# Use of 2n Pollen in Generating Interspecific Derivatives of Groundnut

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Numerically unreduced gamete called 2n pollen is a product of meiosis that bears sporophytic rather than the gametophytic chromosome number. Abnormalities in the division during meiosis or during spore wall formation result in 2n pollen. Often, such pollen are fertile (Christopher 1971).

The presence of dyads and triads at the microspore tetrad stage indicates the presence of 2n gametes. One of the main reasons for 2n pollen formation is meiotic nuclear restitution, which was first proposed by Rosenberg (1927). It is defined as the formation of a single nucleus with unreduced chromosome number, and the failure of the first or the second meiotic division. In the first division restitution, abnormal meiosis takes place with the formation of many univalents, and according to Wagennar (1968), it is a cellular mechanism for terminating the prolonged first division. Nevertheless the resultant restitution forms unreduced pollen. Restitution following the second meiotic division in pollen formation also yields 2n pollen. In some plant species there can be double restitution, although rarely, resulting in the formation of giant pollen. Triad formation occurs as a result of second division restitution (Sosa and Hernandez de Sosa 1971). Here, one group of chromosomes resulting from first meiotic division undergoes normal second meiotic division whereas in the other group, there is restitution nucleus.

During the development of interspecific hybrids in groundnut (*Arachis hypogaea*), cytological-tetrad analysis of $F_1$ hybrids revealed the presence of dyads, triads and tetrads. Detailed cytological analysis revealed the restitution of second division. This meant that the first meiotic division was normal, but the cytokinesis in the second division was impaired, resulting in the formation of dyads and triads. Formation of 2n restitution nucleus or the 2n pollen was observed in crosses with wild species from section *Arachis*, to which cultivated groundnut belongs (Singh and Moss 1984). Formation of 2n pollen in $F_1$ hybrids from crosses *A. hypogaea* × *A. chiquitana* (Figs. 1a and 1b), *A. hypogaea* × *A. kretschmeri* (section *Procumbentes*), and *A. duranensis* × *A. glabrata* (section *Rhizomatosae*) is a new finding. The 2n pollen from the cross *A. hypogaea* × *A. chiquitana* and *A. hypogaea* × *A. kretschmeri* were used to cross with *A. hypogaea* and develop tetraploid hybrids without going through the hexaploid route of backcross.

Use of 2n pollen in *Arachis* crossing program requires a large number of pollinations, but the process amply compensates by the development of tetraploids in one step, without the need to double the chromosome number of triploids and the laborious backcrossing program of the hexaploids to generate tetraploids. By this method it was possible to develop interspecific tetraploid derivatives from the crosses *A. hypogaea* × *A. chiquitana* and *A. hypogaea* × *A. kretschmeri.*

Dyads were observed as a result of restitution of both the groups of chromosomes at anaphase II. The number of dyads formed was low compared to the total number of pollen grains (Table 1), but the advantage of dyads is that they are fertile, which is evident from the acetocarmine stainability and in vivo pollen germination studies. Crosses using triploid pollen (*A. hypogaea* × *A.*

**Table 1. Formation of dyads, triads and tetrads in interspecific derivatives of groundnut (*Arachis hypogaea*).**

| Cross | No. of dyads formed | No. of triads formed | No. of tetrads formed | Pollen stainability |
|---|---|---|---|---|
| *A. hypogaea* × *A. hoehnei* | 48 (5)[1] | 123 (13) | 773 (82) | 28 |
| *A. hypogaea* × *A. cardenasii* | 53 (5) | 205 (20) | 759 (75) | 26 |
| *A. hypogaea* × *A. chiquitana* | 16 (1) | 150 (12) | 1091 (87) | 15 |
| *A. hypogaea* × *A. kretschmeri* | 10 (2) | 53 (12) | 366 (85) | 10 |
| *A. diogoi* × *A. glabrata* | 15 (6) | 23 (9) | 209 (85) | 30 |
| *A. duranensis* × *A. glabrata* | 30 (32) | 8 (9) | 56 (60) | 38 |
| *A. hypogaea* × *A. glabrata* | 9 (3) | 16 (5) | 299 (92) | 26 |

1. Figures given in parentheses are percentage values.

*cardenasii*) (Table 1) gave rise to a few pegs and pods, which is a further confirmation that some of the triploid pollen are fertile. Reciprocal crosses using the triploid (*A. hypogaea* × *A. cardenasii*) as the female parent and *A. hypogaea* as the pollen donor, gave rise to 6% peg formation as a result of 500 pollinations, resulting in 7 pods. The resultant hybrids, obtained using *A. hypogaea* × *A. cardenasii* as the female parent and *A. hypogaea* as pollen donor, were tetraploids, which was confirmed by pollen diameter analysis. It is fairly simple to observe dyads and triads in tetrad analysis, which may not be the case with megasporogenesis, as the eggs are embedded deep in the ovular tissues.

Singh and Moss (1984) reported the formation of pegs and pods in triploid interspecific derivatives from the crosses *A. hypogaea* × *A. chacoense* and *A. hypogaea* × *A. cardenasii*, which were obtained as cuttings from the University of Reading, Reading, UK. Interestingly in Reading, the triploids were sterile, but some of the plants grown at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India were partially fertile with the formation of a few pegs and pods. Cytological analysis of triploids showed that there were meiotic irregularities and the formation of restitution nucleus. Second division restitution was observed in the interspecific hybrids which were developed at ICRISAT (2000–05) between *A. diogoi* and *A. glabrata*, *A. hypogaea* and *A. hoehnei*, *A. duranensis* and *A. glabrata*, and *A. hypogaea* and *A. cardenasii* (Table 1). The action of restitution nucleus was evident by the presence of dyads and triads in pollen tetrad analysis (Figs. 1c and 1d). Dyads and triads have been observed in the tetraploid cross *A. hypogaea* × *A. glabrata* (Fig. 1e), which may not be of use in the improvement of *A. hypogaea*.

The report by Singh and Moss (1984) shows that environment may have a role to play in the formation of restitution nucleus in *Arachis* interspecific hybrids obtained as a result of crossing wild *Arachis* with cultivated groundnut. The results from our study show that 2n pollen can be effectively used to obtain tetraploids interspecific derivatives, without the use of colchicine to double the chromosome number of triploids and avoid the laborious hexaploid route to obtain tetraploids.

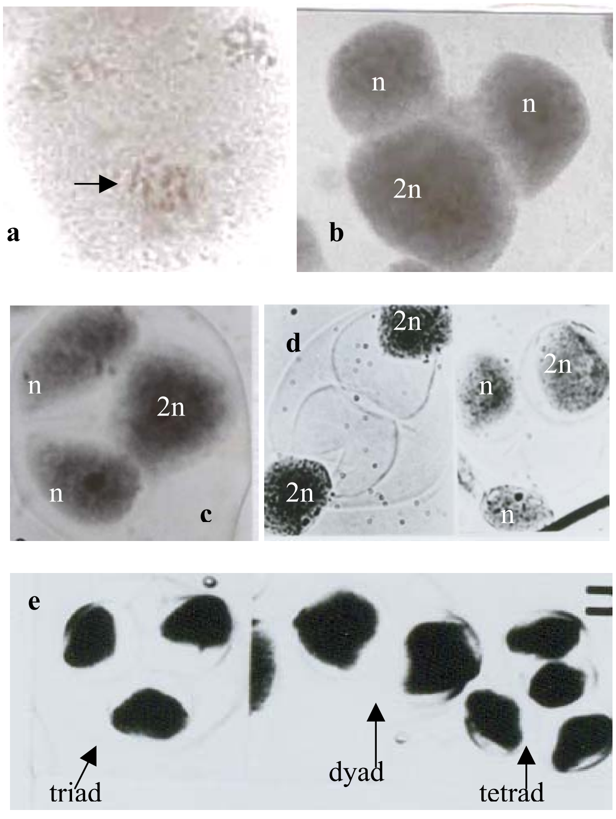


**Figure 1.** Formation of dyads and triads in *Arachis* interspecific hybrids: (a & b) Telophase with a normal separation of chromosomes and a restitution nucleus, leading to the formation of a triad in *A. hypogaea* × *A. chiquitana* (Note: Arrow points towards restitution nucleus); (c) Triad formation in *A. hypogaea* × *A. cardenasii*; (d) Dyads and triads in *A. duranensis* × *A. glabrata*; and (e) Dyads, triads and tetrads in the hybrid *A. hypogaea* × *A. glabrata*.

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