PRELIMINARY EVIDENCE OF A GENETIC CAUSE FOR THE FLORAL ABNORMALITIES IN SOME OIL PALM RAMETS

Keywords: *Elaeis guineensis*, oil palm clones, floral abnormalities.

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In some ramets of oil palm clones the normally vestigial gynoecia and androecia of male and female flowers respectively develop abnormally, resulting in floral dysfunction. Such abnormal development has also been observed in the open-pollinated progeny of these ramets. This evidence of, most probably, matroclinal sexual transmission suggests that the abnormalities may have arisen from changes to extrachromosomal hereditary determinants during tissue culture.

INTRODUCTION

Abnormal flower development in oil palms propagated by tissue culture has inhibited the use of this potentially powerful method of multiplication. The abnormality may occur in either male or female inflorescences or in both (Corley *et al.*, 1986). In the female inflorescence it is conspicuous as a parthenocarpic development of the gynoecium of each flower, often accompanied by similar growth of the surrounding, normally vestigial, staminode ring. The latter results in a characteristic mantle of one to six (and occasionally up to ten) supplementary carpel-like structures around the central parthenocarpic fruit. In the abnormal male inflorescence the normally vestigial gynoecium of each male flower develops parthenocarpically into a small fruitlet. In some of these androgynous inflorescences the stamens of each flower may also develop into a ring of tiny carpel-like structures.

The severity of the abnormality varies among the ramets of a clone — from those with near normal inflorescences except for a few parthenocarpic fruits, to an extreme of ramets with only mantled parthenocarpic fruitlets on both male and female inflorescences.

Parthenocarpy and androgyny also occur in seed-propagated oil palms but less extensively and, as noted by Williams and Thomas (1970), more frequently in early inflorescences. In abnormal ramets, however, such abnormal floral developments are extensive and, hitherto, apparently permanent. Mantled fruits, mostly infertile, are an even more distinctive feature of abnormal ramets. They do not occur in seed-grown palms except in the very rare 'genetic' mantled palms. The latter, found at a low frequency in the wild (Zeven, 1973), produce fully fertile mantled fruits uniformly throughout each female inflorescence by the action of a dominant gene (Hartley, 1988). Male inflorescences in these palms are all normal.

The floral abnormalities of oil palm ramets have serious consequences. Male flowers do not produce pollen and female flowers develop parthenocarpically into small infertile mantled fruitlets. The insufficiency of fertile fruits to sustain bunch development results in total bunch failure and loss of yield.

Attempts to differentiate abnormal from normal ramets, using biochemical and molecular techniques, have so far been unsuccessful. Oil palm tissue culture laboratories are, meanwhile, examining and experimenting with culture protocols that may avoid inducing the abnormalities and thereby, possibly, pin-point their cause.

Plant breeders are using classical breeding methods to ascertain whether the abnormalities have a genetic basis. Some important observations emerging from preliminary breeding work, conducted independently by PORIM Kluang and Pamol Plantations Sdn. Bhd. (PPSB), are reported below.

MATERIALS AND METHODS

The possibilities of a dominant gene mutation or extrachromosomal maternal inheritance could be quickly explored by growing seedlings from fertile open-pollinated mantled fruits of abnormal ramets. Using open-pollinated seeds saves about 8-18 months as compared with controlled crosses.

At PORIM Kluang

Eighteen such seedlings were raised, twelve from abnormal ramets of clone 115E and six from abnormal ramets of clone 31A.

The germinated seeds were sown in small polybags in June 1986, raised in the usual manner and transferred to very large polybags about a year later. Flowering commenced from mid-1988 and to date 17 of the 18 palms have flowered. The types of inflorescences produced were regularly recorded.

At PPSB Pamol Estate

Seventeen germinated seeds from an abnormal ramet of clone 115E and two from an abnormal ramet of clone 90A were sown in December 1986 and transferred to large polybags in June 1987. Flowering commenced in late 1988. Developed female and androgynous inflorescences were harvested and examined for mantled fruits. If they were present the number of supplementary carpels on each fruit was also recorded. In March 1989 the seedlings, having outgrown their bags, were field planted. Observations were continued when flowering resumed. To date seven palms out of the seventeen from the abnormal 115E ramet, and both palms from the abnormal 90A ramet have flowered. For comparison, inflorescences from young, 40 months old field-planted D × P palms grown from seed were similarly examined.

Recently, eleven palms from the selfings of three normal ramets of clone 115E produced their first inflorescences. Preliminary observations from these are also reported below.

OBSERVATIONS

In general the open-pollinated progeny were all vegetatively normal, albeit relatively small because of the extended period in polybags.

At PORIM Kluang

Field observations of the types of inflorescences produced by each palm since first flowering are summarized in *Table 1*. On the basis of these data the 17 palms may be classified into the following groups:

- Normal flowering palms with all normal inflorescences or bearing a few small androgynous inflorescences and/or parthenocarpic bunches similar to those found in seed-grown palms. Palms numbered 1, 2, 5, 12 and 14 fall into this group.

- Abnormally severe androgyny in the inflorescences *(Figure 1)* but the parthenocarpic fruitlets without supplementary carpels: palms 3, 13 and 15.

- Many parthenocarpic bunches *(Figure 2)*: palms 4, 6, 7 and 10.

- Palms producing inflorescences with one or more mantled fertile or infertile fruit *(Figure 3)*: palms 8, 9 and 17.

- Palms producing male and female inflorescences with mantled fruitlets on both: palms 16 and 18.

A notable observation in all the above groups, however, is that more recently produced inflorescences, so far all male, appear increasingly normal. Indeed they constitute a large proportion of the normal male inflorescences shown in *Table 1*. The open-pollinated seeds were collected from ramets with abnormal female but normal male inflorescences. Whether the present observation stems from this or is suggestive of decreasing expression with age, as observed for seedling palms (Williams and Thomas, 1970), can only be ascertained after a few more cycles of male and female inflorescences.

At PPSB, Pamol Estate

Mantled fruitlets *(Figure 4)* were found in the inflorescences of seven of the nine flowering palms *(Table 2)*. The number of mantled fruits in each inflorescence varied between inflorescences within a palm and among the seven palms. Interestingly, most of the mantled fruitlets had only one or two supplementary carpels.

Though at first sight this again seems to suggest reduced expression in the first sexual generation, it could also merely be a reflection of poor germination of the more severely mantled but apparently fertile open-pollinated fruits. Unfortunately no individual fruit germination records, which might have answered this question, were kept when these investigations were first started.

No mantled fruitlets have been found in the inflorescences produced to date by the eleven selfed palms of the three normal 115E ramets *(Table 3)*. Similarly the nine androgynous inflorescences examined from five young D × P seedlings all had parthenocarpic fruitlets but none was mantled.

DISCUSSION

The observations reported above implicate an hereditary determinant or determinants in the induction of abnormal flower development in some ramets of oil palm clones.

A dominant nuclear gene, as in genetic mantled palms, and arising through mutation during culture is unlikely for the following reasons. First, the abnormalities are irregularly expressed within and between bunches in many abnormal ramets. Normal, parthenocarpic and mantled fruits may all occur in the same bunch, the proportions varying between bunches and between ramets. In all genetic mantled palms, on the other hand, all bunches and all the fruits in them are similarly mantled. Second, it is unlikely that the same mutation event occurred in large numbers of cultures in many clones within a relatively short time. Nuclear gene mutations, according to classical ideas, are rare sporadic events and there are no apparent reasons to suggest that oil palm tissue culture protocols are exceptionally mutagenic. Third, the apparent reduction in the extent of the abnormality within and between

TABLE 1. TYPES OF EARLY INFLORESCENCES PRODUCED BY SEEDLINGS OF ABNORMAL RAMETS

Ramet of clone No.	Palm No.	Number of male inflorescences: Normal	Number of male inflorescences: Androgynous	Number of male inflorescences: Androgynous with mantled fruitlets	Number of female inflorescences/fruit bunches with: Fertile and parthenocarpic fruits	Number of female inflorescences/fruit bunches with: Parthenocarpic fruits only	Number of female inflorescences/fruit bunches with: Parthenocarpic and mantled fruits
115E	1	-	2	-	-	-	-
	2	3	2	-	-	-	-
	12	11	-	-	1	-	-
	14	3	-	-	-	1	-
	15	-	17	-	-	-	-
	18	11	6	-	-	-	1
	3	10	4	-	2	-	-
	4	3	1	-	-	8	-
	5	1	3	-	2	1	-
	6	2	-	-	-	4	-
	7	1	-	-	7	4	-
	8	-	-	-	-	-	1
	9	12	4	-	-	1	2
	10	2	4	-	-	8	-
	11[a]	-	-	-	-	-	-
	13	10	7	-	-	1	-
	16	2	9	4	-	-	2
	17	-	6	-	-	-	1

[a]Not flowering yet

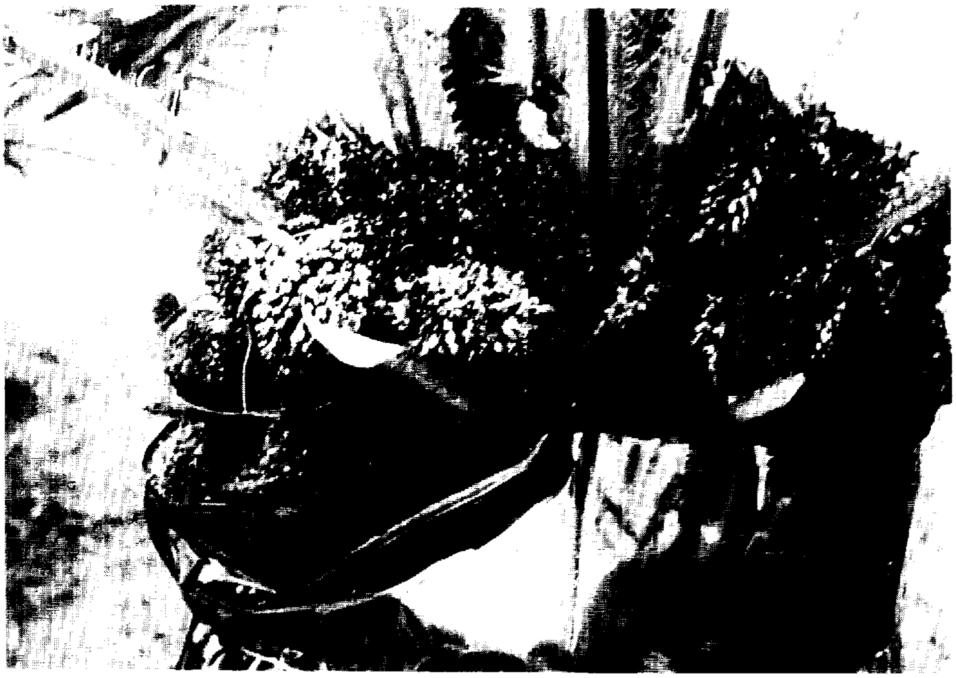

Figure 1. Severe androgyny in an open-pollinated palm derived from an abnormal ramet.

Figure 2. Large number of parthenocarpic bunches in an open-pollinated palm derived from an abnormal ramet.

Figure 3. Bunch with mantled fruits from an open-pollinated palm derived from an abnormal ramet.

Figure 4. Mantled fruitlets from an androgynous inflorescence of an open-pollinated palm derived from an abnormal ramet.

TABLE 2. PROPORTION AND TYPE OF MANTLED FRUITS IN INFLORESCENCES OF SEEDLINGS OF ABNORMAL RAMETS

Clone	Palm No.	Inflorescence No.	Number of fruits examined	Number of fruits mantled	Distribution of mantled fruits by number of extra carpels					
					1	2	3	4	5	6
1115E	2	1	382	0						
		2	2057	0						
		3	203	0						
	3	1	43	4	2	2	-	-	-	-
	6	1	198	0						
	10	1	3659	435	245	132	46	10	2	-
		2	4460	123	87	34	2	-	-	-
	11	1	598	82	41	21	12	6	2	-
		2	214	58	50	5	3	-	-	-
		3	733	143	84	46	12	1	-	-
		4	219	1	1	-	-	-	-	-
		5	2003	244	140	71	27	4	2	-
		6	345	18	12	-	-	4	2	-
	14	1	515	10	8	2	-	-	-	-
	15	1	277	9	6	3	-	-	-	-
90A	1	1	811	0						
		2	452	5	1	4	-	-	-	-
	2	1	87	23	5	18	-	-	-	-
		2	1256	16	16	-	-	-	-	-

TABLE 3. ABSENCE OF MANTLED FRUITS IN SELFED PROGENY OF NORMAL RAMETS

Palm No.	Inflorescence No.	Number of fruits examined	Number of fruits mantled
4/1	1	672	0
	2	242	0
4/2	1	72	0
	2	56	0
	3	116	0
	4	284	0
4/4	1	132	0
	2	147	0
	3	253	0
	4	378	0
	5	439	0
	6	38	0
	7	56	0
5A/1	1	344	0
	2	849	0
	3	698	0
5A/3	1	262	0
5A/4	1	99	0
5B/5	1	794	0
5B/6	1	169	0
5B/7	1	591	0
5B/11	1	671	0
	2	353	0
5B/12	1	605	0

palms of the first sexual generation contradicts the hypothesis of a dominant gene mutation.

However, complete rejection of the hypothesis must await more positive reasons in view of Janssen's (1959) observations on anomalous, inexplicable segregations in some crosses involving genetic mantled palms. Indeed, even where a dominant gene is involved various suggestions can explain irregular and incomplete expression. More definitive evidence for or against may be available soon from the results of a second set of investigations now in progress.

An alternative explanation for the present observations is that the pollen parents of the open-pollinated seeds were also all abnormal ramets. Such a possibility would suggest mutant recessive nuclear genes or, even more remotely, paternal extrachromosomal factors as a cause of the abnormalities. This is, however, highly improbable in the present instance. The ramets were from relatively small clonal trials of normal and abnormal ramets and simultaneous anthesis of a male inflorescence from one abnormal ramet and a female inflorescence from another would be extremely rare. And even if it sometimes occurred, it is inconceivable that all the open-pollinated seed arose thus. The surrounding estate palms were the more likely source of pollen in these open pollinations.

Incidentally, the absence of floral abnormalities in the self progeny of normal ramets *(Table 3)* argues against the suggestion of a recessive nuclear gene, unless, of course, the three normal ramets were all homozygous dominant for the normal homologue.

The foregoing discussion reduces the possible explanations to one likelihood: that extrachromosomal hereditary determinants, matroclinally transmitted, may be a cause of the abnormalities. It is speculated that the tissue culture environment, which is possibly unfavourable, induced changes in the extrachromosomal system and affected material with genetic continuity. Unambiguous proof for or against this suggestion should be forthcoming soon from the reciprocal crosses recently field-planted at PORIM Kluang.

Assuming for now the above hypothesis, of the various extrachromosomal induced changes that have been described the present phenomenon apparently resembles dauermodifications (Jinks, 1964), especially if the reduced expression in the first sexual generation is confirmed. The latter may be explained by two, not mutually exclusive, mechanisms. Selection – at the gamete, cell, tissue or plant level – for any extrachromosomal material unchanged during the original induction, and gradual back-mutation of changed extrachromosomal material to normal.

The above explanation implies that the abnormal ramets and their maternal progeny were variegated for normal and mutant homologues of extrachromosomal determinants. Such variegation may explain the spectrum of differences between abnormal ramets. And, if selection and back-mutation to normal homologues are indeed taking place, then the abnormalities should disappear over succeeding generations, the speed depending on the initial state and the rates of back-mutation and selection.

There is still one puzzle however, and that is the irregular expression of the abnormality within and between the bunches of each abnormal ramet. Interestingly, the irregularity is less obvious in ramets that are almost completely normal or in those that are severely abnormal. It can only be speculated at this stage, and in view of the complex process of gene expression, that in palms variegated for normal and mutant homologues the expression is incomplete and variously modified by non-genetic factors.

CONCLUSIONS

Abnormal flower development in the sexual progeny of abnormal ramets of oil palm clones suggests changes in an hereditary determinant or determinants during tissue culture. Matroclinal transmission suggests that extrachromosomal genetic material is involved.

A second set of investigations now in progress includes selfings of abnormal ramets and their reciprocal crosses with normal and genetic mantled palms. These investigations would confirm or disprove the above suggestions. Hopefully they might also shed some light on the nature of the hereditary determinants.

ACKNOWLEDGEMENTS

The authors are grateful respectively to the Director-General of PORIM, Dato' Dr Hj Abdul Halim Hassan, and the Management of Pamol Plantations Sdn Bhd for permission to publish this paper. The senior author is appreciative of the diligent work and meticulous observations of Mr Erakanaidu and Mr Noh Ahmad of the Plant Breeding Section of the PORIM Research Station, Kluang. The second author appreciates the assistance of Mr Michael Ng and the staff of Pamol Plantations.

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