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Tetraploids, triploids, and 2n pollen from diploid interspecific crosses with cassava

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Summary. Interspecific crosses of five cultivated cassava varieties (2n = 36) were made with two related *Manihot* species, *M. epruinosa* (2n = 36) and *M. glaziovii* (2n = 36). From these diploid interspecific crosses, four spontaneous tetraploids (2n = 4x = 72) and two triploids (2n = 3x = 54) were isolated for the first time in cassava. Occurrence of relatively high frequencies (0.1% – 35.6%) of 2n pollen and of apomixis seems to be associated with sexual polyploidization. The tetraploids and triploids were very vigorous and one of the tetraploids performed as well as the best variety in uniform yield trials conducted in Nigeria. These spontaneous polypoloids provide greater genetic variation and offer an opportunity to breed radically new cassava varieties. Approaches for isolating and utilizing the polyploid cassava clones for varietal and population improvement are discussed.

Key words: Cassava – Polyploidy – 2n Pollen – Apomixis – Interspecific crosses

Introduction

Cassava (*Manihot esculenta* Crantz) of the family *Euphorbiaceae* is an important staple food crop in the tropics, particularly in tropical Africa. The importance of cassava is increasing in Africa owing to its tolerance to stresses such as drought, soil acidity, and low soil nutrient levels. Other factors confirming its importance include its relatively high productivity (Hahn and Keyser 1985) and the diverse uses to which it can be put (Hahn 1989).

Cassava is normally a vegetatively propagated and monoecious crop, but sexual propagation frequently occurs through seed. It is an agronomically annual or biennial crop but could be botanically regarded as perennial. It was first domesticated in the Americas between 5,000 and 7,000 B.C. (Lathrap 1970). The amount of variation within cassava and in the genus *Manihot* is great. Rogers and Appan (1973) reported as many as 98 *Manihot* species confined to the tropical Americas, of which 80 are native to Brazil. Most of the *Manihot* species are also monoecious like cassava but some are hermaphroditic. The wild *Manihot* species are normally propagated by seed, and usually produce tuberous roots of minor economic importance.

Cassava is generally considered, based on regular meiosis, as a diploid with 2n = 36 chromosomes (Graner 1941; Allem 1984). However, Bai (1987) reported meiotic irregularities such as laggards, delayed separation of bivalents, nonorientation, and noncongression of bivalents, restitution nuclei, monads, dyads, and polyads. Cassava was reported as an allopolyploid with a basic chromosome number of x = 9 (Perry 1943; Jennings 1963; Umanah and Hartmann 1973). A segmental allotetraploid origin of cassava with a basic chromosome number of x = 9 has also been postulated based on the number of nucleolar chromosomes and the pachytene karyology, suggesting that cassava is derived from crosses between two closely related parental taxa (Magoon et al. 1969a).

However, the ancestry of cassava is not known, although it has been speculated that several *Manihot* species are its ancestors (Renvoize 1972; Rogers and Appan 1973; Allem and Hahn 1988). A total of 27 *Manihot* species hitherto examined all have chromosome number 2n = 36 (Allem 1984). *M. glaziovii,* which has been most extensively used by cassava breeders (Nichols 1947; Cours 1951; Abraham 1957; Jennings 1963; Magoon et al. 1966; Hahn et al. 1980) for interspecific crosses with cultivated cassava, was reported to be similar to cassava in its number of chromosomes and karyotype (Magoon et al. 1966; Umanah and Hartmann 1973).

Several wild *Manihot* species have been used in the cassava breeding program of the International Institute of Tropical Agriculture (IITA) to introduce their desirable genes into cassava, particularly for resistance to diseases such as African cassava mosaic virus (ACMV) and bacterial blight (CBB), and to pests such as cassava green spider mite (CGM) and the cassava mealybug (CM), as well as for low cyanide content. Resistance to ACMV and CBB and such characteristics as low cyanide content have been successfully introduced from *M. glaziovii* into locally adapted but disease-susceptible cassava varieties (Hahn et al. 1980; IITA 1987, 1988). Recently, facultative apomixis has been found in several cassava varieties derived from interspecific crosses (IITA 1988) and in several *Manihot* species. IITA has acquired over 50 *Manihot* species from the Americas, and interspecific hybrids of cassava with some of these species such as *M. epruinosa* (2n = 36), *M. leptophylla* (2n = 36), and *M. brachyandra* (2n = 36) have been raised, in addition to the interspecific hybrids with *M. glaziovii*.

Tetraploids (2n = 4x = 72) of cassava were obtained through somatic doubling by use of colchicine (Abraham et al. 1964; Graner 1941; Magoon et al. 1969 b) and are referred to as autotetraploids. They do not exhibit as much variation as natural polyploids and have undesirable characteristics, including stunted growth and breakdown of polyploidy during clonal propagation and low yields (Bai 1987). Triploids (2n = 3x = 54) obtained from the cross between the autotetraploids and normal diploid cassava (2n = 2x = 36) performed better than the diploid and tetraploid parents (Abraham et al. 1964; Jos et al. 1970, 1987).

No spontaneous polyploid at the chromosome level of 2n = 72 has so far been reported within the genus *Manihot* (Allem 1984). This paper reports for the first time the occurrence of spontaneous tetraploids and triploids and of 2n pollen (unreduced gametes) from the diploid (2n = 36) interspecific crosses, as well as from the open-pollinated progenies of the interspecific hybrids.

Materials and methods

Interspecific crosses of five cassava (*M. esculenta*) varieties (2n = 36) with their related *Manihot* species, *M. epruinosa*-1 (2n = 36) and *M. glaziovii* (2n = 36), were made in 1981, 1983, and 1986 as shown in Table 1.

The cassava varieties used are progenies sharing a common genetic background with 58308, a derivative of an interspecific cross between cultivated cassava and *M. glaziovii* (Beck 1982). *M. epruinosa*-1 was introduced in seed form from Brazil and *M. glaziovii* (ceara rubber) was obtained from Nigeria. The latter was introduced into Nigeria initially as a rubber tree, probably in the 1930s, and has been extensively used as a shade tree for cocoa plantations. It often grows wild on the edges of forests in the country.

The F_1 progenies (Table 1) were examined for morphological characteristics, pollen stainability, percentage of 2n pollen, and cytological behavior. Flower buds of appropriate stage were fixed in a mixture of 3 parts ethyl alcohol to 1 part propionic acid for 2–3 days, after which they were transferred to 70% alcohol. The pollen grains were stained in 2% propiono-carmine, the deeply stained pollen being considered as viable. For cytological analysis, PMCs (pollen mother cells) were smeared in 2% propiono-carmine following the method described by Swaminathan et al. (1954). The counts per unit area and size of the stomata on abaxial leaf surface were determined, adopting the fevicol-technique described by Bai and Jos (1981).

Results and discussion

Four tetraploid and two triploid cassava clones were isolated using cytological techniques. On the basis of gross morphological characters and stomatal counts, one tetraploid and two triploids have additionally been identified, but these are yet to be confirmed through cytological techniques. Information on their origin is presented in Table 1.

Several characteristics, such as lamina length and breadth, petiole length, lamina thickness, stomata number and size, and pollen stainability of the spontaneous polyploid (tetraploids and triploids) cassava clones in comparison to the normal diploid cassava variety TMS 30572 are given in Table 2.

The leaves of the polyploids were generally broader and thicker than those of the diploid (Fig. 1, Table 2). The stomata counts per unit leaf area and their size were in general lesser and larger, respectively, for the poly-

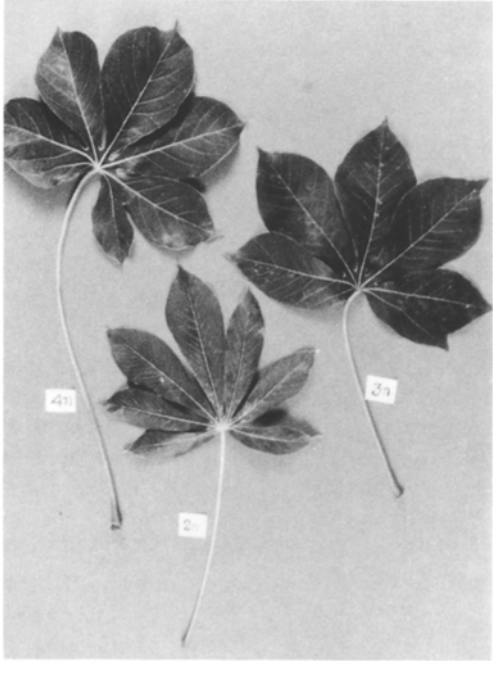

Fig. 1. Fully expanded leaves of tetraploid (top left) and triploid (top right) in comparison with that of diploid (bottom) cassava plants

Table 1. Cross-combinations and year of crossing for interspecific hybridization of cassava with its related *Manihot* species: *M. epruinosa-1* and *M. glaziovii*

Cross-combination		Year of crossing	F_1 plants obtained	Plants examined	Clones with[a]	
Female parent cassava variety	Male parent *Manihot* species				4×	3×
TMS 30555	× *M. epruinosa-1*	1981	185	109		
TMS 4(2)1425	× *M. epruinosa-1*	1983	177	1	1	
TMS 30572	× *M. epruinosa-1*	1986	14	5		
TMS 30572	× *M. glaziovii*	1986	49	20		
TMS 63397	× *M. epruinosa-1*	1986	138	87	1[b]	2[b]
TMS 63397	× *M. glaziovii*	1986	59	25	1	1
TMS 91934	× *M. epruinosa-1*	1986	36	2		
TMS 91934	× *M. glaziovii*	1986	32	6		
TMS 85/00302	× (1-15 × 1-19)[c]	1987	16	2		1
TMS 30572 OP		1980	1020	1	1	
TMS 1525 OP		1983	1022	1	1	

[a] 4× stands for tetraploid and 3× for triploid
[b] to be confirmed
[c] a clone resulting from the cross between two progenies of TMS 30555 × *M. epruinosa-1* of 1981

Table 2. Characteristics of the spontaneous tetraploid and triploid cassava clones

Plant no.	Source/origin of plant	Pollen stainability (%)	Lamina[a]		Petiole length (cm)	Lamina thickness (μ)	Stomata		
			Length (cm)	Breadth (cm)			No./unit area	Size (μ)	
								Length	Breadth
Tetraploids									
TMS 84/00316	TMS 4(2)1425 × *M. epruinosa-1*	49.9	13.0	6.6	27.6	146.5	37	32.5	40.8
TMS 87/00018-42	TMS 63397 × *M. glaziovii*	55.8	10.1	3.2	11.3	136.8	73	25.5	29.8
TMS 81/01623	TMS 30572 OP[b]	64.7	13.1	6.6	24.7	149.0	38	30.3	41.8
TMS 84/00136	TMS 1525 OP	48.6	11.0	5.0	19.7	135.8	68	25.0	27.3
Triploids									
TMS 87/00018-28	TMS 63397 × *M. glaziovii*	11.6	10.5	4.8	15.7	119.0	58	25.0	31.8
TMS 88/112-7	TMS 85/00302 × (1-15 × 1-19)	10.5	15.2	7.3	25.9	130.8	41	28.3	35.5
Diploid									
TMS 30572		75.1	11.0	3.5	14.3	116.8	77	24.5	26.8

[a] Lamina here refers to middle leaflet only
[b] OP – open pollination

ploids because cell size is larger in polyploids than in diploids (Fig. 2A–C; Table 2). These morphological characteristics can be used for initial screening of progenies for polyploidy of cassava.

Pollen stainability of the spontaneous tetraploids isolated was relatively low compared to diploids, and that of the triploids was even lower because of unequal distribution of chromosomes to their gametes. However, the pollen stainability with a range of 49.9% to 64.7% for these spontaneous tetraploids is very high compared to the range of 22% to 38% reported by Magoon et al. (1969b) for the autotetraploids produced by colchicine treatment.

Chromosome association at metaphase was observed for the spontaneous tetraploid TMS 81/01623 and the spontaneous triploid TMS 88/112-7, as well as for the diploid TMS 30572. In the diploid TMS 30572, the chromosomes paired as 18 II at M–I (Fig. 2D).

In the tetraploid TMS 81/01623, the chromosomal associations at M–I consisted of quadrivalents and bivalents and occasionally two univalents (Fig. 2F); their number varied in different cells. The chromosomal asso-

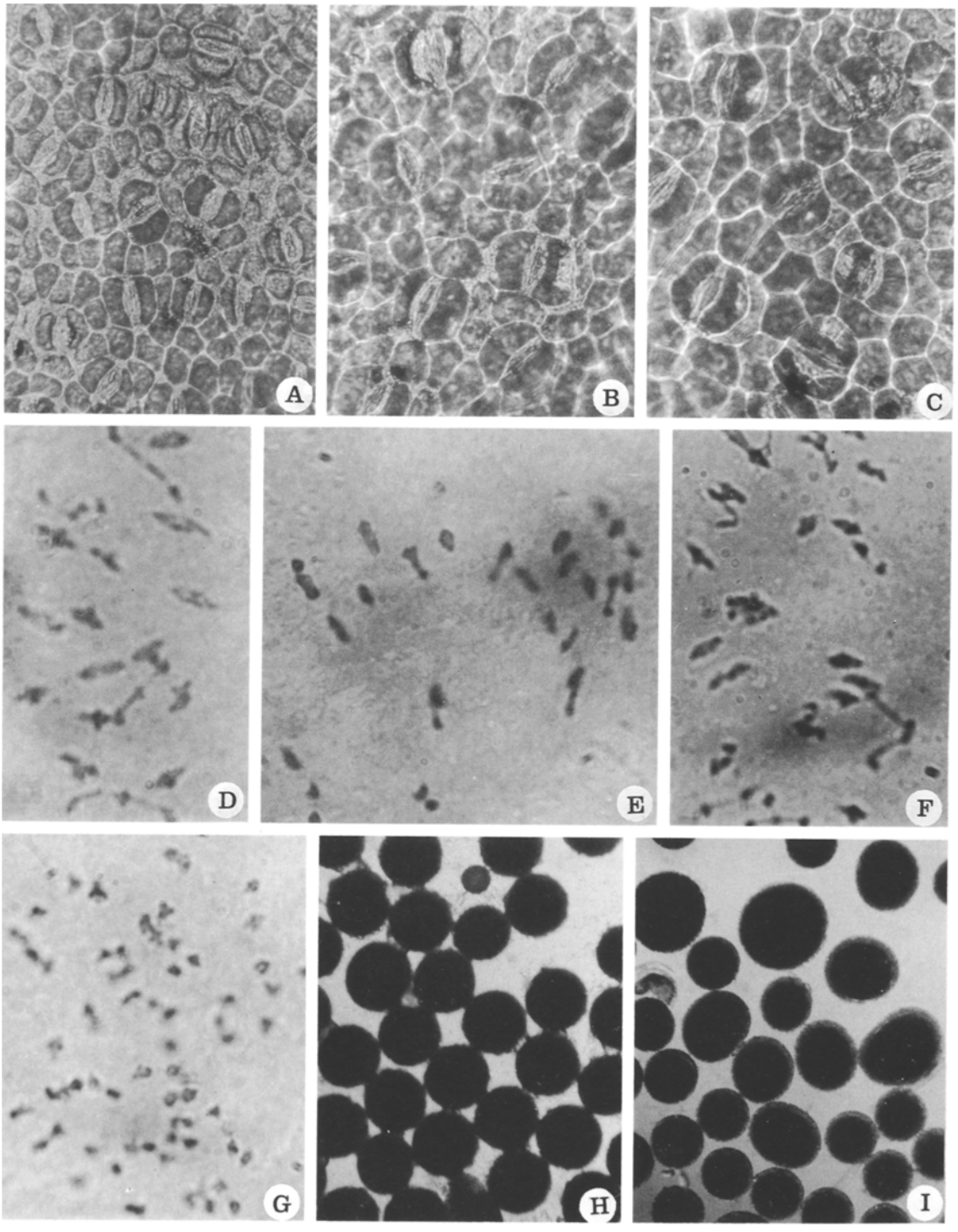

Fig. 2 A–I. Size and number of stomata per unit area of abaxial leaf surface for **A** diploid, **B** triploid, and **C** tetraploid cassava plants. Cytology of: **D** diploid-metaphase I showing 18 II, **E** triploid-metaphase I showing 9 III + 10 II + 7 I, **F** tetraploid-metaphase I showing 3 IV + 30 II, **G** tetraploid anaphase I showing normal separation. Normal pollen (n) **H** from diploid cassava plants in comparison with the pollen **I** from diploid cassava plants producing some 2n pollen

Table 3. Frequency of 2n pollen in various interspecific hybrids of cassava

Cross-combination	Clones examined	2n pollen producing clones	Frequency interval (%)							
			<1.0	1.0–4.9	5.0–9.9	10.0–14.9	15.0–19.9	20.0–24.9	25.0–29.9	>30
1. TMS 30555 × *M. epruinosa*	116	37	11 (29.7)	17 (46.0)	5 (13.5)	3 (8.1)	1 (2.7)	– –	– –	– –
2. TMS 63397 × *M. epruinosa*	84	21	12 (57.1)	5 (23.8)	3 (14.3)	– –	– –	1 (4.8)	– –	– –
3. TMS 30572 × *M. epruinosa*	5	4	–	2 (50.0)	2 (50.0)	– –	– –	– –	– –	– –
4. TMS 91934 × *M. epruinosa*	2	1	–	1 (100.0)	– –	– –	– –	– –	– –	– –
5. TMS 63397 × *M. glaziovii*	25	12	6 (50.0)	5 (41.7)	1 (8.3)	– –	– –	– –	– –	– –
6. TMS 30572 × *M. glaziovii*	20	17	–	5 (29.4)	7 (41.2)	1 (5.9)	1 (5.9)	1 (5.9)	1 (5.8)	1 (5.9)
7. TMS 91934 × *M. glaziovii*	6	6	–	6 (100.0)	– –	– –	– –	– –	– –	– –
8. TMS 4(2)1425 × *M. leptophylla*	4	3	–	3 (100.0	– –	– –	– –	– –	– –	– –

Figures in parenthesis are percentage of number of clones falling in respective frequency classes over total number of 2n pollen producing clones examined

ciation of 16 cells at M–I revealed a mean frequency of 2.6 IV + 30.7 II + 0.2 I per cell. In the triploid, TMS 88/112-7, the chromosome association at M–I consisted of trivalents, bivalents, and univalents (Fig. 2E), the distribution of which varied in various cells. An analysis of 21 PMCs at M–I revealed a frequency of 7.3 III + 11.5 II + 9.1 I per cell. Both the tetraploid and the triploid showed a mixture of autosyndetic and allosyndetic pairing. This also indicates that cassava and its related *Manihot* species, *M. epruinosa* and *M. glaziovii,* have some chromosomes and segments in common. Therefore, *M. epruinosa* and *M. glaziovii* seem to be close relatives of cassava or they may have a common ancestry with cassava. They could all be segmental allopolyploids. Since the related *Manihot* species are strongly apomictic (IITA 1989), they could maintain their own genetic identity through apomixis. But they can be expected to hybridize naturally with cassava.

In the tetraploids, the anaphase I separation and disjunction of chromosomes varied from normal to nearly normal (Fig. 2G). Subsequent meiotic divisions were also nearly normal in a majority of the PMCs observed, explaining the relatively high pollen stainability of the spontaneous polyploids. Three tetraploids set fruits and seeds under open-pollinated conditions. Out of 133 seedlings raised from open-pollinated seeds of three tetraploid clones, 50%–90% per clone exhibited typical tetraploid characteristics.

In the triploids, anaphase I separation and disjunction of the chromosomes to the poles was irregular, as expected. Subsequent meiotic divisions were also irregular, resulting in low pollen stainability.

To identify a plausible mechanism associated with the relatively frequent occurrence of these spontaneous polyploids, the frequencies of 2n pollen for several interspecific cross combinations were examined and the data are summarized in Table 3. A majority of the interspecific hybrid diploid clones produced 2n pollen, but their frequencies varied with cross-combinations and also with clones within respective cross-combinations. The hybrid clones resulting from interspecific crosses involving *M. glaziovii* as a male parent produced the highest percentage of 2n pollen producing clones followed by hybrids with *M. epruinosa*-1. The 2n pollen ranged from 0.1% to 35.6% of the total stainable pollen per flower bud. Out of 101 clones producing 2n gametes, 21 produced between 5% and 10% 2n gametes, and four clones produced above 20% 2n gametes.

The size of the stainable 2n pollen of the polyploids was larger than that of the diploids. In the tetraploids, the diameter of the stainable pollen ranged from 175 μ to 195 μ with a mean of 182 μ, whereas in the triploid it ranged from 155 μ to 190 μ with a mean of 163 μ. The diameter of the 2n pollen of the diploid hybrid clones (Fig. 2I) ranged from 165 μ to 286 μ, with a mean of 187 μ compared to the normal n pollen (Fig. 2H), which ranged from 110 μ to 143 μ with a mean of 121 μ.

The mechanisms of 2n pollen formation in diploid cassava are being investigated. In diploid potatoes, three mechanisms (parallel spindles, premature cytokinesis I

and II) of 2n pollen formation were reported (Mok and Peloquin 1975). The frequencies of microspores observed at the tetrad stage for the families of the present diploid interspecific crosses with cassava seem to indicate more than one mechanism involved.

It seems that the tetraploid cassava clones must have been produced from matings between diploid cassava varieties and their related diploid *Manihot* species that produce 2n eggs and 2n pollen (bilateral sexual polyploidization). Triploids would have been produced by matings between diploids that produce n eggs and 2n pollen, respectively, and vice versa. The mode of 2n egg formation needs to be investigated through progeny testing. The facultative apomixis that was previously reported to occur in some cassava varieties (IITA 1988) may, however, play an important role in 2n egg formation.

The procedure for screening the segregating cassava breeding material for polyploidy will consist of the following.

(1) Visual observation of gross morphology, particularly leaves (the leaves of polyploids are generally broader, thicker, and darker green). The leaf characteristics of the tetraploids are distinct even at the early seedling stage, thus permitting early screening.

(2) Observation of stomata. The stomata of polyploids are generally larger in size but are fewer in number per unit area.

(3) Observation of pollen grain size. The stainable pollen grains of tetraploid plants are quite uniformly large; some stainable pollen grains of triploid plants are large, but some are small and others are often unstainable.

(4) Cytological confirmation of those that are selected, based on the morphological characteristics indicated above.

(5) Further confirmation based on progeny test.

The spontaneous polyploid cassava clones provide greater genetic variability associated with the increased number of genes, and they give an opportunity for radically new cassava varieties to evolve and diverge from the present ordinary cassava. They also provide a new approach to make use of the related *Manihot* species for introducing desirable genes from different *Manihot* species into cultivated cassava.

The polyploids isolated from the progenies of the diploid interspecific crosses with cassava all turned out to be cassava types, probably because of a tendency of the progenies to segregate toward the cassava parents, although the possibility of having the polyploids of wild species types cannot be ruled out. The presence of multivalents in the present polyploids suggests that pairing and crossing-over are taking place between the chromosomes of cassava and its related *Manihot* species. These phenomena can facilitate cassava breeding because not many backcrossings are required to recover desirable traits, and yet introgression of desirable genes from the related species can be enhanced. Our spontaneous cassava polyploids are very vigorous, unlike the autotetraploids produced by colchicine treatment.

A further significant fact is that, since cassava is a vegetatively propagated crop, the polyploid cassava can take advantage of the vigor, which is probably due to heterotic effects, and of the greater range of tolerance to extreme environmental conditions as shown in many other interspecific hybrids (Clausen 1947) and polyploids (Stebbins 1950). Polyploidy may thus help extend the range of adaptation and the area of cultivation of the crop to much wider ecological conditions.

The tetraploid clone TMS 81/01623 was advanced to uniform yield trials conducted under a wide range of environmental conditions from the high-rainfall, acid-soil area to the dry savannah zone in Nigeria. It gave an average yield of 19 tons/ha, which is comparable to that of TMS 30572, a leading improved cassava variety in the country. It was resistant to cassava mosaic virus and bacterial blight disease, and the food quality of its root was acceptable. This provides a strong indication that primary tetraploids can be directly used as varieties without being further improved. In future, attempts will be made at IITA to further improve them through the introduction of a new gene pool via unilateral ($4x \times 2x$) and bilateral ($2x \times 2x$) sexual polyploidization, recombination, and selection. The cassava breeding populations will be improved at the tetraploid level with respect to yield and tolerance to environmental stresses. From the populations, it is hoped to extract much better tetraploid cassava varieties, which are superior in performance to ordinary diploid cassava under the wide range of environmental conditions in Africa. The improved tetraploids can then be used to produce improved triploids by hybridization with the improved diploids. The polyploids will also be used as a step in the process of transferring single valuable characteristics, such as low cyanide and resistance to diseases and pests from the related wild species to cassava. The polyploids, particularly triploids, can be used to produce an aneuploid series for genetic studies with cassava, which has been difficult owing to the nature of polyploidy and genetic heterozygosity of cassava.

In conclusion, spontaneous tetraploid and triploid cassava clones with enormous vigor and genetic variation have been produced. The potential of the polyploids as new types of cassava and for new cassava breeding strategies seems to be great and needs to be further explored. The strategies for isolating polyploids and improving cassava breeding populations at the tetraploid level are briefly discussed.

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