, round and yellow seeds. For the type (b) pollinator the genotype 'P174' was selected from the germ plasm stocks since it carries a number of dominant markers such as purple colour of flowers, pods, seeds and nodes and can be easily identified phenotypically.

For both (i) and (ii) maternal parents pollinated with either (a) or (b), the following methods of matromorphic induction of seed development were employed.

*1. Delayed pollination.* Ten emasculated buds were pollinated every day from 0 to 10 days on each type of maternal parent. Normally pollinations are done on the second day following emasculations.

*2. Gamma radiation.* Eight doses of 5 Kr to 40 Kr with an interval of 5 Kr were applied to the pollen grains along with anthers of (a) and (b) from a $Co^{60}$ source shortly before pollination (10 buds for each treatment).

*3. Growth regulator, IAA:* Five concentrations of 0.3125 ppm, 0.625 ppm, 1.25 ppm, 2.5 ppm and 5.0 ppm were tried on 5 buds in each case, on the 5th day following pollination with (a) and (b). An additional 5 buds were not pollinated but treated with IAA on the fifth day following emasculation.

To explore the possibility of spontaneous parthenogenesis in pea, control emasculations were made on all the genotypes used as maternal parents without subsequent pollination. For additional information the variety 'PG9' was also emasculated as a control.

The attempts to induce matromorphs in peas were begun in 1978–1979 and the matromorphically produced seeds were identified by examining the appearance of recessive seed markers of the maternal parent immediately following pollination. In general, the matromorphic seeds were smaller in size and were borne in shrivelled pods which could be easily differentiated from the normally developed pods. The $M_1$ progeny was raised from only the suspect matromorphically developed seeds carrying the recessive seed markers during 1979–1980 and the appearance of dominant plant markers in the $M_1$ progeny was examined. The $M_2$ progeny was studied for the recessive seed markers in order to establish the genetic status of the matromorphs.

## Results and discussion

The type and frequency of matromorphic families are presented in Table 1. All those cases where there was no pod set subsequent to pollination with (a) or (b) have been omitted. The success of pod set immediately following pollination varied from zero to 28 percent; the highest being for the homozygous maternal parent pollinated with type (b) pollinator following pollination after two days subsequent to emasculation. A pod set of 20% was observed for the homozygous maternal parent pollinated with *Lathyrus odoratus* at 10 days following emasculation and pollinated with type (b) pollinator

**Table 1.** A summary of matromorphic induction methods which exhibited matromorphic progeny in $M_1$ and $M_2$ generations of *Pisum sativum* L.

| Description | Maternal parent | No. of pods | Total no. of seeds | Matromorphic seeds ($M_1$) | % pod set | No. of plants in $M_1$ | Plants with $M_2$ seed markers | Remarks for $M_1$ plants |
|---|---|---|---|---|---|---|---|---|
| a) Pollination with *Lathyrus odoratus* | | | | | | | | |
| 1. Delayed pollination | | | | | | | | |
| 0 days | 'Bonneville' (BV) | 1 | 1 | 1 | 10 | 1 | 1 | Like BV |
| 9 days | 'Bonneville' (BV) | 1 | 2 | 2 | 10 | 2 | 1 | One plant with markers |
| 10 days | 'Bonneville' (BV) | 2 | 5 | 5 | 20 | 4 | 0 | Dominant markers |
| 2. Irradiated pollen | | | | | | | | |
| 30 Kr | BV | 1 | 1 | 1 | 10 | 1 | 0 | Dominant markers |
| b) Pollination with 'P174' variety | | | | | | | | |
| 1. Delayed pollination | | | | | | | | |
| 0 to 8 days | BV | 22 | 77 | 3 (2 days) | 28 | 2 | 1 | One plant with markers |
| | $F_1$ (BV×'P23-2') | 2 (0 days) | 7 | 5 | 20 | 3 | 3 | all like BV |
| 2. Irradiated pollen | | | | | | | | |
| 15 Kr | BV | 1 | 1 | 1 | 10 | 0 | 0 | |
| 35 Kr | BV | 1 | 2 | 2 | 10 | 2 | 2 | Like BV |
| 3. IAA application | | | | | | | | |
| 0.3125 ppm | BV | 2 | 4 | 2 | 20 | 1 | 0 | Dominant markers, no seed set |
| 0.625 ppm | BV | 2 | 8 | 0 | 20 | 0 | 0 | – |
| 2.5 ppm | BV | 1 | 2 | 2 | 10 | 1 | 1 | Like BV |
| c) Control emasculation | BV | 1 | 1 | 1 | 10 | 0 | 0 | No germination |
| | 'T163' | 1 | 4 | 4 | 10 | 4 | 4 | All like 'T163' |
| | 'PG9' | 1 | 1 | 1 | 10 | 1 | 1 | Like 'PG9' |
| | $F_1$ ('T163'×BV) | 1 | 2 | 2 | 10 | 1 | 1 | Like BV |

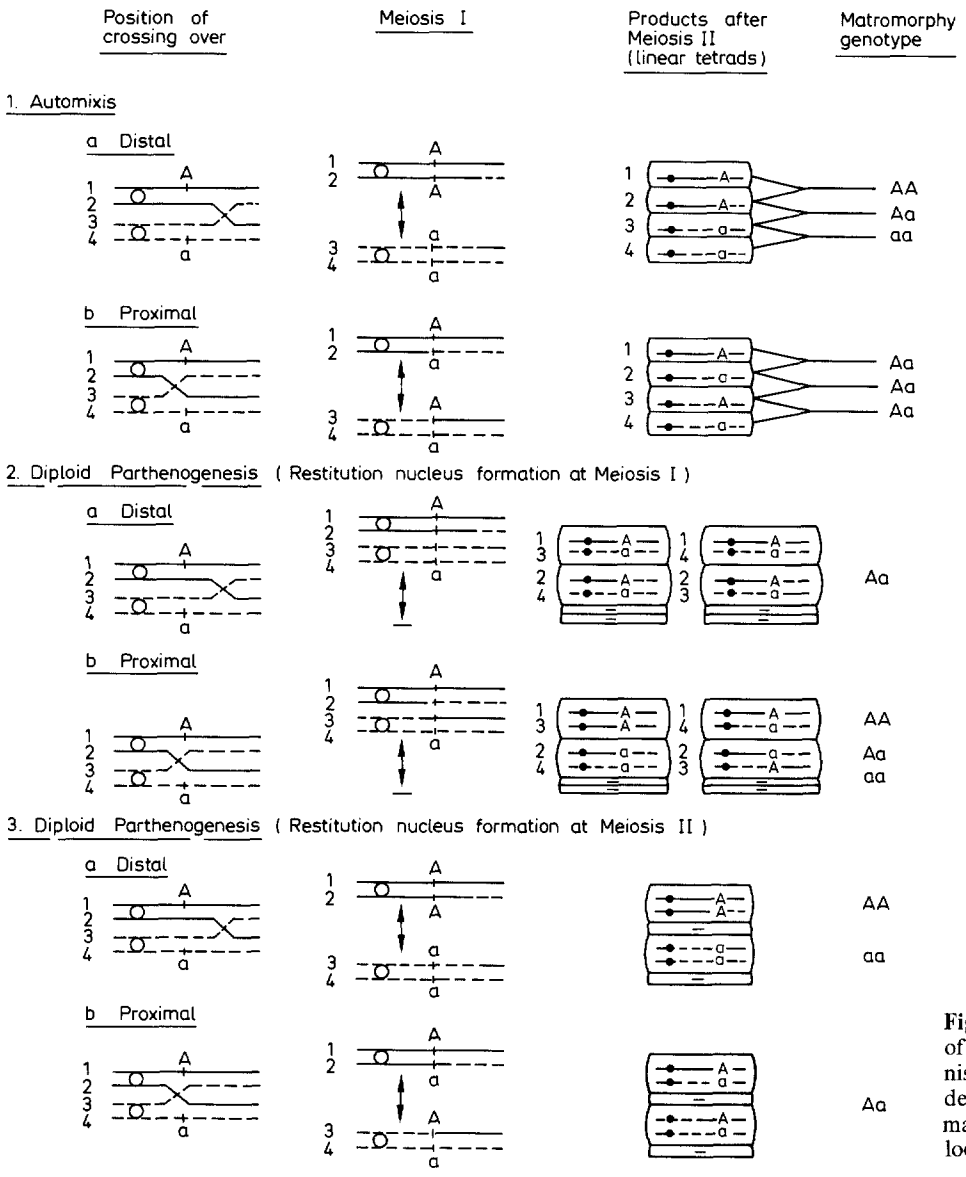


**Fig. 1.** Diagramatic representation of some of the possible mechanisms for matromorphic seed development from a heterozygous maternal parent (*Aa*) for a single locus *A*–*a*

along with 0.3125 ppm and 0.625 ppm of IAA treatment. Pollination of the type (ii) heterozygous maternal parent with type (b) pollinator in the early bud stage (same day) also resulted in 20% pod set. The rest of the cases presented in Table 1 showed at least setting of one pod out of the 10 pollinated. The frequency of the $M_1$ seeds carrying the recessive seed markers favouring their matromorphic development varied from 1 to 5. The results of the behaviour of their progeny in the $M_2$ generation have also been presented in Table 1.

On the basis of consistent appearance of the recessive seed markers in the $M_1$ and $M_2$ generations and the presence of the recessive plant markers in the $M_1$ progeny the homozygosity of the matromorphs was detected. The progeny of BV×*L. odoratus* following pollination on the same day and ninth day subsequent to emasculation, BV×'P174' following pollination on the second day subsequent to emasculation, pollen irradiation with 35 Kr and 2.5 ppm IAA treatment met all the criteria for a purely maternal origin. The progeny of $F_1$ (BV×'P23-2')×'P174' following pollination on the same day subsequent to emasculation also showed homozygous maternal.

The development of homozygous matromorphs could take place through automixis, diploid parthenogenesis, endomitosis and adventitious embryony. In automixis, diploid plants arise from the fusion of the two cells of the immediate product of meiosis or by the fusion of two haploid nuclei within the embryo sac. The immediate products of meiosis are arranged in linear tetrads and the fusion can occur between the adjacent cells only. The results of such a fusion will

depend upon the position of the crossover relative to the marker gene and hence on the nature of the reduction process- pre-reduction or post-reduction. Homozygous plants from a heterozygous maternal parent (type ii) are only expected if pre-reduction is involved (Fig. 1). The possibility of fusion of the two haploid nuclei of the mature embryo sac which would result in completely homozygous plants can also not be ruled out. In endomitosis, the doubling of the reduced maternal gametes takes place which leads to the development of diploid homozygotes. The matromorphic seed induction through diploid parthenogenesis results from the development of an unreduced egg from a diploid plant or a reduced egg from a tetraploid plant. Diploid parthenogenesis will result from the formation of a restitution nucleus through restitutional meiosis. The restitution nucleus can be formed at either the first or the second meiotic division. The final outcome from the homozygous maternal parent will be a homozygous matromorph but the genetic status (hetero- or homozygous) of the matromorph originated from the heterozygous maternal parent will depend upon the time of restitution nucleus formation and the position of crossingover (Fig. 1).

Partial homozygosity in the matromorphs arising from heterozygous maternal parents has been observed by Virk et al. (1977) in *Nicotiana* and Mackey (1972) in *Brassica*. They attributed it to the formation of a restitution nucleus and with the faillure of the second division, resulting in homozygosity for all loci between the proximal crossover point and the centromere and heterozygosity for all the loci beyond the crossover point. In adventitious embryony a somatic cell from the nucellus or integuments develops and hence can lead to the origin of homozygous matromorphs from the homozygous maternal parents. This, however, would produce a heterozygous matromorph from a heterozygous maternal parent. The possibility of adventitious embryony for the development of the homozygous matromorph from the $F_1$(BV×'P23-2') parent can, therefore, be safely ruled out. The other possibilities, such as diploid androgenesis and true hybridisation are also excluded.

The genetic contribution from the male parent was apparent in BV×*Lathyrus odoratus* upon pollination after 9 and 10 days and irradiation of pollen with 30 Kr treatment. Also, dominant plant markers appeared in the $M_1$ progeny of BV×'P174' with pollination after two days and 0.3125 ppm IAA treatment. The $M_1$ seeds of all these plants were, however, wrinkled and green and the appearance of dominant plant markers in the $M_1$ progeny showed their partial heterozygosity. The homozygosity of the seed markers and the segregation for the plant markers could arise if, during automixis or diploid parthenogenesis, crossingover resulted in the exchange of genes other than those controlling seed shape and colour. These results are opposite to those observed by Pandey (1975) and Virk et al. (1977) where single genes were transferred from the pollen parent. In the present case, however, seed marker genes have been contributed by the female. Pandey (1975) hypothesised that single gene transfer from irradiated pollen could result from the pulverisation of the generative nucleus to produce a mass of fine chromatin fragments which, when discharged into the egg, acts as a false fertilization. Consequently, the egg starts dividing but the presence of disorganised pollen chromatin prevents a normal first zygotic division. These chromatin fragments are subsequently lost and this enables the normal mitotic division to occur, resulting in parthenogenetically diploid embryo. Occasionally the fragments of the disorganised chromatin may associate with their homologues of the egg chromatin. During further replication, restitution or addition may occur. Depending upon the pairing, with or without attachment of the fragment as an exosome, the substitution, addition, or substitution plus addition can be heterozygous or homozygous. In the present case the effect of prickle pollination and pollen irradiation resulting in partially heterozygous progeny may be visualised in terms of Pandey's (1975) theory where degenerated nuclei from the prickle pollination or pulverised generative nuclei from the irradiated pollen produce a mass of chromatin network leading to false fertilization.

The possibility of spontaneous parthenogenesis was assessed by control emasculations. One pod each on BV, 'T163', 'PG9' and $F_1$ ('T163'×BV) was observed. Except for the pod on 'T163', which was normally developed with full size seeds, the seeds and pods of the other maternal parents were undersize, favouring their matromorphic development. There is, therefore, evidence in favour of occurrence of natural parthenogenesis in peas.

## Conclusions

If true maternals, inducible at reasonably high frequency, are produced by any of the induction mechanisms or causal stimuli, it is essential that we should be able to detect them and to isolate them from such other novel genotypes as aneuploids, haploids, heterozygous maternals and true hybrids which might arise simultaneously in the $M_1$ progeny. A number of tests can be used but the most important and easily performed test is the use of a marker gene as has been exemplified in the present experiment. The use of a single marker gene is sufficient for detecting and testing the homozygous or heterozygous status of the maternals. The homozygosity or heterozygosity with respect to the marker gene, in some cases however, may be misleading because plants homozygous for one marker but not for the whole genotype may be obtained from the heterozygous female parents. To distinguish between partial and complete homozygosity it will be necessary to use many, preferably linked and or unlinked, gene markers such as the seed colour, seed shape, plant colour, flower colour, pod colour in our experiment and the 10 unlinked loci used by Sarkar and Coe (1971) in maize. The qualitative evaluation may be complemented by biometrical ganetical analyses for quantitative characters such as that shown by Virk et al. (1977).

Matromorphy is of practical value only if maternals can be induced on heterozygous maternal parents of the type (ii). Upon selfing a complete heterozygote, the probability of obtaining a particular homozygote amongst the progeny will be $(¼)^k$, where k is the number of loci controlling the character. The frequency of homozygotes derived from the same heterozygous source by matromorphy will be the same as the frequency of the gametes that carry the allele of the character i.e. $(½)^k$. Therefore, the frequency of pure breeding lines through matromorphs is considerably raised. Furthermore, the lines derived through matromorphy will be completely homozygous as against those derived by conventional procedure where some degree of heterozygosity persists for a long time. However, the occurrence of heterozygous maternals, as observed by Mackey (1972) and Eenink (1974 a, b, c, d, e) should not be taken as evidence for the occurrence of this phenomenon unless it can be confirmed on homozygous parents of type (i) where tests for the absence of the pollen parent contribution to the $M_1$ progeny are more efficient.

The results presented herein have shown that the induction of matromorphs appears to be a viable proposition for producing instant pure-breeding lines in peas. Further experiments are necessary to detect suitable combinations for obtaining matromorphs since large differences in parthenogenetic ability and parthenogenetic inducing ability exist between genera, species, varieties and accessions of plants (Eenink 1974 b). In the suitable combination the female must contain genes which can be switched off under normal pollination conditions but will be switched on by the appropriate male inducer. The later has a genotype which produces a specific chemical substance which on reacting with the specific gene product of the female produces maternals. In order to detect such suitable combinations it is therefore necessary to pollinate different female genotypes by a number of different species. With this in view pollinations were made with *Vicia faba* and *Phaseolus* species. Preliminary observations have shown pod and seed setting in crosses of BV, 'PG-3', 'Hara Bauna', 'PG-4', 'PL-1', 'PG-2', $F_1$ ('P179' × BV) and $F_1$ ('P183' × BV) with *Vicia* species as the male parent.

The homozygosity for a few recessive gene markers, seed colour and shape in the present experiment, is an interesting outcome of our studies. The practical implication of this phenomenon of retaining few genes is similar to that of transferring a few genes for the specific purpose since there is the possibility of affecting selective improvement by retaining some genes and at the same time transferring specific genes into the desired background in a shorter period than required by the usual procedure of successive backcrossing. Furthermore, the production of lines differing at many loci except for a few major genes may be possible.

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