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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 old¹ 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 techniques², squashes³ and progeny tests with qualitative⁴, quantitative⁵, isozyme⁶ RFLP and RAPD markers⁷. 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 R473¹⁰. 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 facultative¹², and of self-incompatibility as cross sterility¹³, sporadic occurrence of fusion of nuclei in the embryo sac (the terms used for such fusions may vary from author to author as is common in apomixis literature¹⁴), an exploratory concept of using facultative apomicts as hybrids¹⁵, development of genetic stocks carrying simply inherited recessive markers¹⁶, a review of sorghum apomixis up to 1982 clarifying the tardiness and periodic clarifications of earlier reports¹⁷, a cytological finding that explained the reason for non-true breeding nature of R473 for apomixis¹⁸ and a review on the present status of apomixis research in sorghum¹⁹, are some of the products of this research. The review¹⁷ of 1982 is exhaustive and had answers/clarifications to the points raised by Bala Ravi.

Based on these studies, the present knowledge on apomixis in derivatives of R473 can be summarized as follows: (a) It is a facultative apomict with an apomictic frequency varying from 0 to 80% in different seasons, different locations, different progenies and even different plants of the same progeny. (b) The mechanisms of apomixis comprise apospory, diplospory and a few others. (c) It is subjected to $G \times E$ interactions. (d) The original line is heterozygous for a chromosomal deficiency or duplication and hence does not breed true as far as reproductive behaviour is concerned.

The details are given in a recent paper¹⁹. The author of the present review has conveniently ignored this paper although he had seen it and had participated in the departmental seminar at NRCS. The figures (1 and 2) presented by the author are too textual, fundamental and fragmentary.

There is growing interest in apomixis for its possible use in several cereal grain crops like rice, wheat, maize, sorghum and pearl millet. All the studies are at the exploratory stage. The complexities inherent in the understanding of the phenomenon and its transmissibility are brought out in the studies on maize²⁰⁻²²

The apomictic materials reported have been made available on requisition to research workers both in the country and abroad.

The article on apomixis under reference is based on the analysis of the author who has been a part of the same organization. It is not based on any research effort or a reinvestigation. The statement in the abstract that the research is to uphold an erroneous initial claim is a negation of the results presented in the review itself and is not warranted. The purpose of this note is to place before the readers, our present understanding of apomixis in sorghum and not to enter into any dialogue.

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Addendum

Clarifications on the issues raised by Bala Ravi

The readers of the review may profitably refer to a 11-year-old paper of the authors (Murty, U. R., Rao, N. G. P., Kirti, P. B. and Bharathi, M., in *Sorghum in the Eighties*, ICRISAT, Hyderabad, 1982, 361-372) where all clarifications were given. Further clarifications were given in a similar article (Murty, U. R., *Apomixis Newsl.*, 1992, 5, 50-60); still the following specific points were given below for ready reference.

Embryological aspects

Production of hybrids when pollinated by an alien line BP 53 in the presence of self pollen only establishes the existence of some sexual potential and does not necessarily rule out the possibility of obligate apomixis in a functional sense, i.e. production of all the seed from only aposporous embryosacs. Absence of ovules with more than one embryosac does not contradict existence of obligate apomixis since it was shown that the single embryosac in most of the ovules was produced by somatic apospory.

Emasculated spikelets of R473 do not set seed to any appreciable extent even with pollen from the same earhead or a sister line. This is because once the spikelet is emasculated, the absence of pollen/anthers in the same spikelet in some unknown manner hinders the capacity of the embryosac to develop further with self- or cross-pollen.

In subsequent studies, as the author himself has pointed out, the presence of sexual potential and consequently operation of facultative apomixis has been

established with greenhouse-grown plants under a different set of environmental conditions (College Station, Texas, USA) from seed derived R473 plants after a few more generations. It should be noted that during each generation changes could have taken place in the reproductive system of the plant. It would not be wise to assume a static reproductive behaviour in a biological entity. The versatility of reproductive behaviour characteristic of facultatively apomictic flowering plants depends upon the side-by-side persistence of two alternative pathways, one the normal sexual and the other the apomictic. The factors determining the success of one system rather than the other in any particular ovule remain unknown. The drastic effects of environment on the frequency of apomixis are well known (Knox and Heslop Harrison; *Botan. Noti.*, 1963, 116, 127-141; Burton, G. W., *Crop Sci.*, 1982, 22, 109-111).

Self-incompatibility

In the early studies, pollen germination could not be observed when pollinated stigmas were squashed and examined with cotton blue under the light microscope. Subsequently, pollen tubes could be observed in some ovules of greenhouse-grown plants studied in the USA (Murty, U. R., Schertz, K. F. and Bashaw, E. C., *Indian J. Genet.*, 1979, 39, 271-278). The phenomenon was later thoroughly analysed and termed as cross sterility (Rao, N. G. P. and Murty, U. R., *Indian J. Genet.*, 1980, 40, 562-567) and its mechanism was very clearly established. The earlier conception of self-incompatibility and the role of cytoplasm has been modified in the light of more detailed findings. Criticising the earlier observations ignoring the later reports only proves the motives of the author of the present 'review'. Cross sterility in sorghum is a real phenomenon. It tends to be 100% during kharif and gets broken down during rabi. Such instability in reproductive events (apomixis, cross sterility and male sterility) due to genotype environment interactions has been clearly established in sorghum (Murty, U. R., Rao, N. G. P., Kirti, P. B. and Bharathi, M., in *Sorghum in the Eighties*, ICRISAT, Hyderabad, 1982, pp. 361-372; Murty, *Curr. Sci.*, 1992, 63, 142-144) and in rice by Chinese and Japanese workers. In fact, one such environmentally sensitive male sterility is economically used by the latter in commercial hybrid production (Yuan, personal communication, 1992).

Marshall and Downes (1977) paper

Concluding reproductive behaviour of selected progeny plants under environmental conditions entirely different

from field grown plants of R473 after several generations in no way can be used to disprove the original findings. It was very clearly demonstrated that R473 is heterozygous for a deficiency or duplication (Kirti, P. B., Murty, U. R. and Rao, N. G. P., *Genetica*, 1982, 59, 229-232). It was also indicated from this that 50% progeny plants of R473 are identical to the parent and this can be achieved by selecting from cross sterile progeny plants only. Hence the study of Marshall and Downes in no way can conclusively demonstrate the non-operation of facultative apomixis in R473. Marshall and Downes themselves did not 'rule out the operation of a low frequency of apomixis in R473'.

The different types of apomixis in R473

Over the years, the investigations on R473 brought to light different mechanisms in the production of diploid/aposporous embryos. Existence of automixis or synkaryogenesis could also be detected as a rare event and from this only the possibilities were suggested. The terminology of apomixis is always controversial and no uniform terminologies exist for such rare events as semigamy, automixis, synkaryogenesis, etc. Therefore stressing this point by the author in great detail with emphasis on mechanism and terminology, is not warranted. This is a rare event and was cited as a possible mechanism for the occurrence of uniform progenies from heterozygous plants in early generation (see page 366, column 2, item 3 in Murty, U. R., Rao, N. G. P., Kirti, P. B. and Bharathi, M., in *Sorghum in the Eighties*, ICRISAT, Hyderabad, 1982, 361-372. 'These terms are used *sensu lato*').

Progeny tests

There are no 'standard' or nonstandard progeny tests in the apomixis literature. Progeny tests basically rely on the identification of uniformity of progeny from heterozygous plants to detect and estimate the frequency of apomixis using either qualitative or quantitative traits (Young, B. A., Sherwood, R. T., Bashaw, E. C., *Can. J. Bot.*, 1979, 57, 1668-1672; Reddy, P. S. and D'Cruz, R., *Stain Technol.*, 1967, 42, 237-240; Burton, G. W. and Forbes, I., Proc. VIII Grassland Congr. Reading (England), 1960, 66-71; Marshall, D. R. and Brown, A. H. D., *Heridity*, 1974, 32, 321-333; Kojima, A., Nagato, Y. and Hinata, K., *Jap. J. Breeding*, 1991, 41, 73-84; Carman, J. G., *Apomixis Newsl.*, 1992, 5, 47-50). Attempts were repeatedly made in sorghum to make such tests with the available stocks and development of genetic stocks carrying simply inherited qualitative markers. In fact with the availability of shrivelled endosperm, tan plant type and bloomlessness in

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facultative apomicts, attempts are being made to detect and isolate stable apomicts in sorghum.

The progeny test made by Reddy *et al.* (Reddy, C. S., Schertz, K. F. and Bashaw, E. C., *Euphytica*, 1980, 29, 223-226) relied on converting R473 into genetic male steriles. It is not clear how the study of progeny of male sterile mutants with normal R473 (as male) can give insight into the reproductive behaviour of R473. Full explanation of these findings has already been given in Murty *et al.*, 1982 (Murty, U. R., Rao, N. G. P., Kirti, P. B. and Bharathi, M., in *Sorghum in the Eighties*, ICRISAT, Hyderabad, 1982, 361-372).

Vybrids

The concept of vybrids (facultative apomicts) was only of an exploratory nature with the objective of using facultative apomixis for partial fixation of heterosis. It was never the objective of this work to develop vybrids to compete with commercial hybrids of sorghum.

Relevant recent work in cereal grain crops

There has been growing interest globally in the exploitation of apomixis in heterosis breeding of rice, maize, wheat, sorghum, pearl millet, etc. During 1992 itself three International Workshops on Apomixis were conducted, IWAR (International Workshop on Apomixis in Rice in China), ABCI (Apomixis Biology and Crop Improvement, at Atlanta, Georgia, USA) and APONET

(First International Workshop on Potential use for Apomixis in Tropical Plant Breeding at Montpellier, France) which reflect the current interest. Our work on apomixis in sorghum was presented and discussed at the First APONET Workshop in France.

We realize that our own embryological and cytogenetical studies, reported in well-refereed journals periodically as they were observed, did alter the initial finding. In this context, it may not be out of place to conjecture that such alterations have been inherent in analyses aimed at enhanced understanding of this complex phenomenon.

As examples, Petrov and his colleagues introduced apomixis in maize which was reported to have been lost over generations. Savidan writes: 'Petrov's material is a great contribution to apomixis research. The fact that they lost apomixis in later generations is no dishonour. Petrov and his colleagues worked with the tools they had and based their information on the information available to them at that time' (Savidan, Y., *Apomixis Newsl.*, 1991, 3, 26-27). Similarly the existence of apomixis and its exploitation in rice has been widely reported in China but confirmatory evidence has been lacking (Chen, J. S., *Apomixis Newsl.*, 1991, 3, 8).

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RESEARCH COMMUNICATIONS

Influence of α -tocopherol on the antitumour potential and the toxicity of doxorubicin

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α -tocopherol reduces the lipid-peroxidation induced in doxorubicin therapy. It is aimed at here in this paper to furnish evidence for the protecting effect of α -tocopherol over doxorubicin-induced toxicity. Such protection is of value only if the antitumour potential of doxorubicin is not significantly reduced by α -tocopherol.

DOXORUBICIN is an antitumour antibiotic clinically active against a wide spectrum of experimental and human solid tumours¹. The therapeutic usefulness of doxorubicin is seriously limited by its cardiotoxic and

other side effects². The chronic effects include insidious onset of cardiomyopathy which often leads to congestive heart failure³.

Doxorubicin is known to generate superoxide radicals either enzymatically or non-enzymatically and to stimulate the formation of lipid peroxides⁴. Since lipid peroxidation induced by doxorubicin has been considered as the proposed mechanism for the deleterious side effects, various antioxidants have been tried along with doxorubicin⁵. α -tocopherol has been shown to reduce the lipid-peroxidation induced in doxorubicin therapy⁶.

Serum, heart and liver LDH levels and its isoenzymes are of clinical interest because they can be used as molecular markers of tissue damage. Since liver is the major organ involved in drug metabolism and heart the target organ for this drug, the LDH total activity and its isoenzymes are assessed in liver, heart and also in serum. The present study is aimed at furnishing