

## Pollen preservation and germination studies in *Arachis* species

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(Received: January 2008; Revised: March 2008; Accepted: May 2008)

Wild *Arachis* is a native of South America, with its cultivated species *Arachis hypogea* L. grown world-wide. One of the surest ways of broadening the narrow genetic base of the crop is by introducing variation through wide hybridization. If methods can be developed to preserve *Arachis* pollen for international transfer and their use later in crossing program, it might be an alternate method to import wild species germplasm from other countries. Pollen preservation can also improve the efficiency of breeding program and aid incorporation of unique traits from diverse sources of germplasm [1].

In order to develop methods for pollen preservation and to check the viability of pollen grains before and after preservation, 25 accessions of wild *Arachis* belonging to 14 wild species and two accessions of *A. hypogea* were used in this study.

### Pollen staining

Pollen samples were collected and dispersed on a drop of 2 % aceto-carmin stain placed on separate slides and warmed gently until the coloration was intense enough for a distinction to be made between stained and unstained pollen grains. Ten areas were viewed for each slide and the average percentage fertility was calculated.

### Pollen germination

Pollen from the flower was dusted evenly on the germination media within 15 min of collection. The germination media comprised of 10% sucrose added to a basal medium containing 250 mg/ml  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 100 mg/ml  $\text{H}_3\text{BO}_3$ , 200 mg/ml  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and 100 mg/ml  $\text{KNO}_3$  in deionized water (Niles & Quesenberry, 1992). 2-3 drops of germination media

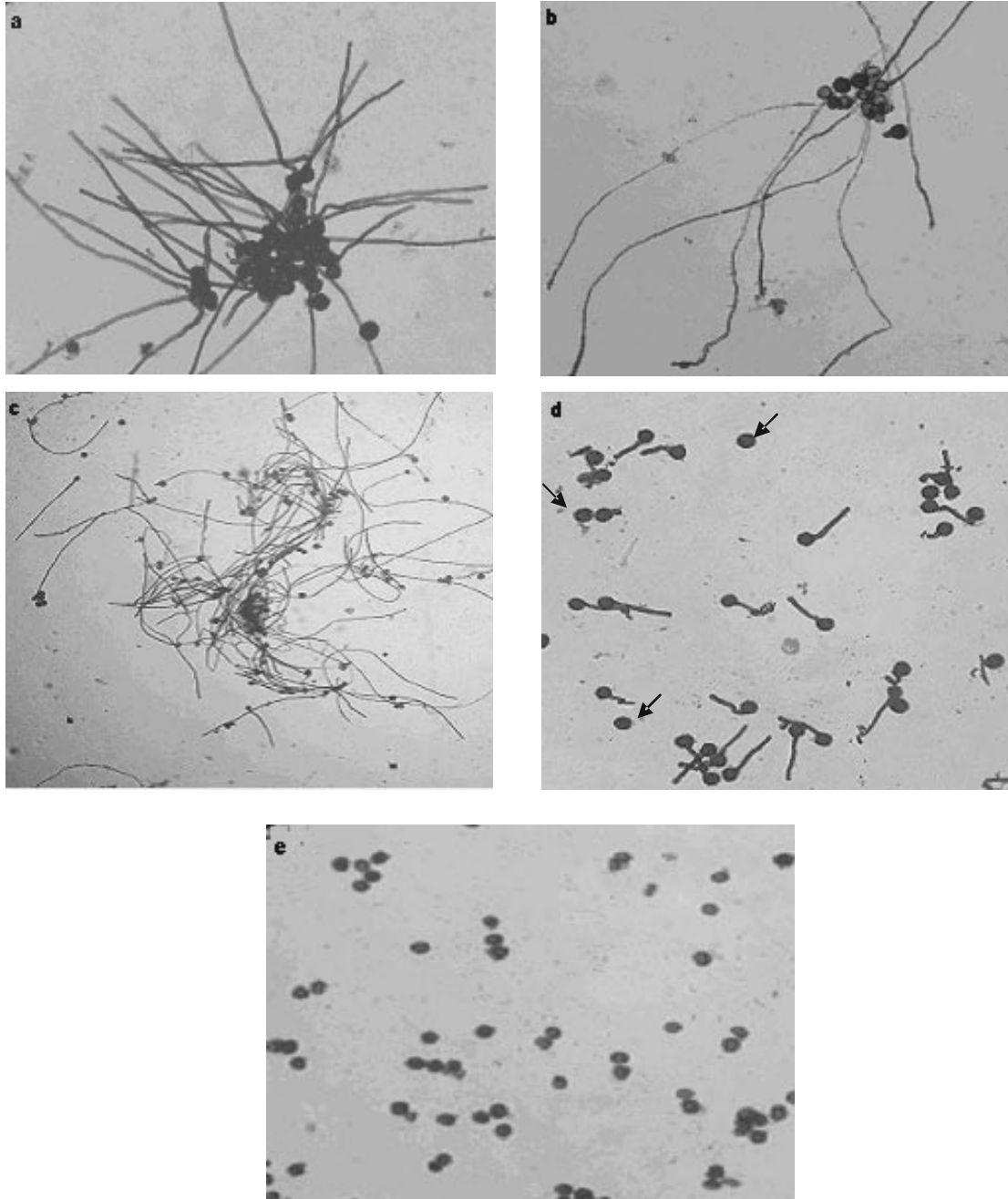
was placed on the slide and the inoculated slides were placed in a petridish lined with a moist filter paper. Percentage germination was ascertained by viewing several areas of a drop under a microscope after 240min (4 hr) of incubation at room temperature. A pollen grain was counted to have germinated when tube length exceeded the grain's diameter.

### Pollen preservation

Flowers from the plants were collected on the day of anthesis before 9 a.m. Pollen grains were isolated from flowers and collected in a vial. The experiments were set in triplicates. Pollen grains were collected by pressing the keel of the flower. Freshly isolated pollen were desiccated in a desiccator containing silica attached to a suction pump for one hour and stored at 6-8°C in the desiccator. Aliquots of pollen were retrieved at 7 days intervals and germinated *in vitro*. Analysis of variance (ANOVA) for pollen stainability, pollen germination, and pollen storage was performed to find the extent of variation of the traits between the accessions.

After incubating pollen grains in germination media for 4 hours and calculating percent germination, pollen grains were stained with acetocarmine. Remarkable differences were observed between percentage stainability and percentage germinability. Pollen stainability as found on the day of collection varied from 86% in *A. duranensis* (ICG 8123) to 96% in *A. stenosperma* (ICG 8137), and it was 91% in the *A. hypogaea* cultivars, whereas only 60.6 % (*A. duranensis* ICG 8138) to 88.4 % (*A. stenosperma* ICG 8137) of the grains germinated on the day of collection ( $p > 0.001$ ). Values of pollen stainability as well as germinability were highly significant at 0.1% ( $p < 0.001$ ). In no case was

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**Fig. 1. *Arachis* species pollen germination: a-*Arachis cardenasii* ICG 8216; b-*Arachis hypogea* cv.JL-24; c-*Arachis stenophylla* 0<sup>th</sup> day of pollen preservation; d-*Arachis stenophylla* 14<sup>th</sup> day of pollen preservation (arrow indicating stained but not viable pollen grains); e-*Arachis cardenasii* ICG8216 stained but not viable pollen grains on 25<sup>th</sup> day of preservation**

pollen germination greater than or equal to percent pollen stainability.

Wild species showed high pollen germination with notable variation from species to species. *A. duranensis* had the lowest percent germination and *A. stenosperma* showed the highest pollen germination. *A. hypogea* also

exhibited high pollen germination with long pollen tubes. (Fig .1c). On 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 25<sup>th</sup> day germinability reduced significantly where number of stained pollen grains remained the same.

There was drastic reduction in percent pollen germination when the pollen grains were preserved for

**Table 1.** Pollen preservation and germination studies

Arachis species	Days of pollen preservation					% of stained pollen grains (unpreserved pollen grains)
	0 days <sup>1</sup>	7 day	14 day	21 day	25 day	
<i>A. hypogaea</i> cv ICGS 44	89.0	39.9	9.9	1.3	0.0	91.0
<i>A. hypogaea</i> cv JL-24	80.3	41.2	10.1	1.0	0.0	91.0
<i>A. batizocoi</i> 8211	82.6	31.2	6.9	0.0	0.0	94.0
<i>A. batizocoi</i> 8958	77.3	33.1	7.1	0.1	0.0	91.0
<i>A. batizocoi</i> ICG 8124	84.0	2.7	0.7	0.0	0.0	93.0
<i>A. cardenasii</i> ICG 8216	87.9	56.1	0.3	0.0	0.0	92.0
<i>A. cardenasii</i> ICG 11564	80.5	46.4	13.1	1.3	0.0	92.0
<i>A. cardenasii</i> ICG 11563	77.6	48.3	12.9	1.0	0.0	91.0
<i>A. diogoi</i> ICG 4983	83.1	49.3	6.9	1.0	0.0	90.0
<i>A. duranensis</i> ICG 8123	72.1	1.7	0	0.7	0.0	86.0
<i>A. duranensis</i> ICG 8195	79.1	15.7	5.1	0.0	0.0	96.0
<i>A. duranensis</i> ICG 8138	60.6	12.9	2.1	0.0	0.0	91.0
<i>A. hoehnei</i> ICG 8190	74.4	15.2	14.2	2.0	0.0	90.0
<i>A. ipaensis</i> ICG 8206	88.3	53.8	13.9	2.2	0.2	91.0
<i>A. kempff-mercadoi</i> ICG 8959	75.1	28.8	11.3	0.0	0.0	84.0
<i>A. stenosperma</i> ICG 8137	88.4	10.1	1.0	0.0	0.0	96.0
<i>A. stenophylla</i> ICG 8215	80.3	60.4	44.9	16.3	2.4	92.0
<i>A. chiquitana</i> ICG 11560	71.4	42.2	13.0	3.0	0.0	90.0
<i>A. major</i> ICG 13162	77.2	19.6	10.0	2.9	0.0	93.0
S.E.	0.92	1.9	0.9	0.5	0.1	1.1
LSD at 1%	3.6	7.5	3.5	2.0	0.6	4.1
C.V.	2.0	10.8	15.4	37.2	125.8	2.0

<sup>1</sup>Percent pollen germination on the day of collection; Values significant at 0.1% level of probability ( $p < 0.001$ )

a week (CV = 10.8). Percent pollen germination ranged from 2 to 56 % after 7 days of preservation. Twenty-one days of preservation affected the viability of the pollen grains and the percent germination ranged from 0 to 16 % (CV = 37.19) and by 25 days of preservation except for *A. stenophylla* (ICG 8215) (Fig. 1C & 1D) none of the species had any viable pollen grains (CV = 125.8) (Table 1). There was no change in pollen stainability, however.

It is thus possible to preserve the pollen grains of *Arachis* species effectively for 2 weeks (14 days) in a desiccator at a temperature of 6°-8°C beyond which the grains lose their viability. Many a time wild species take longer to flower and sometimes they flower for a short while, and at times there is a mismatch in flowering days of wild species and the cultivar. If the pollen grains have to be preserved for use in crossing programme, it can be done so for not more than 2 weeks. Thus this information can help in the development of strategies

for wide crosses using pollen preservation techniques.

Information regarding pollen germination was available for only *A. glabrata*, a wild species distantly related to cultivated groundnut [2]. The present investigation also gives the relationship between pollen stainability and pollen viability. It is presumed that pollen stainability in acetocarmine can be taken as an indicator of pollen fertility, present investigation shows that this holds true for the fresh pollen taken on the day of anthesis (Fig. 1e). For preserved pollen grains the observation does not hold true.

## References

1. **Hanna W. W. and Towill L. E.** 1995. Long-term pollen storage. *Plant Breeding Rev.*, **13**: 179-207.
2. **Niles W. L. and Quesenberry K. H.** 1992. Pollen germination of Rhizoma peanut cv. Glorigraze. *Peanut Science*, **19**: 105-107.