Apomixis in sorghum line R473—Truth or myth? A critical analysis of published work

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The only report on the occurrence of obligate apomixis and self-incompatibility in grain sorghum and its subsequent change to facultative apomixis linked with cross-sterility evoked interest as well as incredulity. A critical review of material published on this apomixis and the reported fixation of heterosis by using this apomixis indicates that this two decade-long research was an effort to uphold an erroneous initial claim with many misleading and fallacious evidences.

OCCURRENCE of obligate apomixis in sorghum was reported by Rao and Narayana[1] in the F_4 progeny of a cross, Aispuri × IS 2942, which was designated as R473. Both the parents used in the cross were normal and belonged to the durra and kafir groups respectively. This apomixis was termed as 'stable with the entire seed in the panicles being aposporous'[1] (see Box 1). Origin of this apomixis was attributed to the genetic divergence between the parents of R473 and to the operation of self-incompatibility mechanism[1,2]. Studies on the development of embryosacs and germination of pollen were used as evidences to substantiate these deviations from the normal sexual reproduction.

Embryological evidences on obligate apomixis

The presence of obligate apomixis in R473 was inferred based on the observations such as degeneration of megagametocytes before meiotic division or at different stages of megagametophyte development (Figure 1), formation of aposporous (nucellar) embryosacs in all ovules with degenerated sexual cells and development of embryo and endosperm from aposporous embryosacs after receiving a 'stimulus' from the self-pollen[1]. However, other findings such as presence of 7-nucleate embryosacs, absence of ovules with more than one embryosac (see Box 1), and ability of R473 to produce hybrids with a pureline, BP 53, also reported by this study[1] contradicted the obligate apomixis theory.

Hanna et al.[3] stated that occurrence of 7-nucleate embryosacs in R473 indicated formation of sexual megaspores rather than the claimed aposporous development of embryosacs from the nucellus and the latter event should have resulted in some of the ovule

having multiple embryosacs. In a subsequent study Rao and Murty[4] reported eight nuclei in all mature embryosacs of '5000–6000 ovules' examined and also double embryosacs in 72 of them (a frequency of about 1.4%). It was also stated that pollination of emasculated R473 with 'as many as 50 genotypes resulted in only one or two grains from five cases'[4]. No details on these crosses or comments on the discrepancies between these results and those of Rao and Narayana[1] were available.

Rao and Murty[4] reconfirmed existence of obligate apomixis citing evidences such as absence of sexual embryosacs in all the 5000–6000 ovules examined, diploid chromosome number in egg and polar nuclei of all 8-nucleate unfertilized embryosacs and development of embryo and endosperm soon after receiving the 'stimulus' from self-pollen without pollen tube growth. Based on these evidences and on the presumed existence of self-incompatibility in R473, it was argued that the ability of this line for normal seed set under selfing was a strong evidence in favour of the obligate apomixis[4].

Self-incompatibility in maintenance of apomixis

Apart from its role in the origin of apomixis in R473, self-incompatibility was considered an essential mechanism for its maintenance[1,2,4]. Evidences put forth in support of self-incompatibility were the normal pollen fertility in R473 and its ability to fertilize other cultivars and to effect complete seed set in male-sterile lines[1]. Through more comprehensive results based on examination of 100 styles from ten R473 plants belonging to each of the five generations advanced under selection and selfing, Rao and Murty[4] confirmed that 'in no case the pollen tube traverses the styles' and the 'number of plants showing pollen germination decreased gradually and consistently as generations advanced'. They concluded that continuous selfing practiced in the R473 progenies for five generations (subsequent to F_4) resulted in complete self-incompatibility and simultaneous 'elimination of all traces of sexuality'.

The nexus between self-incompatibility and apomixis was supported with additional embryological evidences by Murty and Rao[5]. In this study where more than

Box 1

Sexual and apomictic reproduction

Sexual reproduction in angiosperms involves fusion of egg and sperm through fertilization or syngamy. Development of egg or megaspore starts with meiotic division of megagametocyte in the ovule (Figure 1). One of the cells of the tetrad thus produced undergoes three mitotic divisions without cytokinesis and forms an eight-nucleate *Polygonum* type megagametophyte (embryosac). These eight nuclei are differentiated into egg and two synergids (egg apparatus) at one pole, three antipodals at opposite pole and two polar nuclei in between (Figure 1). Sorghum produces this type of embryosacs and the polar nuclei remain separate until fertilization (Hanna *et al. Science*, 1970, **170**, 338). The microspore or male gamete develops after meiotic division of microsporocyte or pollen mother cell in the anther (Figure 1). The haploid pollen nucleus mitotically divides to produce a vegetative and a generative nuclei. The former regulates pollen tube growth and the latter divides again to produce two sperm nuclei. One of them fertilizes the egg and the other fuses with the two polar nuclei through triple fusion and produces triploid endosperm nucleus.

In apomixis, reproduction takes place without syngamy. Apomictic reproduction through seed is called agamospermy and most often apomixis refers to this phenomenon. Agamospermy is classified into various types on the basis of whether development of egg requires a megagametophyte (gametophytic agamospermy) or is directly from a somatic cell without an embryosac (adventive agamospermy). Gametophytic apomixis is further differentiated as diplosporous and aposporous. In diplospory, the megagametophyte originates from megagametocyte (Figure 1). Depending on the nature of deviations in meiosis and mitosis different types of diplosporous apomixis are distinguished. One of them which totally skips meiosis is called *Antennaria* type (H. O. Juel, *Ber. Bot. Zbl.*, 1898, **74**, 369).

In aposporous apomixis, somatic cells from nucellus or integument of the ovule develop into embryosacs through mitotic divisions (Figure 1). Simultaneous development of more than one nucellar initials into as many embryosacs in the same ovule is common in apospory. Similarly simultaneous development of aposporous and diplosporous embryosacs as well as sexual and apomictic embryosacs are also possible in the same ovule. Usually the most competitive among them reach maturity and develop into seed.

Apomicts may produce either normal fertile pollen or sterile pollen at different frequencies. Although the apomictic egg can directly develop to embryo without fertilization (parthenogensis), good pollen fertility is often important for seed set. Development of embryo and endosperm from an apomictic embryosac may be autonomous or pseudogamous. The former requires no pollination. The latter requires pollination and germination of pollen or even fusion of sperm with polar nucleus. Pseudogamy invariably ensures good seed set in cereals (Y. H. Savidan, *Apomixis Newsl.*, 1989, **1**, 47).

Obligate and facultative apomixis

When reproduction is exclusively apomictic, it is obligate apomixis. Facultative apomixis, on the other hand, allows variable frequencies of sexual reproduction. The two types differ in their behaviour and genetic basis. Facultative apomixis is more unstable, particularly under variable environments (G. L. Stebbins, *Chromosomal Evolution in Higher Plants*, Edward Arnold, London, 1971). Apomixis is usually recessive to sexuality (G. L. Stebbins, *Variation and Evolution in Plants*, Cambridge Univ. Press, New York, 1950). While obligate apomixis in almost all cases is governed by two or three major recessive genes (C. M. Talioferro and E. C. Bashaw, *Crop Sci.*, 1966, **6**, 473; G. Almgard, *Lantbr. Hogsh. Ann.*, 1966, **32**, 3; W. W. Hanna *et al., Crop Sci.*, 1973, **13**, 695; L. Powers, *Genetics*, 1945, **30**, 323). Facultative apomixis is invariably controlled by polygenic system (H. Y. Savidan, *Apomixis Newsl.*, 1989, **1**, 22). Failure of fertilization in a facultative apomict may lead to degeneration of unfertilized sexual embryosacs and thus partial seed sterility (C. Crane, *Apomixis Newsl.*, 1989, **1**, 51).

Apomixis in cereals

While obligate apomixis is common in many grasses, cereals are notable for the absence of this reproduction in their primary and secondary gene pools. A claim on obligate apomixis in cultivated rice made from China remains unconfirmed (G. S. Khush, personal communication, 1990) and is considered unfounded (H. Y. Savidan and C. Crane, *Apomixis Newsl.*, 1989, **1**, 25). Presence of obligate apomixis was reported in more distant wild relatives belonging to the tertiary gene pool of pearl millet (H. E. Warmke, *Am. J. Bot.*, 1954, **41**, 5), maize (L. I. Farquharson, *Am. J. Bot.*, 1955, **42**, 737) and wheat (J. B. Hair, *Heredity*, 1956, **10**, 129). Occurrence of sporadic or induced facultative apomixis and erratic tendency to produce ovules with multiple embryosacs are not uncommon in cereals. In sorghum, for instance, there are four reports on facultative apomixis (P. Madhava Menon, P. Devasahayam, and V. S. Raman, *Madras Agric. J.*, 1965, **52**, 454, W. W. Hanna, K. F. Schert and E. C. Bashaw, *Science*, 1970, **170**, 338; C. Y. Tang, K. F. Schertz, and E. C. Bashaw, *Sorghum Newsl.*, 1976, **19**, 124; *ibid, Bot Gaz*, 1980, **141**, 294) and at least one report on consistent production of few ovules with multiple embryosacs (L. S. S. Kumar, *Curr. Sci.*, 1942, **11**, 241) Apomictic frequency in all these cases is less than 25%.
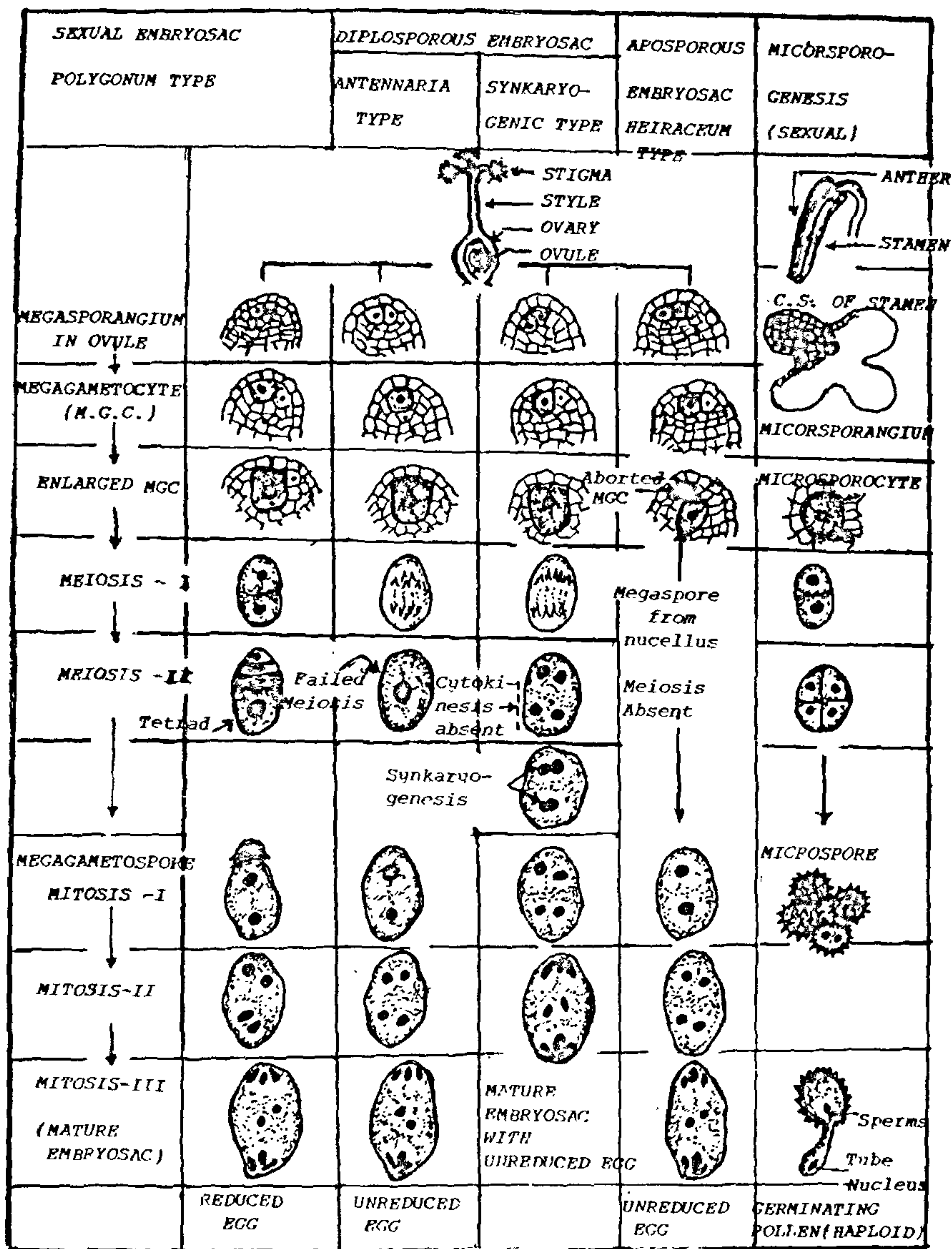
Figure 1. Microsporogenesis and megasporogenesis under sexual, diplosporous and aposporous reproduction.

1500 ovules were examined, the presence of intact tetrads and aposporous initials (see Figure 1) could be seen in several of them. While most of these tetrads degenerated, some of them which developed into embryosacs also degenerated subsequent to self-pollination due to operation of self-incompatibility. In all such cases aposporous embryosacs were found developing from the neighbouring nucellar tissue and this process continued up to six days after anthesis[5]. Such late development of aposporous embryosacs, the need for 'stimulus' from self-pollen for seed development from these embryosacs and the normal seed set in R473 under selfing bring forth certain self-contradictions.

The self-incompatibility claimed in R473 is in conflict with the widely held view of non-existence of such reproductive barriers in sorghum, either in the natural variability or among the derivatives of many crosses involving parents more divergent than those of R473. Realizing this, Rao et al.[6] and Murty and Rao[5] sought to explain the possibility of creating self-incompatibility through interaction between sterile cytoplasm and fertility non-restoring nucleus and a nebulous inter-relationship among cytoplasm-based male sterility, self-incompatibility and apomixis. While there was no basis for this wild speculation, it has no relevance to the self-incompatibility claimed in R473 as neither of its parents were reported to have sterile cytoplasm. All these claims were, nevertheless, firmly maintained until a first independent study contradicted them[7,8].

Independent genetic analysis invalidates apomixis

Marshall and Downes[8] from Australia studied the reproductive behaviour of R473 using fast-moving (FF) and slow-moving (SS) isozymes of Mdh 1 locus as the genetic marker. Among the 73 plants grown in a phytotron under 8- and 16-hour photoperiods, 49 were homozygous for isozyme FF, 20 homozygous for isozyme SS and four heterozygous (FS) for them. The selfed progeny comprising of 201 plants derived from the four heterozygotes were found segregating for FF, FS and SS genotypes in 1:2:1 ratio. This strongly invalidated the self-incompatibility and obligate apomixis claimed in R473. Moreover, the goodness of fit between the expected and observed segregation ratios in all the four progenies excluded any detectable facultative apomixis.

Arbitrary change to facultative apomixis

Genetical evidences such as the above are more reliable and conclusive than the embryological evidences in ascertaining the type of reproductive behaviour. The conclusions emerged from the first genetical study[8] on

R473 were in complete disagreement with those based on a series of embryological studies[1,4,5]. This major contradiction was sought to be resolved by Murty et al.[9] through arbitrary change in the nature of apomixis from obligate to facultative (see Box 1). On the other hand, the clear evidence obtained[8] for normal sexual reproduction in R473 was arbitrarily termed as 'very inconclusive'[10]. All these raise serious doubts on the embryological evidences put forth to support the apomixis and self-incompatibility claimed in R473.

The only reason put forth by Murty et al.[9] for changing the nature of apomixis was that 'R473 did not function as an obligate apomixis in all cases and it functioned as a facultative apomixis in crosses'. This reason is not convincing and also misleading due to the following considerations. First, the change was not conditional to the crosses alone. Since this change, R473 has always been termed as facultative apomict, irrespective of the status of its pollination. Secondly, this change contradicted their earlier conclusion on the 'gradual and consistent elimination of all traces of sexuality' during a few early generations advanced under selfing[4]. Thirdly, it is an elementary fact that no true obligate apomict frequently produces hybrid seeds on pollination with purelines or heterozygotes. Murty et al.[9] did not explain how an obligate apomict becomes facultative under cross-pollination. On the contrary, all embryological evidences confirming obligate apomixis were put forth without irrefutably disproving the demonstrated ability of R473 for producing hybrid seeds in good numbers[1,4,5]. Finally, the genetic basis of obligate and facultative apomixis in almost all known cases is different (see Box 1). A polygenic basis speculated for this facultative apomixis[11] is inconsistent on a consideration that it originated from an erstwhile obligate apomixis[9].

Presence of self-incompatibility becomes ambiguous

When the presence of self-incompatibility in R473 was categorically disproved by Marshall and Downes[8] and the nature of apomixis was shifted to facultative[9], it was necessary to re-confirm self-incompatibility and to define its role in view of the fact that facultative apomixis essentially involves certain frequency of sexual reproduction. The very ability of R473 to produce normal seed set under selfing even after a shift in the nature of its apomixis also disproved the presence of self-incompatibility (see Box 1). This brought to focus the unreliability of all embryological and other evidences put forth to substantiate this mechanism. In view of these serious contradictions, Murty et al.[9] neither re-confirmed the presence of self-incompatibility nor defined its role in facultative apomixis but maintained the status quo.

Box 2

Role of apomixis in crop improvement

Continuous recombination of genes through sexual reproduction is essential for crop improvement. However, introduction of apomixis in sexual species has limited advantage such as fixation of hybrid vigour (D F. Petrov, *Doklady Akad. Nauk SSSR*, 1957, **112**, 954; W. W. Hanna and E. C. Bashaw, *Crop Sci.*, 1987, **27**, 1136).

Apomixis introduced into occasionally or usually out-crossing crops can lead to its lateral spread and eventual decreased genetic variability in the gene pool to jeopardise the long-term improvement prospects of these crops. Transfer of apomictic genes into co-habitating weedy wild relatives may enhance their competitive ability which could pose a threat to the delicate equilibrium in the eco-agricultural system.

Fixation of hybrid vigour in sexually reproducing crops would be possible only when stable and simply inherited obligate apomixis is available in their primary gene pool. It is also important that this obligate apomixis is governed by apospory or *Antennaria* type diplospory and does not interfere with normal seed set. Other types of diplospory which allow genetic segregation are not suitable for fixing hybrid vigour. Facultative apomixis, even with high frequency of apomixis may at best be useful in this respect, with its inherent limitations, in crops where grain yield, grain quality and agronomic uniformity are not very critical. A facultative apomixis having a frequency not less than 80%, for instance, was used to retain hybrid vigour in Kentucky bluegrass used for turf (G. W. Pepin and C. R. Tunk, *Crop Sci.*, 1971, **11**, 445; 1974, **14**, 356).

The controversial apomixis reported in R473 is not suitable for fixing hybrid vigour in grain sorghum because of its facultative nature, high inconsistency and assumed polygenic inheritance. Despite these serious limitations, a major breeding programme was taken up to develop permanent sorghum hybrids, which were termed as 'vybrids', using this apomixis (U. R. Murty and N. G. P. Rao, Applicability of apomixis in grain sorghum breeding, Scheme for National Fellowship and Professorial chair, ICAR, 1978).

'Vybrid' breeding method (U. R. Murty, N. G. P. Rao, P. B. Kirti, and M. Bharathi, *Cereal Res. Commun.*, 1981, **9**, 239) involves mating between a sexual line (female parent) and a facultative apomictic line The hybrid is backcrossed with the same or another facultative apomictic line. A large ear-to-row $BC_1 F_2$ progeny is raised Plants or rows are selected on the basis of agronomic merit, cross-sterility and high apomictic frequency as determined by embryological methods. Selected plants are intermated in $BC_1 F_3$ Elite progenies from these intercrosses are selected and advanced to F_2 and F_3, from which plants resembling their F_1 (on the presumption that they are apomictic) are selected. These F_2 or F_3 selections having similarity are constituted as a 'vybrid' and propagated like a pureline It is important to note that this breeding method did not prescribe any threshold level of apomictic frequency for the male parent used in the initial cross or backcross, selecting plants for intermating and constituting the 'vybrid'.

It is not clear how the term 'vybrid' is appropriate to the product described above. This, in fact, is neither a hybrid nor a pureline Moreover, this breeding method is incapable of harnessing the hybrid vigour of the initial cross. And intermating between heterozygous, cross-sterile sister lines is not expected to result in any worthwhile heterosis. The cross-sterility and the embryological methods used for determination of apomictic frequency are not well founded in R473 (see text for details). And even if some elite selections are fortuitously found, their maintenance is difficult due to the facultative nature and inconsistent behaviour of this apomixis.

Notwithstanding these serious limitations in the 'vybrid' breeding, success in the development of several 'vybrids' which were claimed to have yielding ability comparable to the popular hybrid like CSH 9 and superior to varieties such as CSV 8R and SPV 232 were reported (U. R Murty and P. B. Kirti, *Cereal Res. Commun*, 1983, **11**, 229). On the basis of these claims, it was predicted that these 'vybrids' offered attractive additional advantage (over hybrids) under the rainfed conditions under which sorghum is cultivated in India and Africa (U. R. Murty, P. B. Kirti, M. Bharathi and N. G. P. Rao, *Z. Pflanzenzuchtg*, 1984, **92**, 30). These claims were found to be baseless when the best three of the 'vybrids' were subjected to a multi-location yield evaluation under the All India Coordinated Sorghum Improvement Project (AICSIP) The yield levels of 'vybrids' obtained from these trials were significantly far lower than those of varietal checks (AICSIP Annual Report, 1983–84). They were also adjudged as unfit for further evaluation. In addition, they lacked agronomic uniformity due to the segregation of residual heterozygosity. It appears, these results forced the 'vybrid' breedin termination.

While it was abundantly clear that there was no scientific reason to embark on this breeding, it appears this breeding was taken up and claims about immediate successes were made to provide another support for this controversial apomixis

It is noteworthy that the embryological study on the 'facultatively apomictic R473 plants' could neither detect any sexual embryosac nor any event to suggest occurrence of self-fertilization[9]. On the other hand, this study revised the endosperm development as pseudogamous (see Box 1). While it also described the entry of self-pollen tube into embryosacs, discharge of sperms, their fusion with polar nuclei or between themselves, it also maintained that the self-pollen did not reach many embryosacs as evidenced from the persistent synergids[12] (see Box 1). This implied existence of self-incompatibility at embryosac level and this embryological criterion was widely used for estimation of apomictic frequency in R473. Another significant point to be noted is the complete avoidance of any reference to self-incompatibility in all their subsequent studies on R473. Instead, the emphasis was shifted to a newly detected incompatibility mechanism called 'cross-sterility'[9,10].

New embryological evidences to support apomixis

All the earlier discussed embryological evidences on the nature and mechanism of apomixis and the frequency of ovules with multiple embryosacs were obtained from studies based on examination of more than 8000 ovules sampled from F_4 to F_8 progenies using serial section method and ovule squash method[1,4,5]. In view of this, it is surprising that a subsequent study[9] of 194 ovules sampled from later generation plants using the same ovule squash method led to new deviations in the embryological results. The new evidences included absence of ovules revealing either meiosis or meiotic products (tetrads), presence of different types of embryosacs with 1–5 nuclei, discharge of sperms from self-pollen in the embryosacs, their fusion with polar nuclei and fusion between two nuclei in the egg apparatus. On the basis of these new evidences involvement of diplospory and synkaryogenesis as additional apomictic mechanisms was put forth[13]. Apart from the inadequacy of these evidences to support the newly suggested apomictic mechanisms, the evidences and the claim on synkaryogenesis provide an excellent illustration on the convenient use of embryology for creating evidences for apomixis. This is discussed later.

Frequencies of ovules with multiple embryosacs reported before and after the change to facultative apomixis provide another instance of doubtful results. Ovules with multiple embryosacs do serve as an embryological proof for apomictic tendency, although its frequencies may not accurately reflect the apomictic frequency due to competition between sexual and apomictic embryosacs. In R473, when apomixis was presumed to be obligate, ovules with multiple embryosacs were either absent or its frequencies were less than 2.5%[1,4,5]. Interestingly, subsequent to the change to facultative apomixis, the frequencies of such ovules showed a sudden increase to 18% in one study[9] and to 30% in another[12]. These embryological results tend to indicate either an amazing ability of R473 plants in revealing qualitatively different results in different studies or an unusual skill behind the embryological studies for providing evidences suited to support apomixis, notwithstanding the contradictions and inconsistencies it may make with earlier ones.

Synkaryogenesis: An illustration on convenient use of embryology

Synkaryogenesis is a type of diplosporous apomixis[14-16]. Here the megagametocyte undergoes normal first and second meiotic divisions without cytokinesis, resulting in two pairs of nuclei at opposite poles of the cell (Figure 1). These nuclei may not be genetically identical due to heterozygosity common in apomictic plants and the recombination during meiosis. Nuclei of each pair then fuse. The resulting diploid nuclei undergo two mitotic divisions to produce 8-nucleate diploid embryosac (Figure 1). Due to meiosis and fusion of its products genetic segregation occurs under synkaryogenesis.

Reporting a nuclear fusion in R473 Murty et al.[9] stated that 'in some ovules 8-nucleated embryosacs were surrounded by one or more enlarged cells resembling embryosac. Presumably these cells originated in the nucellus and were capable of functioning as aposporous embryosacs. In two cases, the *nuclei of the unorganized egg apparatus in the extra sac appeared to be fusing*'. It is clear from this result that the fusion was in the egg apparatus and in the aposporous embryosacs. It was, however, not clear whether the egg was one of the nuclei involved in the fusion, whether the fusion occurred before differentiation of egg and synergids in the egg apparatus or after pollination, whether these aposporous embryosacs having fused nuclei had any selection advantage over sexual embryosacs, and what was the structure of these extra embryosacs before and after fusion. Another report from Murty et al.[17] on the occurrence of similar fusion between antipodal nuclei was also equally vague and with no data on its frequency.

This fusion was, however, described as synkaryogenesis and claimed that it resulted in the production of 'remarkably uniform' progenies identical to 'dihaploids'[13] (semantically double haploids). It was also claimed that the synkaryogenic potential of this apomixis could be exploited for the mass production of 'dihaploids' more easily and economically than their production from tissue culture[18,19].

Murty et al.[13] justified the occurrence of this synkaryogenic fusion with the support of a pre-

pollination fusion reported in *Rubus*[20]. This fusion was, in fact, called automixis and not synkaryogenesis and both differ in many respects. Automixis also occurs in embryosacs derived from megagametocytes. Here, after the first meiotic division, one of the dyads undergo three mitosis to produce 8-nucleate haploid embryosacs. Fusion of egg and one of the synergids (automixis) was speculated in these embryosacs on eventual detection of only two nuclei in the egg apparatus. Instant occurrence of complete homozygosity among progenies of heterozygous plants was attributed to this fusion[21].

The entire apomixis literature has only one more instance on automixis[22]. Normal degeneration of one of the synergids just before the arrival of pollen tube near the embryosac was later demonstrated in electron microscopic studies[23,24]. On the basis of this information on synergid, extreme rarity of instances reporting automixis and lack of conclusive embryological evidence on this fusion, the reports on automixis have recently been questioned and the homozygosity attributed to this was explained under regular sexual reproduction[25].

An examination of embryological events leading to synkaryogenesis[14], automixis[20,22] and the sketchy nuclear fusion reported[9] in R473 may categorically suggest that the latter cannot be called synkaryogenesis for any reason. In sorghum also one of the synergids degenerates before pollen tube entry. If at all the vague nuclear fusion claimed in R473 were true, it would be an instance of automixis. Automixis in aposporous embryosacs can by no means produce the so-called dihaploids. Development of such embryosacs into seeds, on the other hand, could have resulted in autotetraploid progenies. The fact that no autotetraploidy was detected among R473 progenies clearly proved the reported nuclear fusion[9,13] was either erroneous or of no consequence.

Progenies of R473 were expected to be more heterozygous because of the origin of this line from genetically divergent parents[1] and the reported shift from normal reproductive behaviour which could be traced to the F_2 if not F_1 of this cross. Contrary to this expectation, progenies of R473 eventually became highly uniform and homozygous under continuous selfing[9,13]. This strongly suggested regular operation of sexual reproduction rather than the claimed apomictic reproduction. It appears that Murty et al.[9,13] sought to accommodate this awkward contradiction by introducing this vague nuclear fusion and making a claim that this apomictic mechanism was responsible for the homozygosity in R473. In this process they mixed up automixis with synkaryogenesis and committed a grave error while structuring the vague embryological evidence. Their further efforts to legitimise this wrong claim by making more and equally vague claims

on the occurrence of similar fusion between antipodal nuclei, advantages of this mechanism for mass production of sorghum 'dihaploids', and recovery of 'cross-fertile' and 'cross-sterile' 'dihaploids' aptly illustrate the style and ethics followed in this research.

The cross-sterility–apomixis nexus

Change to facultative apomixis also involved a new link-up between apomixis and a newly described cross-incompatibility[9]. Apart from Rao and Narayana[1], Quinby[26] had also independently demonstrated the ability of R473 to produce hybrid seeds on pollination with different purelines. Therefore, evidences put forth to establish cross-sterility in R473 provide another interesting instance.

A 'cross-fertilization barrier' with purelines in R473 first reported by Murty et al.[9] was termed as 'cross-sterility' by Murty and Rao[10] with the support of embryological evidences. These evidences in many respects were similar to those provided for substantiating self-incompatibility in this line[4]. The need for fertilization under facultative apomixis arises only in sexual embryosacs. Hence a consideration of this claim along with the earlier discussed stand of Murty et al.[12] on the existence of self-incompatibility takes to a unique combination of two types of incompatibilities in R473, self-incompatibility in the apomictic ovules and cross-sterility in the sexual ovules.

The untenability of these claims and their mutual contradictions were sought to be reconciled by invoking a theory of mutual indispensability between apomixis and cross-sterility[10]. This theory was re-emphasized by Murty et al.[17] stating that 'no sorghum cultivar had ever induced seed set on emasculated spikelets of *genuine R473*'. This implies that only the cross-sterile R473 plants were 'genuine' and apomictic and those plants on which hybrids were produced by using other pureline pollen by Rao and Narayana[1] and by Quinby[26] were 'not genuine'. There are also contradictions between the genetical and cytological evidences put forth to explain the basis of this cross-sterility[27,28]. This intangible apomixis–cross sterility nexus encountered another paradox when they disengaged spontaneously and produced many cross-fertile apomictic plants[19]. A wholistic examination of this cross-sterility, the context of its detection and abrupt disappearance may tempt one to doubt whether this was introduced and insisted with a view to dodging a standard progeny test or to pre-empt adverse conclusions from such tests.

Independent progeny test rejects apomixis

Progeny test is a standard procedure adopted for detection and estimation of apomixis. Under this test,

suspected apomictic plants possessing a simply inherited recessive marker phenotype are crossed with a pollen parent which is homozygous dominant for the marker genotype. All progenies with recessive marker phenotype are considered to have been originated by apomictic reproduction. Care to exclude maternally inherited characters and accidental selfing in female plants are essential safegaurds for this test. Progeny test using male-sterility gene (ms/ms) is illustrated in Figure 2.

According to Murty et al.[9,10,29] progeny test was not possible in R473 as it has no simply inherited recessive trait and was not crossable with homozygotes. In view of this, they justified the use of embryological methods alone for the detection and estimation of this apomixis. The untenability of three embryological criteria, the number of ovules showing multiple embryosacs, the synkaryogenic fusion and failure of pollen tube to enter apomictic embryosacs used for the estimation of this

apomixis were discussed earlier. It must be mentioned here that the two reasons put forth to avoid progeny test on R473 were not correct.

Contrary to their contentions on progeny test on R473, Murty et al.[11,12,19,29] reported different frequencies of apomixis among these plants from different progeny tests. Their first test on four plants reported 100, 50, 37 and 0% apomixis in them[11]. This extreme variation in apomictic frequency among R473 plants and its ready detection from the four plants used by this test appeared as a paradox in view of the fact that obligate apomixis was consistently confirmed in this line during many generations, that this apomixis was reported to be governed by more stable diplospory and apospory and that this line was continuously maintained through selfing. A close examination of this test revealed that it violated the basic rules of standard progeny test and its conclusions were inconsequential.
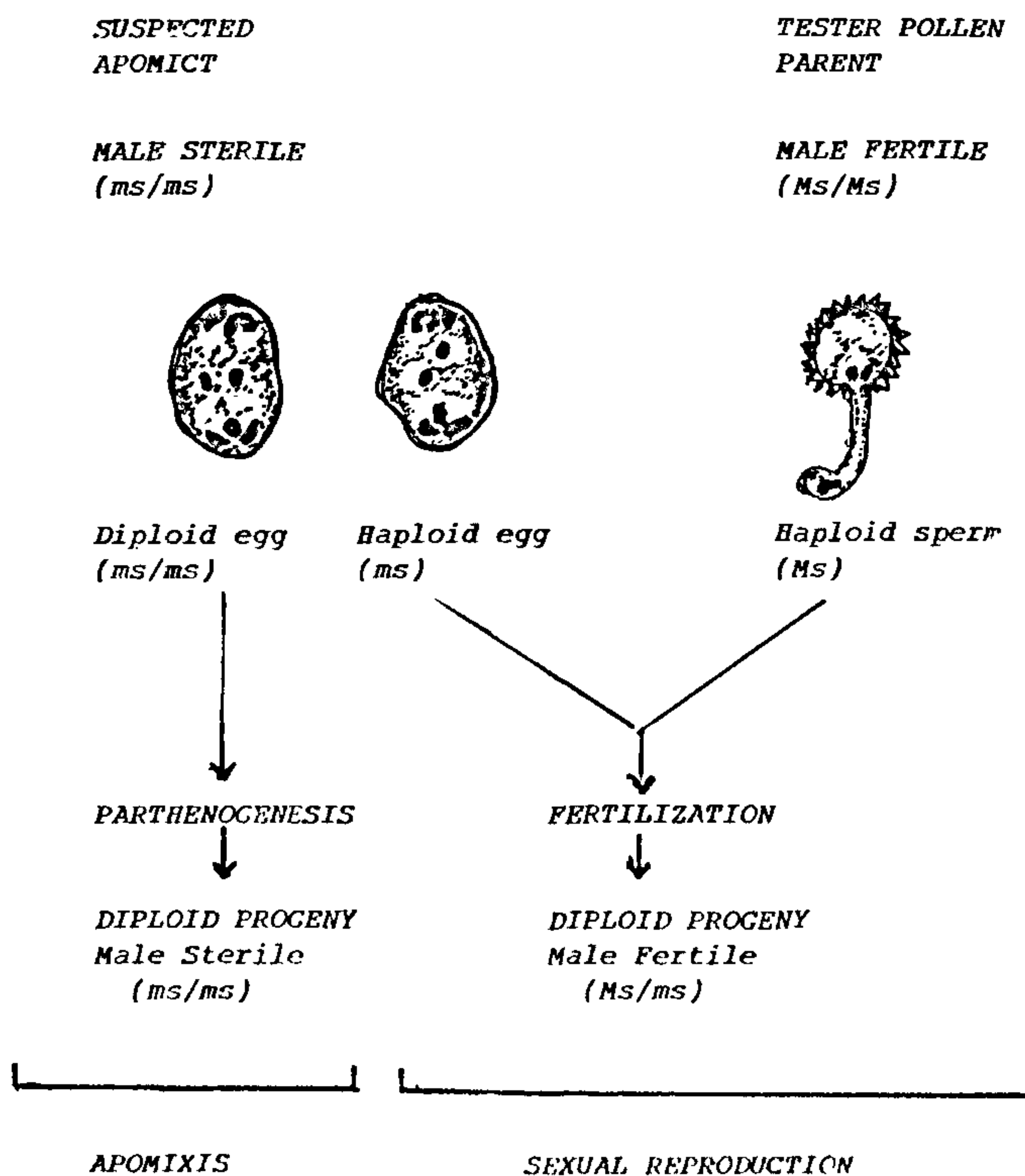

SUSPECTED
APOMICT

TESTER POLLEN
PARENT

MALE STERILE
(ms/ms)

MALE FERTILE
(Ms/Ms)

Diploid egg
(ms/ms)

Haploid egg
(ms)

Haploid sperm
(Ms)

PARTHENOGENESIS

FERTILIZATION

DIPLOID PROGENY
Male Sterile
(ms/ms)

DIPLOID PROGENY
Male Fertile
(Ms/ms)

APOMIXIS

SEXUAL REPRODUCTION

Figure 2. Progeny test using male sterile gene (ms/ms) as marker character

The marker phenotypes chosen for this test were complex quantitative characters, there was no information on the precise genetic differences between R473 plants and the pollen parents for these characters and the pollen parents were either heterozygous or of unknown genetic make-up for the marker traits. There was also no information on the number of progenies examined by this test. From the context of this study, it appears as though these results were published to contest the findings of Marshall and Downes[8].

Two other progeny tests reported by Murty et al.[12,19] used F_2 or BC_1F_1 derivatives of R473 possessing simply inherited recessive marker traits such as tan plant colour or shrunken endosperm associated with high lysine[30]. Tan plant colour was introduced from a facultative apomictic line reported by Tang et al.[31] and the shrunken endosperm trait from a sexual line[29]. These tests, in general, also indicated variation ranging from 0 to 100% in apomixis frequencies among the test plants. These results, more specifically, were used to prove transferability of the apomixis claimed in R473. Absence of full information on the female plants used in these progeny tests limited the number of progenies used by one of these tests[12], increased chances of accidental self-pollination while conducting progeny test on cross-sterile plants and other test methods and interpretations followed to provide a bias towards favourable results render the conclusions drawn from these progeny tests questionable. It is also significant that none of these studies, embryological or progeny tests, could systematically and unambiguously establish whether those R473 plants which were reported to have high apomixis (80–100%) were more effective in producing offsprings with similar apomictic frequencies than those which had low or no apomixis. This ambivalence on the consistency of this apomixis, a polygenic basis arbitrarily attributed to it and the instability usually associated with the facultative apomixis are conveniently used to counter negative conclusions from independent studies and to justify any apomictic frequency or even its very absence.

The first progeny test on R473 was in fact conducted by an independent group from A&M University, Texas[32]. An elegant methodology used by this test and the comprehensiveness of its results render the conclusions drawn by this test most decisive and emphatic. In view of the cross-sterility claimed in R473, Reddy et al.[32] introduced a simply inherited recessive trait, genic male sterility, in R473 through sodium azide mutagenesis. Nine plants homozygous for male sterility were crossed with normal (male fertile) R473 to obtain 128 F_1 plants and 2665 F_2 plants. A perfect monogenic segregation of male fertiles and male steriles (3:1) in F_2 and absence of any male sterile plants among the 128 F_1 plants categorically excluded any trace of apomixis

in the female plants used by this test. The normal F_2 segregation also confirmed that the R473 plants used as the pollen parent also could not transfer any apomictic ability into the F_1 s.

Conclusion

From the foregoing it is clear that apomictic reproduction in the sorghum line R473 could not be established beyond doubt. So also are the claims on self-incompatibility and cross-sterility. All emphatic evidences put forth to establish these rare reproductive deviations became nugatory on contradiction of these claims by other independent studies. A continuously shifting stand taken on the nature and frequency of this apomixis and accompanying abnormalities during a two decade-long research renders this whole exercise infructuous from academic and applied points of view. There was no scientific basis for the attempted vybrid breeding. This is conclusively proved by the evanescence of the vybrids, its hotch-potch breeding and dismal performance. While the initial claims on R473 could possibly be considered as a hasty conclusion from incomplete results, the evidences subsequently put forth to confirm apomixis and self-incompatibility and to claim cross-sterility were of doubtful nature. The structured origin of some of them was unravelled by this review. It appears such evidences were continuously and systematically provided to defend and maintain a claim proved baseless by different independent studies. Later efforts to transfer apomixis to R473 from other facultative apomicts are very significant in this context.

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The response of U. R. Murty regarding R473 is published below.

Appraisal on the present status of research on apomixis in sorghum

Apomixis literature is 150 years old[1] and is flooded with scores of reviews on the classification, mechanisms, terminology and diagrams. Several misconceptions on apomixis exist like the assumption of the existence of obligate apomixis (obligate apomixis does not occur in any plant, even in the so-called obligate apomicts, some amount of sexuality always exists) and the assumption of existence of perfect techniques on detection and estimation of apomixis (classical embryological methods), clearing techniques[2], squashes[3] and progeny tests with qualitative[4], quantitative[5], isozyme[6] RFLP and RAPD markers[7]. As of today there is no single perfect method. Drastic genotype environment interactions[8,9] are often ignored and materials are compared in as wide an array of environments as phytotron, greenhouse and field. Apomixis is not a simple genetic phenomenon. It is a complex series of events starting from unreduced or restituted nuclei till the formation of a viable embryo and endosperm.

During the course of studying several segregating lines of sorghum under selfing, plants with fresh persistent stigmas on developing seeds were observed. Further investigations on such progenies led to the report on apomixis in R473[10]. The phenomenon, thought to be obligate at that time, was concluded from the following observations: (a) the presence of persistent and fresh stigmas on developing seeds, (b) lack of evidence on pollen germination and pollen tube growth under self-pollination in spite of the presence of fertile pollen, (c) lack of seed set on emasculation followed by pollination of self or cross pollen from a few other varieties; (occasional and small amount of seed set does take place) and, (d) evidence from embryological investigations.

Subsequently in 1970, a report on apomixis in sorghum in *Science*[11] generated further interest.

Since the first report on apomixis in sorghum, attempts were made to better understand the mechanism of apomixis to enable its utilization in crop improvement. The investigations have gradually clarified the reproductive events in R473 line of sorghum and its derivatives. Correction of the initial conclusion of obligate apomixis as facultative[12], and of self-incompatibility as cross sterility[13], sporadic occurrence of fusion of nuclei in the embryosac (the terms used for such fusions may vary from author to author as is common in apomixis literature[14]), an exploratory concept of using facultative apomicts as vybrids[15], development of genetic stocks carrying simply inherited recessive markers[16], a review of sorghum apomixis up to 1982 clarifying the tardiness and periodic clarifications of earlier reports[17], a cytological finding that explained the reason for non-true breeding nature of R473 for apomixis[18] and a review on the present status of apomixis research in sorghum[19], are some of the products of this research. The review[17] of 1982 is exhaustive and had answers/clarifications to the points raised by Bala Ravi.