MECHANISM OF CROSS STERILITY IN SORGHUM

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The inability of certain varieties of maize to set seed under cross-pollination was designated as cross sterility (Demeric, 1929). In maize this cross sterility was governed by the gametophytic factor '*ga*'. In *Sorghum bicolor* (L.) Moench, a grain sorghum line, R473 was reported to be apomictic and unable to set seed under cross pollination (Rao and Narayana, 1968; Rao & Murty, 1972; Murty & Rao, 1972). The behaviour of this line in so far as it does not set seed with other lines is more or less analogous to that of corn. In corn, the cross sterile lines set seed with at least some other lines but in sorghum, no other line has so far been found to set seed normally with R 473.

In the first studies on apomixis by Rao and his colleagues, it was thought that neither self-nor cross pollen germinates and/or penetrates the stigmas and styles of R473. However, (Murty *et al.*, 1979) observed that under selfing pollen grains do germinate and enter the embryo sacs, although it is not clear whether they effected fertilization Under crossing, there is no information available on the post-pollination events.

The phenomenon of cross sterility has hampered progress in studies of apomixis in sorghum. Simple progeny tests on R473 could not be made because of the existence of this interfering cross sterility. In addition, the evidences supporting apomixis were looked upon with some amount of skepticism and have prompted some very inconclusive studies (Marshall and Downes, 1977). An understanding of the mechanism of cross-sterility should be helpful in future investigations of apomixis in sorghum. The present study was undertaken to find out the post pollination reproductive events under cross pollination in R473.

Materials and Methods

The following lines of sorghum were used in the present study; 1. R-473 (Bulk); 2. R-473-6-1; 3. R-473-1-2; 4. R-473-1-2-7; 5. R-473-1-2-9; 6. Kafir A, and 7. Kafir B. Spikelets were emasculated one day prior to anthesis and were sibbed or crossed at the time of anthesis. The following materials were employed in making a cytological study of the post pollination reproductive events:

1. R-473 (Bulk) — Cross pollinated spikelets, at 2-hour intervals upto 24 hours and then 1, 2, 3 and 4 days after cross-pollination and 2 hours after sibbing.
2. R-473-6-1 —2 hours after sibbing
3. R-473-1-2 —2 hours after sibbing
4. R-473-1-2-7 —2 hours after cross pollination
5. R-473-1-2-9 —2 hours after sibbing
6. Kafir A —2 hours after crossing by Kafir B.

Seeds of R-473-1-2 and R-473-6-1 were generously supplied by J. R. Quinby. In studying the reproductive events, whole embryo sacs were examined in acetocarmine squashes. Details of the technique are given in Murty *et al.* (1979).

Results

In all the materials examined after sibbing, pollen tubes were found to have entered the embryo sac. This entry of the pollen tube was facilitated by the degeneration of one of the synergids. In all the embryo sacs, the entry of the pollen tube was always through this degenerating synergid. The pollen tube lost its identity after its entry into the synergid. Under the light microscope, it appeared that the degenerating synergid has ruptured towards the inside and discharged its contents into the embryo sac cytoplasm (Fig. A). Male nuclei were seen with difficulty as granular dots. In the sexual material, one

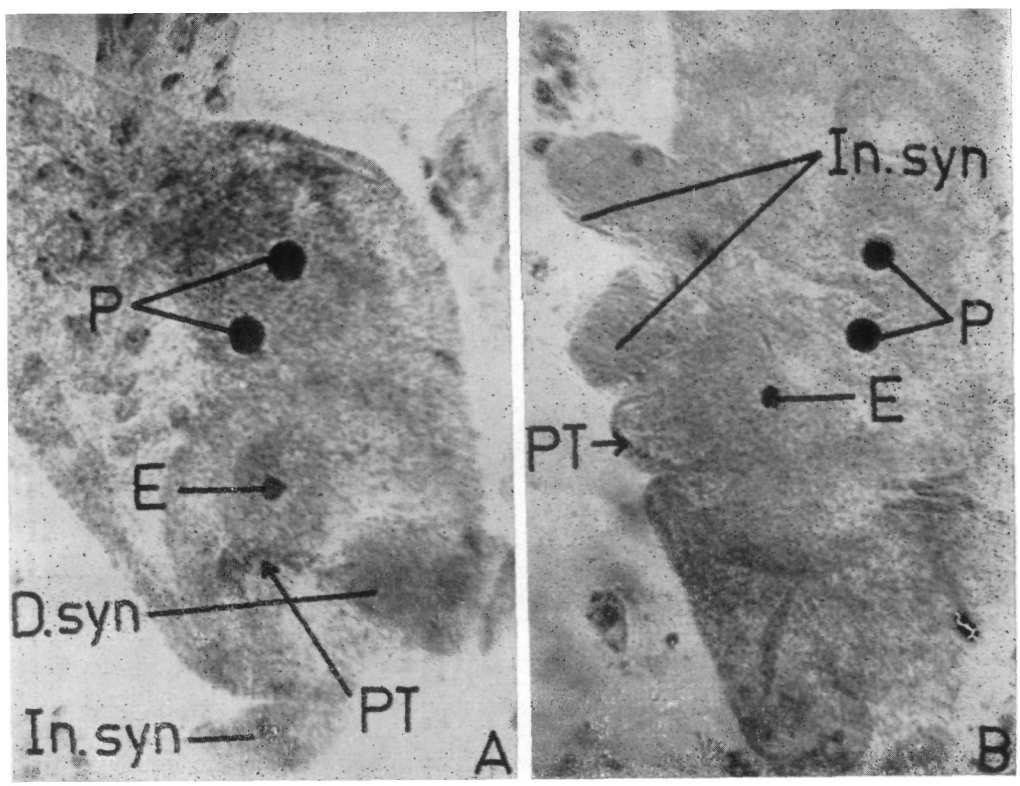

Fig. A. Embryo sac in Kafir B, 2 hrs after self pollination. The pollen tube has entered the sac through the degenerating synergid. ×700.

Fig. B Embryo sac in R-473, 4 hrs after cross pollination, pollen tube penetrating the sac by growing around the egg ×700
(P, polar nuclei; E, egg, D.syn, degenerated synergid, In syn, intact synergid; PT, pollen tube).

male nucleus was observed near the egg and the other near the polar nuclei. Also, in the sexual material, entry of the pollen tube and degeneration of the

synergid were 100%. In the R-473 lines, however, the results were different. Pollen tubes could be seen only in some of the ovules. In addition, these lines exhibited a varying frequency of multiple embryo sacs. The frequency of multiple sacs and of ovules without pollen tubes were more in R-473-1-2 than in R-473-6-1 (Table 1).

TABLE 1

Results of the embryo sac squash study on cross sterile sorghum after sibbing

Line	Ovules with single sac	Ovules with more than one sac	Ovules with pollen tubes	Ovules without pollen tubes	Total
1. R-473-1-2	15 (71.66)*	6 (28.56)	16 (76.19)	5 (23.81)	21
2. R-473-6-1	20 (92.31)	1 (7.69)	20 (80.77)	1 (19.23)	21
3. R-473 (bulk)	29 (87.88)	4 (12.12)	14 (42.42)	19 (57.58)	33
4. R-473-1-2-9	14 (51.85)	13 (48.15)	6 (22.22)	21 (77.78)	27

*Percentages given in parentheses.

Cross pollination: Under cross pollination there appeared to be an inhibition of pollen tube growth, because pollen tubes did not reach the embryo sac as under sibbing. In R-473 (bulk), out of 16 ovules examined at 2 hours, there were no pollen tubes. After 4 hours, pollen tubes were seen in 4 out of 28 ovules examined. In R-473-1-2-7 at 2 hours out of 34 ovules squashed, pollen tubes could be seen only in 2 ovules. The rest were devoid of pollen tubes. In contrast to self-pollination there was no degeneration of the synergids. In the few ovules where a pollen tube was seen, it was observed that the pollen tube has tried to penetrate the embryo sac wherever it could by growing all around the sac (Fig. B). The pollen tube was never seen to enter the embryo sac through the synergid. In a few cases, where it has entered the sac near the synergids, it has penetrated across or in between the cells of the egg apparatus.

In a few cases, development of endosperm could be seen. In such cases, a male nucleus was seen to persist near the egg for as long as 20 hours. An extremely rare development of the embryo is seen 2-3 days after pollination. In 250 ovules examined, 2 days after cross-pollination, 8·37% had both embryo and endosperm and 13·15% had only endosperm. Development did not proceed any further from the 3rd day onwards. Degeneration of the endosperm started at this time. Degeneration of the embryo preceded that of the

endosperm and degeneration was almost complete by the 4th day. The effect of self and cross-pollination on embryo and endosperm formation in R-473 bulk are given in Table 2.

TABLE 2

Embryo and endosperm formation following self and cross pollination in R-473 (bulk)

Stage	Embryo and Endosperm	Endosperm only	Neither
At anthesis	—	—	100.00
1 day after self pollination	93.02	—	6.98
2 days after self pollination	100.00	—	—
1 day after cross-pollination	12.16	36.48	51.35
2 days after cross-pollination	8.37	13.15	78.49

DISCUSSION

The non-functioning of self-pollen in fertilization and its functional normalcy on alien stigmas had led (Rao and Narayana, 1968) to the conclusion that R-473 is self-incompatible. However, in self-incompatible organisms, cross-pollination alone results in seed set while in R-473, the situation is reverse. Seed set is obtained only under selfing although fertilization may be absent. The phenomenon in R-473 is, therefore, better designated as cross sterility.

It is apparent from the present study that the cytological mechanism of cross sterility in *Sorghum* has 3 aspects : 1. an inhibition of the pollen tube growth somewhat similar to that in corn (House and Nelson, 1957) 2. a mechanical obstruction for the entry of the pollen tube brought about by the nondegeneration of one of the synergids under cross pollination and 3. as yet unknown physiological processes. The existence of some physiological factors is suspected since in spite of the first two inhibitions, a few eggs get fertilized but then, no seed is formed and embryo and endosperm degenerate.

The study of the mechanism of cross sterility in R-473 is of significance in the general field of pollination and fertilization in plants. Although several people agree that there exists an intensive metabolic interaction between the growing pollen tube and the conductive tissue of the style, the precise sequence of events leading to fertilisation is not clear. Cass and Jensen (1970) concluded that after pollination some stimulation precedes the pollen tube tip travelling at a much faster rate than the pollen tubes and conveys a message to one of the synergids, that responds by beginning to degenerate.

The differential effect of self-and cross-pollination in cross sterile sorghum has brought about this relation very clearly. Degeneration of one of the synergids after pollination but before fertilization was recorded in sorghum (Vazaart, 1955; Murty *et al*, 1979) and several other plants (Cass & Jensen 1970; Mogensen, 1978). Cross-pollination did not bring about such a degeneration with the result that pollen is unable to enter the embryo sac. Although there is an inhibition of the pollen tubes under crossing, in a few cases, they have tried to reach and penetrate the embryo sac. This observation shows that the factors controlling the growth of the pollen tube are probably independent of those controlling the events leading to fertilisation in cross sterile sorghum. This differential effect of self and cross pollen seems to be the first of its kind so far reported in plants although other differential effects in protein metabolism were noted earlier, presumably, in *Petunia hybrida* (Deuremberg, 1976). A difference in protein metabolism, detected after self-and cross-pollination, indicated that a signal has to be sent from the stigma or style towards the ovary which induces the changes in metabolic activity. Obviously, the signal must be different for cross and self pollination. In R473, probably the response to receive the signal is lost through mutation.

The evolution of cross sterility in sorghum is of significance to plant breeders interested in exploiting apomixis for fixing heterozygosity. If cross sterility has originated before apomixis, the apomictic line R473 may no longer be apomictic in the absence of cross sterility.

Summary

Growth of the pollen tube and its entry into the embryo sac were examined in normal and cross sterile *Sorghum bicolor* (L.) Moench. In normal sorghum, pollen tubes reached the embryo sacs by 2 hrs after pollination. One of the synergids of the embryo sac degenerated before the entry of the pollen tube but after pollination. Entry of the pollen tube into the embryo sac was always through the degenerating synergid. In cross sterile sorghum, growth of the pollen tube was nearly normal following self-pollination. However, pollen tube growth was arrested when foreign pollen was used. Very few pollen tubes reached the embryo sac 4 hrs after cross pollination. The synergids were normal and the pollen tubes could not enter the embryo sacs. In a few cases where they entered the sac, it was not through the synergid. Pollen tubes travelled all over the sac and some of them could enter the sac through the embryo sac wall. Cross sterility in sorghum was brought about by an arresting of the pollen tubes and a mechanical inhibition to fertilization manifested through failure of degeneration of one of the synergids.

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REFERENCES

Cass, D. D. and W. A. Jensen. (1970). Fertilization in barley. *Amer. J. Bot.* **57:** 62–76.
Demeric, M. (1929). Cross sterility in maize. *Zeits. Abst. u. Vereb.* **50:** 281–91.
Deurenberg, J. J. M. (1976). *In vitro* protein synthesis with polysomes from unpollinated, cross and self pollinated *Petunia hybrida*. *Planta* **128:** 29–33.
House, L. R. and O. E. Nelson, Jr. (1958). Tracer study of pollen tube growth in cross sterile maize. *J. Hered.* **49:** 18–21.
Marshall, D. R. and R. W. Downes. (1977). Test for obligate apomixis in grain sorghum R473. *Euphytica*, **26:** 661–65.
Mogensen, H. L. (1978). Pollen tube synergid interactions in *Proboscidea Lousianica* (Martiniaceae). *Amer. J. Bot.* **65:** 953–64.
Murty, U. R. and N. G. P. Rao. (1972). Apomixis in breeding grain sorghums. 'Sorghum in Seventies'. Ed. N.G.P. Rao and L. R. House. Oxford and IBH. New Delhi, 517–23.
Murty, U. R., K. F. Schertz, and E. C. Bashaw. (1979). Apomictic and sexual reproduction in *Sorghum bicolor* (L.) Moench. *Indian J. Genet.* **39:** (in press).
Nelson, O. E. (1952). Non-reciprocal cross-sterility in maize. *Genetics.* **37:** 101–24.
Powers, L. (1945). Fertilization without reduction in guayale (*Parthenium argentatum* Gray) and a hypothesis as to the evolution of apomixis and polyploidy. *Genetics.* **30:** 323–46.
Rao, N. G. P. and U. R. Murty. (1972). Further studies on obligate apomixis in grain sorghum. *Indian J. Genet.* **32:** 383–97.
Rao, N. G. P. and L. L. Narayana. (1968). Apomixis in grain sorghum. *Indian J. Genet.* **28:** 121–27.
Vazart, B. (1955). Contribution a L'etude caryologique des elements reproducteurs et de la fecondation chez les vegetaux angiosperms. *Rev. Cytol. Biol. Veget.* **16:** 209–390.