

BREEDING FOR FUSARIUM WILT TOLERANCE: AN OVERVIEW OF RESEARCH PROGRAMS AND SEED PRODUCTION POTENTIALITIES IN CAMEROON

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Abstract

With an annual global production of 50 million mt equating about 39% of world production of vegetable oils, palm oil is currently the most important vegetable oil commodity in the world. Despite a total production of 230 000 mt in 2010 representing an overall contribution of less than 1% to world production, palm oil production in Cameroon covers about 90% of the demand for edible oil. The country is currently a net importer of palm oil with an estimated output gap of about 60 000 mt/year. The Government of Cameroon has set up a development plan aiming to absorb this gap by increasing palm oil production to 450 000 mt/year by 2020. This can be achieved mainly through increasing total planted area alongside oil yields. As a result, many investors have been trying to secure land to plant oil palm in Cameroon in the upcoming years, and it is believed that about one million hectares of land in the southern forested zone are concerned. However, the occurrence of *Fusarium* wilt disease in most of the planting areas may seriously thwart these efforts, as it is considered to be the main threat to the development of oil palm plantations in Africa and particularly in Cameroon. The disease was first identified in the region in the early forties, and since then breeding programmes designed to produce wilt resistant seeds have been undertaken.

Currently there are two national seed producers, PAMOL PLANTATIONS PLC (formerly owned by UNILEVER) and CEREPAH La Dibamba. *Fusarium* wilt resistant seeds produced by CEREPAH derives from crosses between Dabou Deli x La Me BPROs, particularly DA 115 D x LM 2T selfs. CEREPAH's current annual wilt tolerant seed production is about 800,000 units, but a production capacity of about 5 million per annum could be attained. At PAMOL, *Fusarium* wilt resistant seeds are mainly derived from crosses between Ekona x Ekona and Ekona x Binga BPROs, particularly descendants of Lb 2/2311 and Bg 312/3. For the past years, average annual production was about 200,000 despite a potential of more than 1 million. Further trials involving local BPROs (Widikum, Batibo) are currently going on in both research units, thus leading to expect the production of more efficient *Fusarium* wilt tolerant seeds in the upcoming years.

INTRODUCTION

Since almost half a decade already, the oil palm has overtaken soybean to become the most important oil crop in the world. The boom of palm oil production was mainly supported by intensive plantings during the two last decades in Malaysia and Indonesia which are the two main producers of palm oil. However, due to increasing regulations preventing the clearing of forests and increased scrutiny of land acquisitions in these countries, it becomes more and more difficult to acquire land for palm oil expansion. According to recent forecasts, there will be no more land available for new oil palm plantings in these two countries by 2020. Major oil palm companies from Southeast Asia have therefore gain awareness of the need to diversify their production areas and seek for new lands for further expansion. In this regard, the Western and Central African regions appeared as natural targets, as they were proven to be the cradle of wild oil palm expansion and are therefore suitable for the establishment of palm plantations. According to recent findings (Pauron and Ballong, 2012), it is estimated that about 2.5 million hectares of land have been recently allocated to oil palm majors in about twenty countries of the Western, Central and Southern part of Africa ranging from Gambia to Cameroon, Angola, Mozambique up to Madagascar. In Cameroon, about 500,000 ha of land have reportedly already been allocated to foreign groups for the establishment of oil palm plantations (Hoyle and Levang, 2012). However, particular care must be considered during the implementation of these new plantation projects regarding *Fusarium* vascular wilt, as it is currently considered as the main threat for palm plantations in Africa. *Fusarium* vascular wilt is one of the most important diseases infecting oil palms in Western and Central Africa (Aderungboye, 1982, Corley and Tinker, 2003). It was observed in Ndian Estate of Pamol Plantations, Cameroon where it was spreading rapidly from the neighbouring Cowan Estate in Nigeria as far back as 1940 (Prendergast, 1957). It has been reported in Ivory Coast, Ghana, Benin, Democratic Republic of Congo (DRC or Ex-Zaire) and Liberia (Flood *et al*, 1989; de Franqueville *et al*, 2011). The disease is caused by a soil borne phytopathogenic fungus, *Fusarium oxysporum* f. sp. *elaedis* (Ho and Varghese, 1986; de Franqueville *et al*, 2004). The first description of the disease was provided by Wardlaw (1946) in DRC who isolated the pathogen from diseased palms. Fraselle (1951) later reproduced artificially the disease symptoms on seedlings and thus confirmed the pathogenicity of the isolates.

The significance of the disease in Africa, with particular reference to Cameroon cannot be over-emphasized. The disease is fatal in its acute form causing huge economic losses in mature oil palm plantations and in the chronic form, it retards vegetative growth thus preventing the production of fruit bunches (Wardlaw, 1950; Waterston, 1953; Guldentops, 1962; Renard and Quillec, 1983; de Franqueville and Diabaté, 1995). It is more severe in replanted estates (Renard and Quillec, 1983), as the pathogen and its population in the soil increase during the first planting, hence causing damages to younger palms. Incidences of up to 40% have been recorded

in field planted palms at Ndian Estate in PAMOL, Cameroon (Hornus *et al.*, 1987). However, the disease is widespread throughout the Southwest administrative Region of Cameroon, as industrial plots from the Cameroon Development Company (CDC) also suffer severe attacks. No other areas with high disease incidence have been reported so far, but this may be due to the fact that almost all industrial estates are located in the Southwest and neighboring Littoral administrative Regions. As a result, almost 70% of the country's total land area suitable for oil palm cultivation have never been seriously investigated for the presence of *Fusarium* wilt and may potentially host the disease. This latter assertion is also reinforced by the intensive and above all totally uncontrolled movement of oil palm seedlings throughout the country over the past years which have certainly contributed to spread the disease.

The causal organism of the disease is widely distributed in tropical soils thereby creating practical difficulties to chemical control as such treatment would be too expensive to cover large expanse of planted areas. The complexity of the disease therefore calls for concerted research in the breeding and selection of resistant progenies (Prendergast, 1957; Guldentops, 1962). It is important here to mention that in the case of the oil palm, the strategy adopted was to breed for multiple defense genes involved in partial resistance rather than to breed for total resistance, as the latter can also be bypassed easily by a pathogen. For this purpose, selected progenies are tested with a large diversity of isolates of the pathogens, thus conferring a non specific and partial resistance but with a larger spectrum than the total specific gene-for-gene resistance. In the case of such multiple partial resistances the term "tolerant" is most of the time preferred and is generally used by breeders and planters to refer to planting materials bearing this trait. Significant efforts have been done so far towards the development of *Fusarium* wilt tolerant elite progenies in the breeding programmes of PAMOL PLANTATIONS Plc and CEREPAL La Dibamba which are the two seed producers in Cameroon. The present paper thus gives an overview of the research programmes on wilt tolerance in force in both structures and overall potentialities for the production of wilt tolerant seeds in Cameroon.

FUSARIUM WILT ASSESSMENT

Early long-standing observations on palms in the field have shown that it was possible to detect differences in susceptibility amongst crosses. This was also proven possible at the pre-nursery and nursery stages after contamination of young seedlings by *F. oxysporum* f.sp. *elaedis* strains. Based on these observations, an early test was developed in order to detect and reproduce tolerant strains (Prendergast, 1963). The technique was later adapted to the development of a method for large scale evaluation of the performance of planting materials at the nursery and above all the pre-nursery stages, as the latter allowed significant gains in space, time and precision (Renard *et al.*, 1972). The efficiency of the early screening test was assessed by several authors (De Franqueville, 1984; De Franqueville and Renard, 1990) and showed a good relationship between pre-nursery index and the degree of resistance in the field.

Fusarium wilt assessment is routinely carried out in a pre-nursery and/or nursery wilt test and under natural infection by several strains. Basically, the incidence of wilt in a cross is recorded as the proportion of wilted palms in the cross. The performance of each cross with respect to *Fusarium* wilt is defined by an index (I) whose value is in inverse proportion to the tolerance of the progeny under consideration (Meunier *et al*, 1979; Renard *et al*,1980). The lower the “I” rating, the higher the tolerance of the cross under consideration. An “I” rating of 100 correspond to the mean of the percentages observed for all the crosses in a trial. The index “Ig” of a progeny represents the ratio between the percentage of palms of that progeny affected by vascular wilt and the mean of the percentages of affected palms of all the progenies of the trial. Each parent is attributed the mean of the indices of the crosses in which it is involved a value Ig which indicates its combining ability, that is, the parents ability to transmit to its progeny either a certain degree of tolerance if the value is less than 100, or susceptibility if the Ig value is greater than 100.

BREEDING FOR WILT RESISTANCE IN THE LOBE PROGRAMME, PAMOL PLANTATIONS Plc.

In 1940, a mysterious new disease later identified as vascular wilt disease by Wardlaw (1946) was observed at Cowan Estate in Nigeria where it was spreading fairly quickly and finally moved into Pamol Ndiain Estate in Cameroon. It then became imperative to undertake a breeding and selection programme designed to produce, eventually, varieties of the oil palm resistant to vascular wilt. At the same time, large scale quantities of Dura x Pisifera (DxP) seeds were required for new plantings. Since outside sources of supply were very limited, it became necessary to produce improved wilt resistant seeds at source. Thus, in 1948 a scientific and comprehensive Research programme was undertaken by Pamol for selection and breeding for improved yields and testing for wilt resistance. This breeding work was undertaken through the Family and Individual Palm Selection (FIPS) scheme.

The work started in 1949 with the development of the foundation stock comprising 18 duras and 24 teneras mainly of Ekona and Cowan origins (Table 3) which were used to produce the first breeding cycle in 1954 and 1955. Selections within the F1 plantings were used to create the F2 planted from 1966 to 1971 with some introductions from NIFOR, La Me (IRHO), Bamenda, Binga (DRC or Ex-Zaire), Ecuador, “Sabah breeding programme” exchange and also by exchange with OPGL (Oil Palm Genetics Laboratory) (Rosenquist *et al* 1990). These diverse origins confer to the Pamol planting material, a wide genetic base which is an important feature of the Lobe breeding programme. Records from the second generation were on FFB yield and its components and comprehensive bunch analysis data. Most of the progenies were also tested in the nursery for resistance to vascular wilt. The breeding of oil palms is now in the third generation in Pamol.

Phase one of the third generation breeding cycle included a combined breeding programme for wilt resistance breeding which began in 1985. This involved breeding for wilt resistance using crosses between the *tenera* 2/2311 (wilt index: 47) and its descendants at Lobe and Bg 312/3 (wilt index: 46) and its descendants at Binga (Democratic Republic of Congo, Ex-Zaire) (Fig. 3 and Fig. 4). These two palms appear to transmit a high degree of wilt resistance to their off springs. In addition, the best *pisiferas* at Lobe come from the selfing of 2/2311T.

Table 3 shows the wilt indices of progenies selected for production of wilt tolerant seeds. The progenies all have I values of less than 90 indicating high tolerance to vascular wilt.

Table 1: crossing chart used for the production of *Fusarium* wilt tolerant seeds at PAMOL

DURA	POLLEN GROUP	A	B	E	H
PROGENY		CWN 3	NDN 212	Lb 158	Lb 135
	PARENTAGE	23/10215T Selfed	3AR/7239T Selfed	2/2311T Selfed	1.2229T x 1.2215P
Lb 10	3.417D x 1.2229T				
Lb 130	3AR/7239T x 2/2311T				
Lb 136	2/2311T x 2/5622T				
Lb 158	2/2311T SELFED				
Lb 200	3AR/7239T x 2/2809T				
Lb 214	2/5622T SELFED				
Lb 235	37/1649D x 31.4958T				
Lb 240	2/2319D x 31.4958T				
Lb 242	24-16-12T x 70.14.208D				
Lb 244	35/2718D x 31.4958T				
Lb 256	2/1301T x 1/10920T				
Lb 261	4-26-10T x 24-16-12T				
Lb 263	4-26-10T x 74.11.25.5T				
Lb 267	2/5622T SELFED				
Lb 323	2/11308D x 2/10323T				
Lb 328	L2T x D10D				
Lb 344	2/1301T x 2/2311T				
Lb 349	35/1609T x 35/0911T				
Lb 376	2/2311T SELFED				

BREEDING FOR WILT RESISTANCE IN THE CEREPAH LA DIBAMBA PROGRAMME

La Dibamba is part of the French IRHO/CIRAD network alongside other research stations in Africa such as La Mé in Ivory Coast or Pobè in Benin. From the “International Experiment” after world war II initiated by IRHO which involved exchanges of material and crosses amongst various BPROs (Breeding Populations of Restricted Origins) from research institutes throughout the world, three major findings were outlined:

- The existence of substantial variability between the different BPROs (populations) studied;
- The additive nature of the heredity of the number and average weight of bunches of the different BPROs;
- The superiority of between-origin and generally complimentary crosses compared to within-origin crosses.

Later on, these results were used to develop a RRS (Reciprocal Recurrent Selection) breeding scheme in force in the IRHO/CIRAD network where the different BPROs were divided into two distinct groups based on the complementarities of their traits: Group A comprising origins with a small number of large bunches and group B including origins with a large number of smaller bunches. Group A origins were used as *dura* female parent and included Deli, Angola and some Cameroon populations whereas group B origins were used as *pisifera* male parents mainly made up of African populations (La Mé, Yangambi-Sibiti, N.I.F.O.R., Cameroon). The various stages of the selection process consisted of a succession of screening phases aimed at identifying the best hybrid combinations during comparative inter-group DxT/P testing of parents and genetic mixing phases where the best parents in the same group are recombined to obtain a new improved population. Details of this RRS scheme are provided by Meunier and Gascon (1972).

A first selection cycle was launched in the late fifties and several trials were laid down in the IRHO network research stations. Out of about 529 crosses assessed, only 15 were selected, thus allowing an overall yield improvement of about 18% compared to the starting population. Various interesting combinations were found amongst crosses between Deli x La Mé and Deli x Yangambi populations, but the latter were less considered during the second selection cycle due to their important vegetative growth and high susceptibility to *Fusarium* wilt. So, during the second selection cycle emphasis was put on Deli x La Mé crosses due to their generally high degree of wilt tolerance and slow growth rate (hence inducing longer economic life span of palm plantations) alongside traits relating to yield improvement which was also estimated to about 18% compared to first selection cycle material (Gascon *et al.*, 1988; Nouy *et al.*, 1991). Average yield of this Deli x La Mé 2nd cycle planting material was about 4.5 tons oil/ha/year in moderate water deficit conditions of La Dibamba.

Seed production was launched in the then La Dibamba station in the mid-eighties. Up to the early 2000, commercial seed production involved reproduction of the best 1st and 2nd cycle crosses, with a progressive shift to 2nd selection cycle material. Since 2005, only crosses from the 2nd

selection cycle are reproduced for commercial seed production purpose. The seeds are made up exclusively from crosses between Deli x La Mé BPROs. Most of the progenies used for *Fusarium* wilt tolerant seed production derive from crosses between DA 115 self x LM 2T self (Fig. 2 and Fig. 5). Based on results of nursery wilt tests, the progenies used for the production of *Fusarium* wilt tolerant seeds are classified according to their I rating.

- I<90 => high tolerance
- 90<I<100 => moderate tolerance
- 100<I<120 => susceptible
- I>120 => highly susceptible

Seeds with a good level of tolerance to *Fusarium* wilt can be produced through the following combinations:

- High tolerant dura x High tolerant pisifera (priority 1)
- High tolerant dura x Moderate tolerant pisifera (priority 2)
- Moderate tolerant dura x High tolerant pisifera (priority 2)

Fusarium wilt tolerant seeds are produced at CEREPAH Dibamba according to the crossing plan in table 2 below. It appears that more than 80% of the *Fusarium* wilt tolerant seeds are produced by the combination of progenies from (DA 115 D self) x (LM 2T self), as both parents are renowned for their high tolerance to *Fusarium* wilt. In order to ensure overall good quality wilt tolerant seeds, the proportion of crosses in yellow (priority 2) in table 2 is kept below 33%. Moreover, pollen from at least 7 different *pisifera* palms from each progeny must be used for each progeny of *dura* mother palms in the table below.

Further testings for wilt tolerance are currently going on with improved material from diverse origins (Widikum) and recent prospecting exercises.

Table 2: Crossing plan for the production of *Fusarium* wilt tolerant seeds at CEREPAH Dibamba

Dura	Year plant.	progeny	Parentage	Number of palms	Pisifera	
					LM 2T self	
					LM 16844 ²	LM 19029 ¹
					LM 18978 ²	
	87	LM 12165 ¹	LM 2531 D AF	93	C1001 F	C1001 F
	93	LM 16578 ¹	LM 2509 D x LM 3394 D	115	C1001 F	C1001 F
	95	LM 17114 ¹	LM 3394 D AF	73	C1001 F	C1001 F
	95	LM 18443 ¹	LM 2523 D x LM 2531 D	129	C1001 F	C1001 F
	97	LM 18565 ¹	LM 2523 D AF	102	C1001 F	C1001 F

DA 115 D self	97	LM 18668 ¹	LM 2515 D AF	52	C1001 F	C1001 F
	97	LM 18801 ¹	LM 3394 D x LM 3005 D	129	C1001 F	C1001 F
	97	LM 19016 ¹	LM 2531 D AF	125	C1001 F	C1001 F
	97	LM 19121 ¹	LM 3005 D AF	130	C1001 F	C1001 F
	97	LM 19175 ¹	LM 2509 D AF	110	C1001 F	C1001 F
	87	LM 12565 ¹	LM 2526 D AF	24		C1001 F
LM 269 D x DA 115 D	97	LM 18744 ²	LM 5216 D x LM 5100 D	105		C2301 F
	97	LM 18745 ²	LM 5155 D x LM 5100 D	77		C2301 F
DA 5D x DA 3D	87	LM 13533 ²	LM 3257 D AF	53		C2501 FX

X¹ : Highly tolerant (I < 90)

X²: moderately tolerant (90 < I < 100)

CAMEROON'S WILT TOLERANT SEED PRODUCTION POTENTIAL

PAMOL breeding programme

There is a production potential of 1.4850 million standard seeds, 1.2705 million wilt resistant seeds and 0.1045 million highlands seeds. This potential is not however attained because not all the selected palms are currently used for seed production. Figure 1 shows the actual wilt tolerant seed production from 2000 to 2011. Overall average production for this period is 224 473 wilt tolerant seeds/year.

CEREPAH breeding programme

Significant efforts were done by CEREPAH in the nineties to extend the seed gardens using 2nd cycle planting material. On the basis of number of parent palms available on the field, the current production capacity of CEREPAH is estimated to 9 million seeds/year of which 2/3 are *Fusarium* wilt tolerant. However, due to financial constraints, only about 1/3 of this potential is currently being used. Hence for the past 12 years an average production of 567 971 wilt tolerant seeds/year was recorded. Unlike the PAMOL programme, the production capacity has increased here during the past years, and if appropriate funding is made available this production could be extended to about 5 million seeds/year within a period of 2 to 3 years.

CONCLUSION

Being located in the oil palm belt, Cameroon also hosts most of the pathogens of this crop of which *Fusarium* wilt which is the most serious threat for oil palm cultivation in Africa. As a result, comprehensive breeding programmes in order to control of the pathogen were initiated

early by the two national seed producers Pamol and CEREPAH. Improved planting material with good level of wilt tolerance is available in both structures, with an overall current national production capacity of about 2 million wilt tolerant seeds/year. The room for manoeuvre is more important for CEREPAH which seed garden is largely underused, as its production capacity could be rapidly extended to about 5 million seeds/year. Indeed, the country has a good production capacity and could support massive plantings in the upcoming years.

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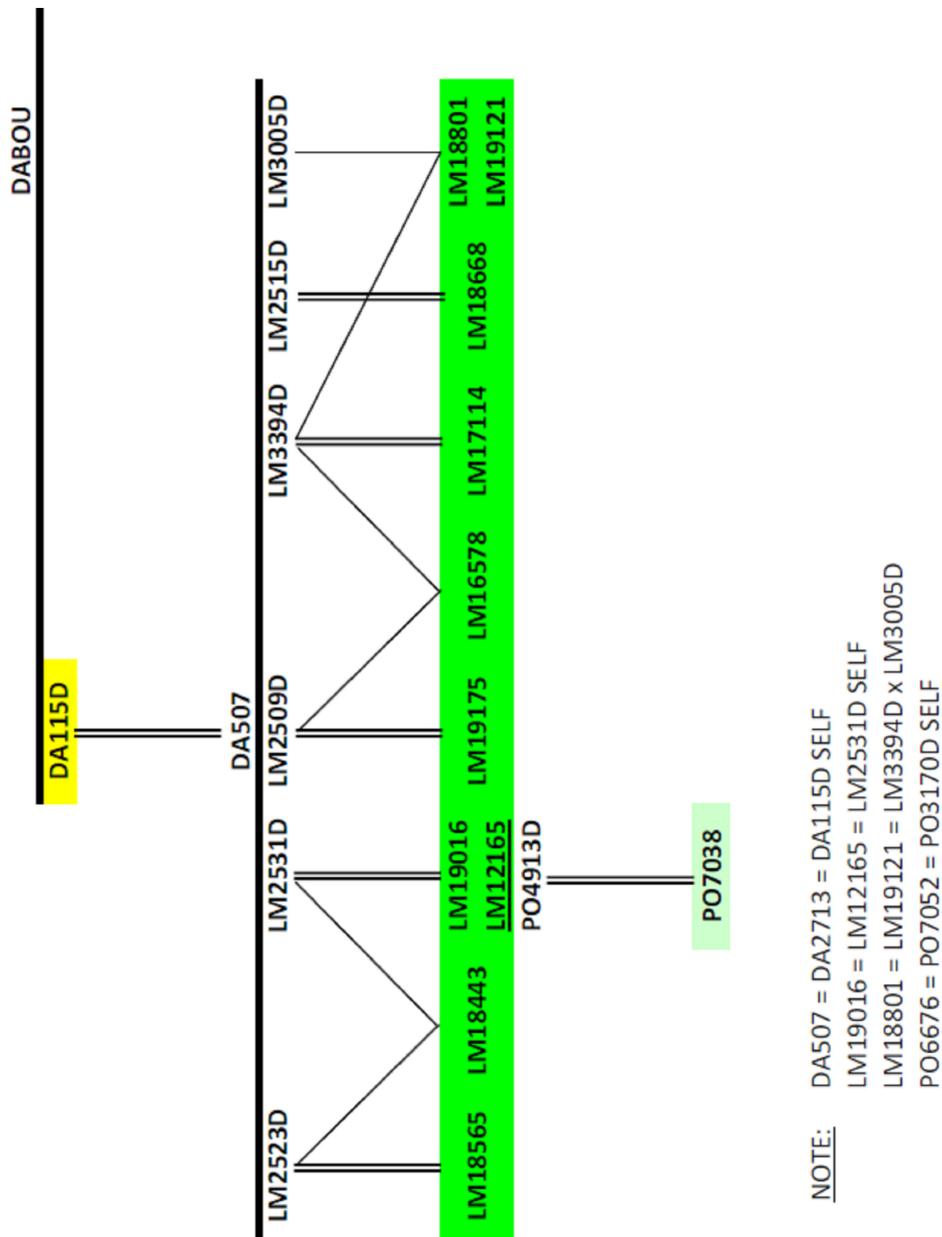
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Fig. 1: *Fusarium* wilt tolerant seed production at PAMOL and CEREPAH during the past 12 years



DA 115 D self wilt tolerant planting material used at CEREPAH

Fig. 2: Pedigree of

Table 3: Parentage, performance and wilt tolerance index of progenies used for seed production at PAMOL

PROG. CODE No.	PARENTAGE	ORIGIN	NUMBER OF PALMS		PERFO
			DURAS	PISIFERAS	FFB/PALM/YR
10	3.417D x 1.2229T	Calabar x Angola	1		109.2
158	2/2311T SELFED	Ekona Selfed	8	4	110
376	2/2311T SELFED	Ekona Selfed	26	1	110
200	3AR/7239T x 2/2809T	Ekona x Ekona	3		85.9
328	L2T x D10D	Lame x Dabou Deli	18		123
256	2/1301T x 1/10920T	Ekona x Ekona	1		103.4
323	2/11308D x 2/10323T	Ekona x Ekona	12		118
130	3AR/7239T x 2/2311T	Ekona x Ekona	23		101.2
244	35/2718D x 31.4958T	Deli INEAC x INEAC	9		99.9
136	3AR/7239T x 2/2311T	Ekona x Ekona	4		105
263	4-26-10T x 74.11.25.5T	Yalingimba x Yalingimba	26		94.5
214	2/5622T SELFED	Ekona Selfed	3		77.3
267	2/5622T SELFED	Ekona Selfed	21		77.3
235	37/1649D x 31.4958T	Deli(NDN) x INEAC	31		87.3
240	2/2319D x 31.4958T	Ekona x INEAC	27		104.6
242	24.16.12T x 70.14.208D	Yangambi x Yangambi	31		92.5
261	4-26-10T x 24-16-12T	Yalingimba x Yalingimba	3		75.1
344	2/1301T x 2/2311T	Ekona x Ekona	8		105
349	35/1609T x 35/0911T	(Deli Dumpy x Ekona) x (Deli x Ekona)	10		104
		TOTAL	265	5	
		MEAN			99.12

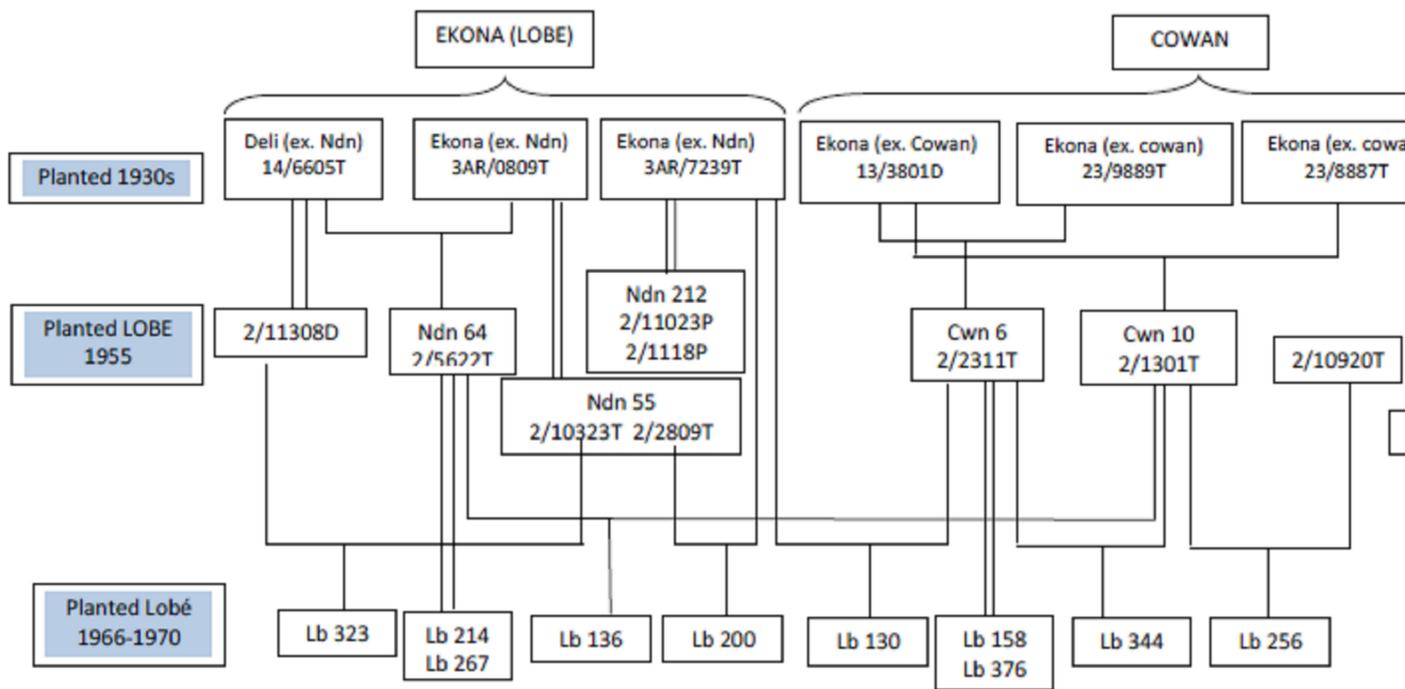


Fig. 3: Pedigree of Ekona planting material used at Pamol

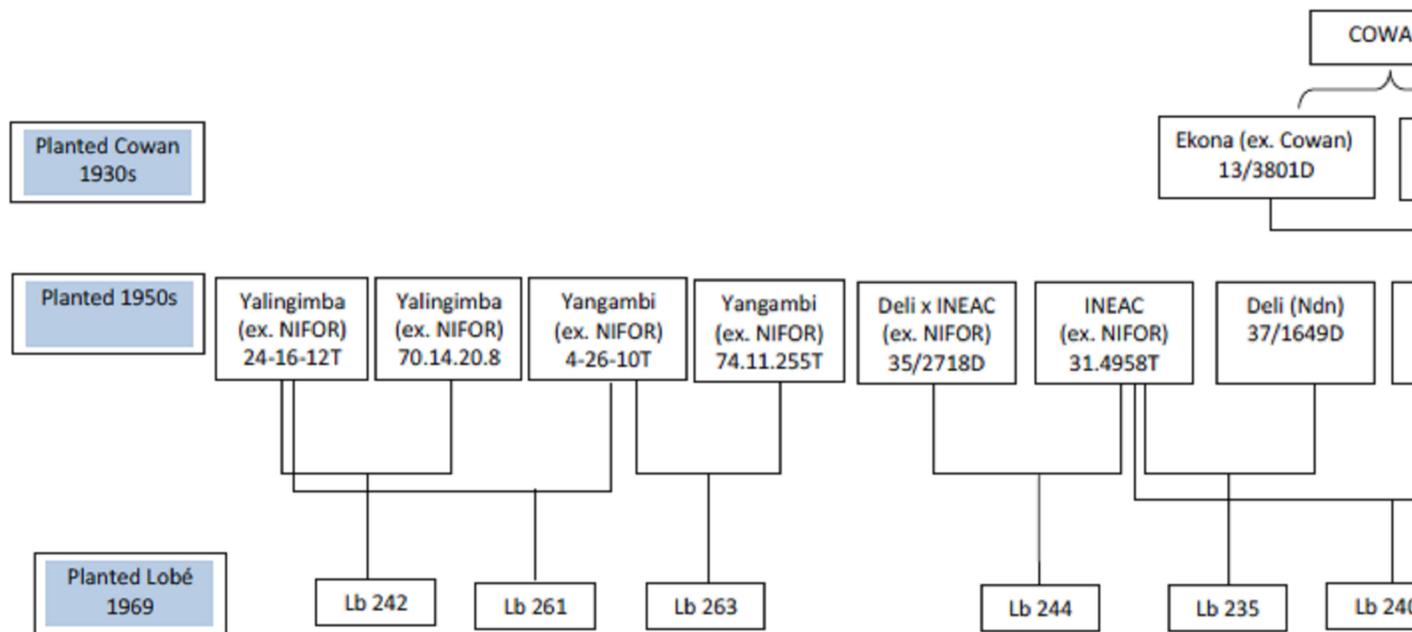


Fig.4: Pedigree of Binga planting material used at Pamol

