Anther culture of groundnut (*Arachis hypogaea* L.) : I. Categorization and selection of flower buds (1)

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Summary. — Flower buds from three varieties of groundnut, Robut 33-1, TMV 3, and ICGS 11, growing in the field and one variety, Robut 33-1, growing in the glasshouse, were collected at different times after sowing and the size of the flower buds, colour of the anthers, and the genotypes were correlated with the stage of anther development. Significant differences were observed among sizes of anthers containing pollen at different stages of development. These sizes differed at the beginning and end of the flowering season, but the correlation of anther colour with the stage of development was independent of the time of flowering. The observations showed that anthers could be harvested for culture by standardizing the sizes of flower buds one day earlier, and by collecting buds only of sizes corresponding to the desired stage (the non-overlapping zone of the graph).

INTRODUCTION

Production of haploids through anther culture has been standardized in many plant species since the report of Guha and Maheswari (1964). The technique has been applied to over 200 species of higher plants (Maheswari *et al.*, 1982 ; Keller *et al.*, 1987). Some solanaceous species have responded very well, but in most species the success rates were disappointingly low, or species were totally recalcitrant. A number of factors affect pollen plant production in anther cultures (Maheswari *et al.*, 1980 ; Heberle-Bors, 1985). Among these, the developmental stage of the pollen at the time of inoculation, and the physiological state of the parent plant play important roles. A few people (Martin *et al.*, 1974 ; Martin and Rabechault, 1976 ; Mroginski and Fernandez, 1979, 1980) have tried anther culture in *Arachis* and secured very low frequency of haploid cells in the callus. Martin *et al.* (1974) tried to correlate the development of flower buds to the stage of microsporogenesis, but they did not mention anything about how to select and which type or size of buds to select for the actual culture of anthers. In the present investigation, attempts have been made to quantitatively correlate the size of flower buds, colour of anthers, age of the donor plants, and varietal differences to the stage of development of anthers.

MATERIAL AND METHODS

Groundnut (*Arachis hypogaea* L.) cultivars Robut 33-1, TMV 3, and ICGS 11 were obtained from the Genetic Resources Unit of ICRISAT and grown in the field at Patancheru in the 1987 rainy season (July-October). Cultivar Robut 33-1 was also grown in the glasshouse under controlled conditions with temperatures 25 °C (day) and 22 °C (night). Flower buds of different sizes were collected from field-grown plants of Robut 33-1 at 40, 45, and 60 days after sowing (DAS), TMV 2 and ICGS 11 at 45 DAS, and glasshouse-grown plants of Robut 33-1 at 40 DAS. Flower buds were collected in the morning between 0800 and 1000 and fixed in Carnoy's fluid II (1 acetic acid : 3 chloroform : 6 alcohol by volume) for 6-12 h, followed by Carnoy's fluid I (1 acetic acid : 3 alcohol by volume) for 24 h, and then stored in 70 % alcohol. Length of flower bud, from the base of the sepals to the tip of the bud, was measured (using eye-piece micrometer) before the anthers were dissected and stained in 2 % aceto-carmine. Some fresh, unfixed flower buds were measured, anthers dissected, and the colour recorded, before staining in aceto-carmine to observe the stage of microsporogenesis. A minimum of 30 and maximum of 60 flower buds were studied under each category. Size and stage of flower buds and colour of anthers were compared, using « t » test, between field-grown and glasshouse-grown plants, between 40 DAS and 60 DAS and between different genotypes.

In a subsequent season, flower buds from glasshouse-grown plants of Robut 33-1 were collected, their sizes were measured, and the stage of development was observed. A graph was plotted to define the sizes that contained maximum number of anthers at a particular stage. The next day, flower buds were collected and measured, and those buds that fell into the defined, non-overlapping zones were fixed and stained.

RESULTS

There was a significant difference at any one stage of microsporogenesis between the size of the flower buds of the field-grown (FG) plants and the glasshouse-grown (GG) plants (Table I). The buds from GG plants were consistently smaller than the buds from FG plants. The mean size of the buds corresponding to a particular stage of development showed significant difference from the buds containing the next stage.

There was also a significant difference in the size of buds at the start of flowering and at the end of the flowering season (Table I). The differences ranged from 0.62 to 1.80 mm. However, the size of buds with a specific stage of development of microspores was consistent at one part of the season. The buds from 60-day-old plants were significantly smaller than those from 40-day-old plants.

There was no significant variation in the size of flower buds in the different genotypes used at any stage of development (Table II). But the differences in sizes between the stages within each genotype were significant.

The colour of the anthers containing a particular stage of microspores was always the same in all the cases — FG, GG,

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TABLE I. — **Size of flower buds and colour of anthers in field-grown (40 and 60 DAS (a)) and glasshouse-grown (40 DAS) plants of groundnut cv. Robut 33-1. ICRISAT Center, rainy season, 1987.**

Stage of development	Colour of anthers	Size of flower buds (mm) Field-grown 40 DAS (1)	Field-grown 60 DAS (2)	Glasshouse-grown 40 DAS (3)
Meiosis				
Early stages	Transparent	2.91	2.29	2.79
Tetrad	White	3.95	3.08	3.76
Pollen grains :				
Uninucleate	Light yellow	5.01	3.94	4.41
Binucleate	Yellow	6.20	4.86	5.25
Mature	Deep yellow	7.81	6.01	6.94
SE mean		± 0.76	± 0.58	± 0.63
t (1, 2) = 3.028* (b)				
t (1, 3) = 5.697** (c)				

(a) DAS = Days after sowing.
(b) * Significant at 5 % level.
(c) ** Significant at 1 % level.

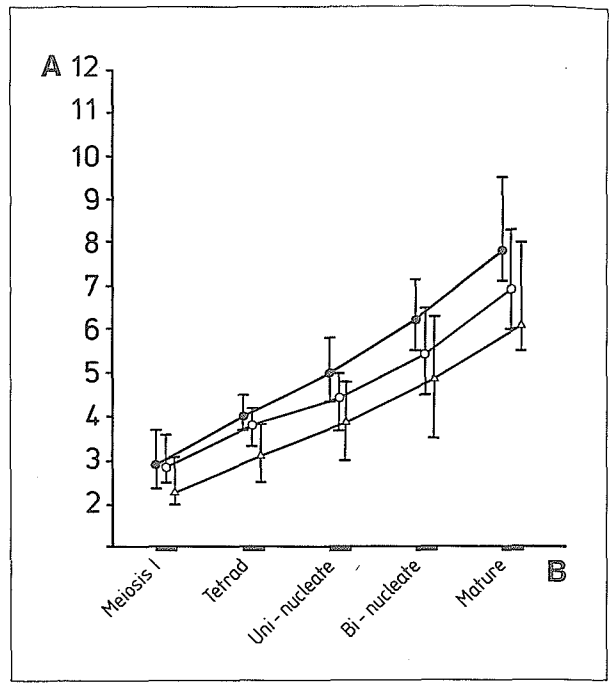

FIG. 1. — Size of flower buds and stages of development of pollen in field-grown (40 and 60 days after sowing) and glasshouse-grown (40 days after sowing) plants of Robut 33-1. ICRISAT Center.
A = ●——● 40 DAS Field-grown plants
○○ 60 DAS
B = △——△ 40 DAS Glasshouse-grown plants.

TABLE II. — **Size of flower buds (mm) and colour of anthers in different genotypes of groundnut grown in the field (45 DAS). ICRISAT Center, rainy season, 1987.**

Stage of development	ROBUT 33-1 (1)	TMV 3 (2)	ICGS 11 (3)	Colour of anthers
Meiosis				
Early stages	2.72	2.81	2.65	Transparent
Tetrad	3.68	3.45	3.85	White
Pollen grains :				
Uninucleate	4.44	4.24	4.12	Light yellow
Binucleate	5.43	5.31	5.41	Yellow
Mature	7.51	6.14	7.16	Deep yellow
SE mean	± 0.73	± 0.54	± 0.69	
t (1, 2) = 1.424				
t (2, 3) = 0.675		... not significant at 5 % level		
t (1, 3) = 1.198				

early, late, or in different genotypes (Table I). However, some overlapping was observed in the size of flower buds of successive stages (Fig. 1), and a few anthers contained microspores at more than one stage of development.

There results were tested in a subsequent season. A graph was plotted (Fig. 2), and the next day flower buds were collected from the non-overlapping zone of the graph. Ninety two, 81, and 100 % of the buds of the selected stages conformed to the uninucleate, binucleate and mature pollen grains, respectively (Table III). Colour of the anthers was in absolute agreement with the stage of development in all the buds observed.

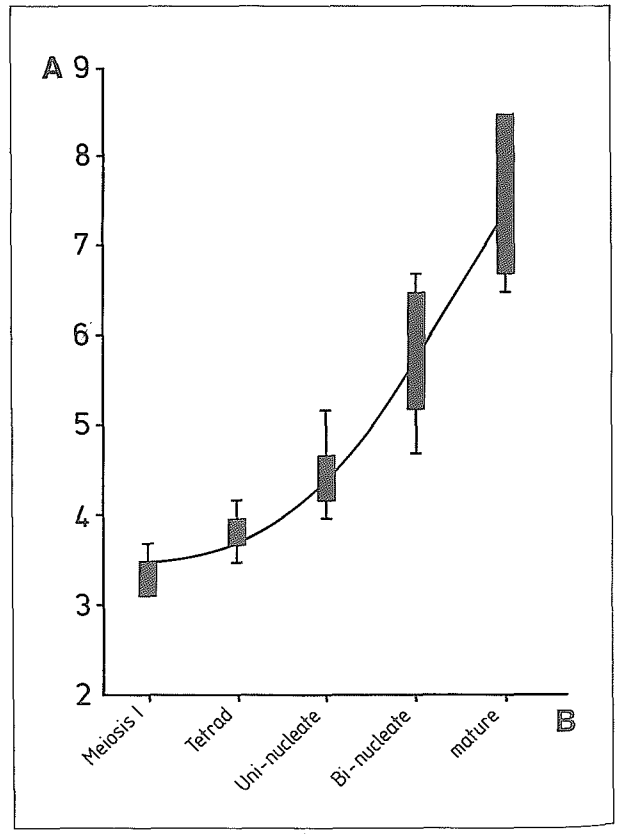

FIG. 2. — Standardization of size of flower buds and stage of development of pollen in Robut 33-1 (glasshouse-grown plants).
Non-overlapping zone.

TABLE III. — **Stage of development of microscpires in the flower buds from the non-overlapping zone of different sizes in Robut 33-1. ICRISAT Center, rainy season, 1988.**

Size of buds (mm) and expected stage of development (a)	No. of buds tested	No. (and percentage) of buds at the required stage
3.1-3.5 (Meiosis-I)	23	18 (78)
3.7-4.0 (Tetrad)	17	10 (59)
4.2-4.7 (Uninucleate)	38	35 (92)
5.2-6.5 (Binucleate)	32	26 (81)
> 6.7 (Mature)	25	25 (100)

Based on cytological observations to set these parameters for this group of plants.

DISCUSSION

In most species, uninucleate pollen has been reported as the most successful to use for anther culture. However, microscopic observation of the anthers is time consuming and breaks any internal sterility of buds. On the other hand, measuring the flower buds is a quick and easy method of selecting buds with anthers at a specific stage of development. Although this is followed in almost all anther cultures, a consistent correlation between size and stage of development has not been established for *Arachis*. Many factors, such as the stage of flowering (early or late), environment of the plants, and genotype, affect this correlation.

In the present investigation, it was observed that there was a correlation between size of bud and stage of development in plants of the same age ; this was true for plants from the field, as well as in glasshouse-grown plants. The buds at the start of the flowering season are larger than those at the end of the flowering season, and there are differences in size between genotypes. The colour of the anthers bearing a particular stage of microspores is independent of size of flower buds, age of plants, genotype, or any of the factors considered. This is in conformity with the results of Martin *et al.* (1974), who have given the biology of flowering in *Arachis* (but they did not stress the use of anther colour in selecting the anthers for culture). However, assessment of colour is subjective, and the bud has to be opened.

Based on the results obtained in the present study, it is suggested that flower buds for anther culture should be collected from one group of plants over a period of a few days. The sizes should be standardized at the start of the experiment and buds that fall into the non-overlapping zone should be collected and used. At the time of excision of anthers, any anthers not conforming to expected colour should be rejected. As the age of the plant advances, the size of the buds changes and this should be monitored. Preferably, anthers should be collected in the beginning of the flowering season as they are larger, and also more responsive than at a later period (Sunderland and Dunwell, 1977 ; Sunderland, 1978). Successive sowings of plants are to be recommended in preference to successive collection of anthers from the same plants.

After selecting buds, the anthers are dissected out for inoculation, when the colour of the anthers is a most useful guide. The difference in colour between anthers bearing uninucleate or binucleate pollen grains is between shades of yellow, but earlier or later stages can be eliminated by rejecting transparent, white, and deep yellow anthers.

Although these observations are more demanding and time consuming then the observations of size and shape of bud, by using these criteria it has been possible to select anthers in which almost all microspores were at the expected stage.

CONCLUSION

In anther cultures, selection of flower buds with the required stage of development, usually the « uninucleate » stage, is difficult and time consuming ; the present study recommends a simplified technique using size of the flower buds and colour of the anthers. There was significant difference in size of flower buds and colour of anthers of a particular stage of development. For the anther culture, the flower buds should be collected over a period of time, preferably at the start of the flowering season, and the sizes should be standardized one day before the actual collection of flower buds. While dissecting the anthers from buds, the colour of the anthers should be considered for discarding the unwanted stage anthers. This technique has been tested to confirm the required stage of development viz, tetrad, uninucleate, binucleate microspores.

REFERENCES

[1] GUHA S. and MAHESWARI S. C. (1964). — *In vitro* production of embryos from anthers of *Datura*. *Nature*, 204, 497.

[2] HEBERLE-BORS E. (1985). — *In vitro* haploid formation from pollen : a critical review. *Theor. Appl. Genet.*, **71**, 361-374.

[3] KELLER W. A., ARNISON P. G. and CARDY J. (1987). — Haploids from gametophytic cells — recent developments and future prospects. *In : Plant Tissue and Cell Culture*. Alan R. Liss. Inc., 223-241.

[4] MAHESWARI S. C., RASHID A. and TYAGI A. K. (1982). — Haploids from pollen grains-retrospect and prospect. *Am. J. Bot.*, **69**, 865-879.

[5] MAHESWARI S. C., TYAGI A. K., MALHOTRA K. and SOPORY S. K. (1980). — Induction of haploidy from pollen grains in angiosperms the current status. *Theor. Appl. Genet.*, **58**, 193-206.

[6] MARTIN J. P., CAS S. and RABECHAULT H. (1974). — Cultures *in vitro* d'étamines d'arachide (*Arachis hypogaea* L.). 1. Stades du développement des boutons floraux et microsporogenèse. *Oléagineux*, **29**, (3), 145-149.

[7] MARTIN J. P. and RABECHAULT H. (1976). — Culture *in vitro* d'étamines d'arachide (*Arachis hypogyaea* L.) II. Etablissement de cultures de tissus et organogenèse. *Oléagineux*, **31**, (1), 19-25.

[8] MROGINSKI L. A. and FERNANDEZ A. (1979). — Cultivo *in vitro* de anteras de especies de *Arachis* (Leguminosae). *Oléagineux*, **34**, (5), 243-248.

[9] MROGINSKI L. A. and FERNANDEZ A. (1980). — Obtencion de plantulas por cultivo *in vitro* de anteras de especies silvestres de *Arachis* (Leguminosae). *Oléagineux*, **35**, (2), 89-92.

[10] SUNDERLAND N. (1978). — Strategies in the improvement of yields in anther culture. *Proc. Symp. on Plant Tissue Culture*, Science Press, Peking, 65-86.

[11] SUNDERLAND N. and DUNWELL J. M. (1977). — Anther and pollen culture. *In : Plant Tissue and Cell Culture* (Ed. H. E. Street) Blackwell Scientific Pubs., Oxford, 223-265.

RÉSUMÉ

Culture d'anthères d'arachide (*Arachis hypogaea* L.) : I. Classement et sélection des bourgeons floraux.

Y. SUDHAKAR et J. P. MOSS, *Oléagineux,* 1990, **45,** N° 11, p. 501-504.

Des bourgeons floraux provenant de trois variétés d'arachide, Robut 33-1, TMV 3 et ICGS 7, cultivées au champ, et d'une variété, Robut 33-1, cultivée sous serre ont été récoltés à des intervalles différentes après le semis, et la taille des bourgeons floraux, la couleur des anthères et les génotypes ont été correlés avec le stade de développement des anthères. Des différences significatives ont été notées entre la taille des anthères contenant du pollen aux différents stades de développement. La taille est différente au début et à la fin de la saison de floraison, mais la corrélation de la couleur des anthères avec le stade de développement est indépendante du temps de floraison. Les observations ont montré que les anthères destinées à la culture peuvent être récoltées en étalonnant la taille des bourgeons floraux un jour plus tôt, et en ne récoltant que les bourgeons dont la taille correspond au stade désiré (la zone de non-enchevauchement du graphique).

RESUMEN

Cultivo de anteras de maní (*Arachis hypogaea* L.) : I. Clasificación y selección de las yemas florales.

Y. SUDHAKAR y J. P. MOSS, *Oléagineux,* 1990, **45,** N° 11, p. 501-504.

Yemas florales de tres variedades de mani, las Robut 33-1, TMV 3 e ICGS 7, cultivadas en el campo, y de una variedad, la Robut 33-1, cultivada bajo invernadero, se recogieron en varios intervalos después de la siembra, y se establecieron correlaciones entre el tamaño de las yemas florales, el color de las anteras y los genotipos, por un lado, y la etapa de desarrollo de las anteras, por otro lado. Se notaron diferencias significativas entre el tamaño de las anteras que contienen polen en las varias etapas de desarrollo. Su tamaño es distinto al principio y al final del período de floración, pero la correlación entre el color de las anteras y el estado de desarrollo no depende del tiempo de floración. Las observaciones han mostrado que las anteras destinadas al cultivo pueden cosecharse contrastando el tamaño de las yemas florales un día antes, y recogiendo sólo las yemas cuyo tamaño corresponda a la etapa deseada (representada por la zona del gráfico sin traslapo).

Information

ALECTRA VOGELII
PHANÉROGAME PARASITE DE L'ARACHIDE AU BURKINA-FASO

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Alectra vogelii Benth., parasite radiculaire de l'arachide, a été identifié dans deux localités du Sud-Ouest du Burkina (province de la Comoe) durant une enquête phytosanitaire en 1987. Cette plante est traditionnellement connue des agriculteurs de la région sous le nom de « striga » de l'arachide, montrant ainsi qu'ils sont conscients de son comportement parasite. *A. vogelii* fait partie de la famille des Scrophulariacées. Les plantes à maturité ont une taille de 20 à 40 cm. Les fleurs sont jaunes et de petite taille, les rameaux s'insèrent à la base de la plante. Le suivi des parcelles contaminées a été poursuivi en 1988 et 1989. Il a montré la persistance de la population d'*A. vogelii.* Durant ces deux années, les plantes apparaissent soit au cours de la seconde quinzaine du mois de septembre, soit en fin de cycle de l'arachide. Au sein des parcelles atteintes, l'infestation n'est pas homogène : *A. vogelii* se répartit par foyers, dans lesquels les pieds d'arachide peuvent être parasités par deux ou trois voire cinq ou six plantes. Ces foyers ont une surface de 2 à 3 m^2 et sont au nombre d'une dizaine pour une parcelle de 500 m^2. Des prospections ultérieures, en 1988 et 1989, n'ont pas permis de découvrir d'autres lieux de présence de ce parasite, *A. vogelii* se limitant aux parcelles antérieurement identifiées. Du fait de sa faible densité au champ ainsi que de son extension très réduite, *A. vogelii* n'est qu'un parasite d'importance mineure au Burkina-Faso. Il convient toutefois de surveiller une éventuelle dispersion à partir des foyers actuels.

PHOTO 1. — Alectra dans un champ d'arachide.

PHOTO 2. — Systèmes racinaires du parasite et de la plante-hôte.

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