# Advances on polyploid breeding in yam *D.alata*

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## Introduction

Yam is an important food security crop in the Caribbean, South Pacific, West Africa and parts of Asia. The greater yam (*Dioscorea alata L.*) is one of the most widely distributed species worldwide. It has a greater potential yield (particularly in poor soils) and keeps well (3 to 5) months. However, several factors limit its development. These include abiotic and biotic constraints, including anthracnose disease that is caused by the fungus *Colletotrichum gloeosporioides*, a constant threat.

A selection and varietal improvement program is conducted by CIRAD (Centre International de Recherche Agronomique pour le dévélopement) in Guadeloupe (French West Indies). The objectives are to create new varieties that combine high and stable yields, anthracnose resistance and tuber characteristics adapted to commercial production (quality, tuber shape, etc.).

*D. alata* is a polyploid species including varieties with three ploidy levels 2n=40, 60 and 80 chromosomes. Recently we demostrated using flow cytometry and microsatellite segregation analysis that accessions with 2n= 40, 60 and 80 chromosomes are diploid, triploid and tetraploid respectively, and not tetraploid, hexaploid and octoploid, as previously assumed (Arnau et al. 2009). Ploidy increase is correlated with growth vigour, higher tuber yield and increased tolerance to abiotic and biotic stress (Malapa et al. 2005; Lebot 2009; Arnau et al. 2010) Although polyploidy has been recognised for a long time, all breeding programmes were exclusively based on the creation of diploid varieties, until 2006. The first polyploid hybrids (triploid and tetraploid) in this crop have been created by CIRAD (Arnau et al., 2006; Arnau et al., 2007). Progress could be achieved thanks to the use of biotechnological tools (cytogenetic techniques, molecular markers and in vitro culture) as a complement to standard techniques. The capacity of some diploid varieties to produce natural unreduced gametes was revealed by flow cytometry analyses, by detection of hybrids with non expected ploidy level in progenies produced.

Polyploid breeding appears promising for the genetic improvement of the greater yam, making it possible to maximise heterozygosity and heterosis. Several ways to obtain polyploids can be exploited and these will be presented.

## Materials and methods

### *Plant materials*

A progeny of 300 individuals obtained by cross between two diploid parents that produce both unreduced gametes was analyzed.

Four different progenies obtained from 2x x 4x crosses were used to understand the origin of triploid hybrids via interploid crosses and also the origin of tetraploid hybrids obtained via gametic non-reduction in the female parent.

A progeny of 300 individuals obtained by cross between two natural tetraploid parents genetically distant have been evaluated over four years to select performing hybrids.

### *Ploidy level: flow cytometry analysis*

Flow cytometry was performed on nuclei solutions obtained from fresh leaf samples as described by Arnau et al. (2009).

### *Microsatellite analysis*

Twelve SSR markers that revealed different allelic profile between two parents, and heterozygote profiles, were used to determine the origin of unreduced gametes (maternal or paternal).

### *Embryo culture*

Embryos obtained by interploid crosses (2x x 4x) were rescued in vitro, 90 days after pollination, and cultured on MS mineral solution supplemented with 30 g $L^{-1}$ sucrose, 0.5 mg $L^{-1}$ biotine, 0.5 mg $L^{-1}$ pyridoxine , 0.5 mg $L^{-1}$ thiamin , 100 mg $L^{-1}$Myo-inositol, 1 mg $L^{-1}$BAP, 2 g $L^{-1}$ charcoal, and 3g $L^{-1}$ gelrite.

## Results and discussion

### *Production of triploid hybrids from 2x x 2x crosses via natural gametic non-reduction in the female parent or in the male parent*

2x X 2x crosses produced diploid hybrids in majority, but also some triploids the origin of which can be explained by a phenomen of non gametic reduction in the female parent or in the male parent.

***Production of triploid hybrids from interploid 2x x 4x crosses***

Interploid 2x X 4x crosses is a second way to obtain triploids. Almost all seeds produced were found to contain embryons but an abnormal development of endorsperm, and it was therefore necessary to obtain seedlings using embryo rescue. The reciprocal crosses (4x x 2x) were not successful.

***Production of tetraploid hybrids from interploid 2x x 4x crosses via natural gametic non-reduction in the female parent***

Through this cross, we normally obtain triploids but we also obtained some tetraploids the origin of which can be explained by a phenomen of non gametic reduction in the female parent.

***Production of tetraploid hybrids from 4x x 4x crosses***

By crossing two genetically distant natural tetraploids it is possible to obtain high heterozygote tetraploid hybrids. Progeny evaluation in the field over 4 consecutive years allowed selection of performing hybrids.

**References**


Arnau, G., Maledon, E., Bachand, I., and Abraham, K. 2006. Production of interploid hybrids and molecular markers heterozygosity determination using microsatellite markers in the greater yam, *D. alata*: Importance for the genetic improvement of the greater yam. In: Proceedings of 14th Triennial Symposium of the International Society for Tropical Roots Crops, Thiruvananthapuram, Kerala, India, p 52-53.

Arnau, G., Maledon, E., and Némorin, A. 2007a. Genetic improvement of the greater yam *D. alata* through polyploidy breeding. In: Colon Wilfredo, Lugo Wanda I. (eds.). Marketing opportunities for agriculture and forestry products in the Greater Caribbean - A challenge for the 21st century. Caribbean Food Crops Society, Annual Meeting Caribbean Food Crops Society, 16-22 September, San José, Costa Rica, p. 112.

Arnau, G., Nemorin, A., Maledon, E., and Abraham, K. 2009. Revision of ploidy status of *Dioscorea alata* L (*Dioscoreaceae)* by cytogenetic and microsatellite segregation analysis. Theoretical and Applied Genetics. 118: 1239-1249.

Arnau G, Abraham K, Sheela MN, Chair H, Sartie A and Asiedu R .2010. Yams. Root and Tuber Crops Handbook of Plant Breeding, Volume 7, pp 127-148

Lebot, V. 2009. Tropical root and tuber crops: cassava, sweet potato, yams and aroids. In Wallingford (ed) CABI, XIX, 413 pp.

Malapa, R., Arnau, G., Noyer, J.L., and Lebot, V. 2005. Genetic diversity of the greater yam (*Dioscorea alata* L.) and relatedness to *D. nummularia* Lam. and *D. transversa* Br. as revealed with AFLP markers. Genetic Resources and Crop Evolution 52: 919–929.