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Wide hybridization in okra

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Summary. Crosses were made between members of the two West African okra types 'Soudanien' and 'Guineen'. All crosses succeeded in both directions and the F_1 plants which showed hybrid vigour for plant stature were partially sterile. Cytological observations of the F_1 plants revealed abnormal meiosis which resulted in the production of microspores of variable sizes. The frequency of viable pollen (as indicated by acetocarmine staining) was low in the hybrids: 35.80% (U.I.92 × U.I.313) and 39.41% (1bk-1 × U.I.215). The number of seeds produced per fruit was low in the hybrids and only a few of these seeds are viable. The possibility of gene transfer between the two okra types was discussed.

Key words: *Abelmoschus esculentus* – Okra – Wide hybridization – Pollen viability – Unreduced pollen

Introduction

There is a wide range of genetic variability for okra (*Abelmoschus esculentus* (L.) Moench) in West Africa where it is an important vegetable grown for its green tender fruit. A numerical taxonomic study on some okra accessions from different parts of the world identified the West African types as belonging to two morphologically distinct groups, while those from elsewhere generally tended to cluster together (Chheda and Fatokun 1982). Siemonsma (1982) also identified two groups among West African okra and called them 'Soudanien' and 'Guineen' types.

In Nigeria, the 'Guineen' type of okra are grown on a smaller scale than the 'Soudanien' because the former generally flower very late. In fact, many are short-day plants (Njoku 1958) and also when cooked their pods do not produce the thick soup favoured by most consumers. However, the bulk of okra available in the markets during the dry season are of the 'Guineen' type which tend to live longer and tolerate drought better. When screening about 300 okra accessions for resistance to yellow mosaic virus, two of the 'Guineen' type consistently showed no disease symptoms.

It appears that scattered within the two main types of okra are several desirable agronomic characteristics. Hence crossing should lead to the production of varieties that combine these attributes. The study reported here describes the morphology and viability of F_1 hybrid plants obtained following crosses between 'Soudanien' and 'Guineen' okra types.

Materials and methods

Four okra cultivars, 1bk-1 and U.I.92 ('Soudanien' types) and U.I.215 and U.I.313 ('Guineen' types) were used for this study. Reciprocal crosses were made between 1bk-1 and U.I.215 and between U.I.92 and U.I.313. At least 20 plants from each cross were sown in drums containing about 120 kg top soil. There were four plants in each drum. The parents were also sown in eight drums with two drums per parent. All drums were watered daily and plants were sprayed against insects, using 1.0 ml Sherpa plus per l water.

Mature and dried fruits were plucked from the plants and the seeds extracted. The seeds were stored in paper bags in the laboratory for about 8 weeks before germination tests were performed. Then 400 seeds from each genotype (parent and reciprocal F_1 hybrids) were placed on a double layer of moist filter papers in petri-dishes. There were 50 seeds in each petri-dish.

For cytological work, flower buds were collected from the hybrids between 7.00–8.00 a.m. and fixed in Carnoy's liquid (6:3:1 of absolute ethanal: chloroform: glacial acetic acid) for at least 72 h before transferring to 70% ethanol. Anthers were then squashed in 1% acetocarmine. Pollen grains were obtained on day of anthesis and placed on slides before staining with acetocarmine.

Table 1. Comparison of some morphological characteristics of 'Soudanien' and 'Guineen' okra types and the F_1 hybrids between them[a]

Characters	'Soudanien'		'Guineen'		Hybrids	
	1bk-1	U.I.92	U.I.215	U.I.313	1bk-1 × U.I.215	U.I.92 × U.I.313
Plant height at maturity (cm)	95.43	132.85	126.32	89.72	189.71	143.66
Epicalyx number/flower	11.25	11.30	6.92	6.61	9.08	9.27
Epicalyx length (cm)	0.79	0.83	1.48	1.51	1.59	1.60
Pollen viability (%)	96.92	95.20	98.12	96.35	39.41	35.80
Fruit length (cm)	8.94	8.79	11.50	10.93	8.82	9.72
Number of seeds/fruit	97.25	93.13	68.12	85.60	14.08	20.75
100 seed weight (g)	4.65	4.54	5.17	4.71	4.80	4.32
Seed germination (%)	88.51	84.67	78.82	85.34	40.03	22.40
Epicalyx shape	Narrow	Narrow	Broad	Broad	Intermediate	Intermediate
Pigmentation of petal base	Both surfaces pigmented	Both surfaces pigmented	Inner surface pigmented	Inner surface pigmented	Both surfaces pigmented	Both surfaces pigmented

[a] No appreciable differences were found between reciprocal crosses, hence their values were pooled

Results and discussion

All crosses between 'Soudanien' and 'Guineen' types of okra succeeded; fruits filled with seeds were produced. When sown, all the hybrid seeds germinated and produced vigorous seedlings. A few seedlings (10%) from the cross 1bk-1×U.I.215 either had three cotyledons each or the two cotyledons differed distinctly in size. None of the parents shows this characteristic. The hybrids showed heterosis through their more vigorous growth and taller stature than the parents (Table 1). Flowers borne by hybrid plants were also generally larger in size than in the parents. The hybrids, however, were intermediate between the parents in some traits, especially epicalyx shape and fruit length.

The hybrids flowered normally and it was observed that number of pollen grains per anther was much fewer than in the parents. Frequency of fruit set per plant did not differ between hybrids and parents when insects are very active in the flowers. Most flowers that opened when insect activity was at a low level, especially on days when insecticide was sprayed on the plants, dropped without developing into fruits. This could be attributed to the low amount of pollen produced in anthers of the hybrids.

Cytology of F_1 plants

Cytological observations revealed that meiosis in the hybrid plants are highly irregular. At the early stage of Metaphase 1, a few chromosomes were found separating from others still arranged on the equatorial plate (Fig. 1). Precocious chromosome movement also continued during Anaphase 1 when several laggards were observed. The randomly moving and laggard chromosomes formed micro-nuclei which gathered some quantity of cytoplasm to give rise to the under sized microspores (Fig. 2 a–e). These small microspores were referred to as 'microcytes' by Rubaihayo and Gumisiriza (1978). The number of microspores per sac at the tetrad stage differed remarkably in the hybrids. There were monads (Fig. 2 e), diads (Fig. 2 g), triads (Fig. 2 b, d) and even some with up to 10 microcytes (Fig. 2 c). The frequency of normal tetrads (Fig. 2 f) in the hybrid 1bk-1×U.I.215 was approximately 20.4% among about 2,000 microspore sacs observed. The variation in the size of daughter microspores is most probably due to haphazard distribution of chromosomes such that each had a different number of chromosomes.

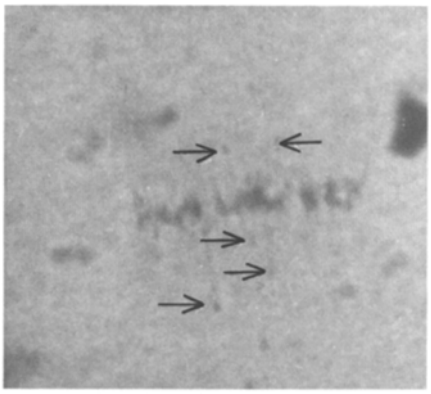

Fig. 1. Precocious chromosome movement (*arrows*) at Metaphase 1 in the hybrid 1bk-1×U.I.215

Pollen size, shape and stainability

The size and shape of pollen varied remarkably in the hybrid plants (Figs. 3 a–d), even among those from the

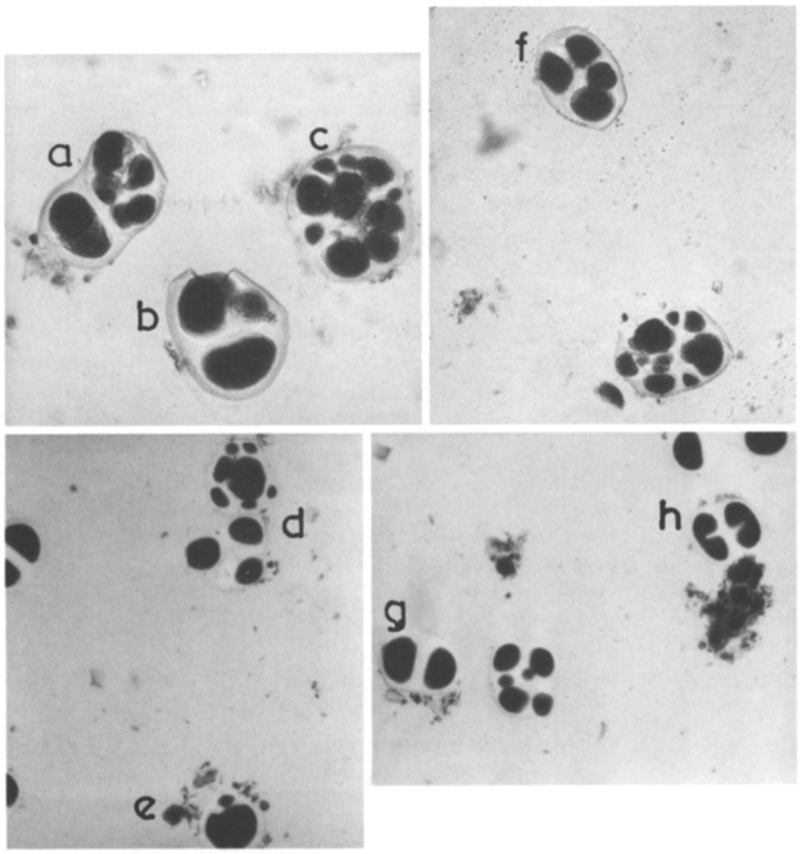

Fig. 2. Abnormal pollen quartets in hybrid 1bk-1×U.I.215. *a* An unreduced pollen with microcytes in same pllen sac; *b* a triad with an unreduced pollen and two unequal sized microspores; *c* a quartet with 10 unequal sized microspores; *d* a triad with unequal sized microspores; *e* a monad with a microcyte; *f* a tetrad with uneqal sized microspores; *g* a diad with unreduced pollen; *h* a diad in which cytokinesis was not completed hence unreduced pollen

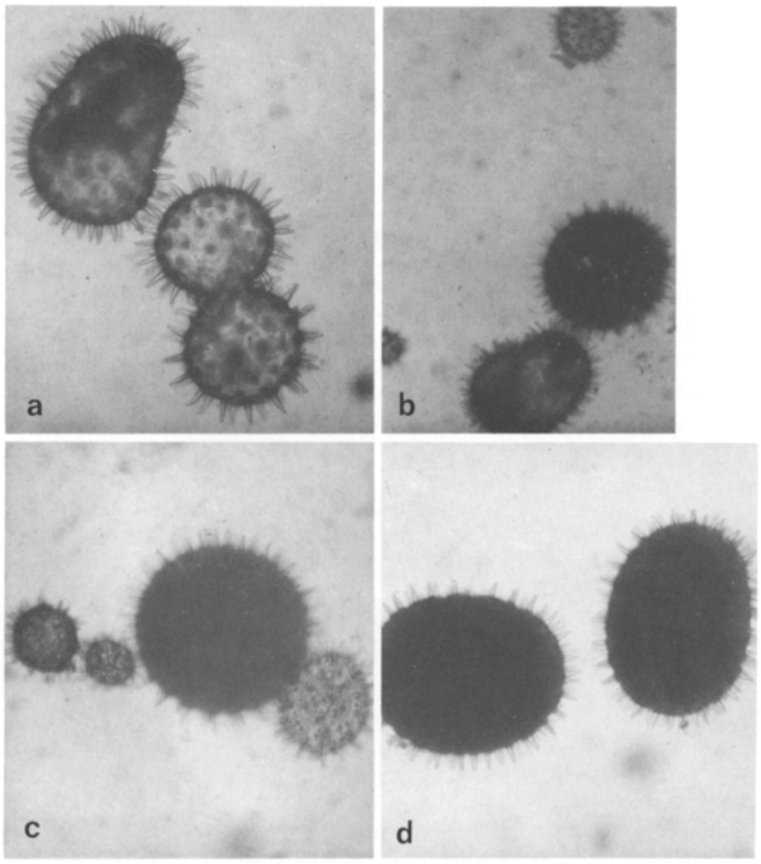

Fig. 3a–d. Normal and abnormal pollen grains in hybrid U.I.92×U.I.313. **a** Unstained and abnormally shaped pollen grains. Failure of Cytokinesis probably caused pollen grains not to separate. **b** Normal (darkly stained) and abnormal (partially stained) pollen grains. **c** Normal (darkly stained) and abnormal (unstained under sized) pollen grains. **d** Pollen grains with abnormal shapes but darkly stained

same anthers. Pollen grains of okra are normally spherical (Fig. 3 c). In the F_1 hybrid plants there were a few oblong pollen grains which also stained dark red with acetocarmine (Fig. 3 d). They were slightly larger than normal ones and may have developed from diads, thus being unreduced pollen grains (Cockerham and Galletta 1976).

Among the four parents used in this study, pollen stainability ranged from 95.20% in U.I.92 to 98.12% in U.I.215. However, in the two F_1 hybrids only 39.41% and 35.80% of pollen were stained in 1bk-1×U.I.215 and U.I.92×U.I.313, respectively (Table 1). This observation suggests that the hybrid plants are partially sterile. It was also observed that some malformed pollen also stained light or dark red. However, none of the under-sized pollen grains stained. The small non-stained pollen appeared empty while some large ones were also empty and others contained granular cytoplasm. The low levels of pollen viability associated with the F_1 hybrids could be attributed to inbalanced dosage of genes from the parents and unequal distribution of chromosomes during meiosis (Duck 1964; Gibson et al. 1971).

Seed production and viability

The quantity of seed produced by the hybrid plants is low with the average number per fruit being 14.08 and 20.75 for crosses 1bk-1×U.I.215 and U.I.92×U.I.313, respectively (Table 1). In the parents, number of seeds per fruit ranged from 68.12 to 97.25. There were slight variations in the size and colour of seeds produced by hybrid plants. In general, empty hybrid seeds were coloured brown. Apart from low quantity, the viability of seeds produced by hybrid plants was low. Although hybrid 1bk-1×U.I.215 produced fewer seeds per pod, more of them germinated than in hybrid U.I.92×U.I.313. The average number of viable seeds per fruit was 5.6 and 4.7 in the former and latter hybrids, respectively. In the hybrid 1bk-1×U.I.215 about 43% of seeds were empty while 12% had small embryos that did not develop to fill the seed cavity. All of these embryos attained the late torpedo stage and could not develop further because of insufficient endosperm. When these seeds were placed on moist filter papers germination did not occur.

The partial sterility of the F_1 plants coupled with their production of only a few viable seeds suggest a reproductive isolation of these two okra types. Gene transfer can, however, be effected between them for improvements. Through a series of backcrosses, it should be possible to transfer some of the attributes of the 'Guineen' type of okra to the more popularly grown and consumed 'Soudanien' type.

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