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Early Sign of Dwarf Stem Trait Inheritance in Third Cycle *Dura* Derived from Nigerian Prospection Material - A Study in Large Polybag Nursery

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Selection for annual increment of short trunk (dwarf) is one of the objectives in this oil palm breeding programme with the expectation of prolonging the economic lifespan of a cycle of field cultivated commercial plantings. Bi-monthly vegetative measurement of seedlings in the main nursery indicates the early inheritance of low height increment trait in the third cycle of duras *developed from Nigerian Prospection Material (NPM) of MPOB trial 0.150 as compared to the third cycle of inbred lines Limited Breeding Programme (LBP)* dura*, which is considered as the traditional Deli* dura *population. The main nursery measurement showed that the third cycle inbred NPM* dura *was shorter and the character could have been inherited from the selected second cycle parents. Other agronomical observations related to palm physiology on the uptake of magnesium recorded during the study is also discussed in this paper.*

Keywords*: Oil palm, Nigerian prospection material, nursery growth measures.*

Oil palm has the potential of sustaining high oil yields for 25 years or more. Yield for commercial oil palm field normally peaks at 8 years after planting and declines after 16-18 years. One of the factors for the decline in potential peak is difficulty in harvesting and maintaining an optimal number of fronds as the height of the palm increases (Chew & Goh, 2003; Mathews *et al.*, 2006; Lee *et al*, 2019). With the acute labour shortage currently faced by the oil palm plantations, the management has little option other than to embrace mechanisation in their daily plantation operations including fresh fruit bunch (FFB) harvesting to increase productivity. Various challenges in mechanising FFB harvesting have been identified and one of them is the high magnitude of annual height increment of the oil palm itself (Abd Rahim *et al*, 2011). Developing progenies with slow height increment for commercial planting is one of the possibilities that can be pursued by oil palm breeders in order to prolong the oil palm economic life and to increase the efficiency of mechanisation in FFB harvesting. Seng *et al.* (2015) reported that the Nigerian Prospection Material (NPM) possesses outstanding vegetative traits especially slow in height increment. NPM is regarded as one of the potential parental sources to produce dwarf commercial planting material (Isa *et al.*, 2008;

Junaidah *et al*., 2008; Musa & Gurmit, 2008; Veriappan *et al.*, 2008). The hybrid DxP progenies derived from this population was reported to be short with an average annual palm height increment of less than 30 cm per year, which is 33-60 per cent less than the typical range in annual height increment of 45-75 cm per year for the current commercial DxP planting material (Arolu *et al*., 2018).

NPM is a part of IOI group's oil palm genetic collections, which was originally prospected together by the Nigerian Institute for Oil Palm Research (NIFOR) and the Malaysian Palm Oil Board (MPOB) [then Palm Oil Research Institute of Malaysia (PORIM)] from Nigeria in 1973 (Rajanaidu, 1985). Subsequent selections from the original accessions and from trial 0.150 were then released to members of the industry in Malaysia by PORIM for further exploitation (Marhalil *et al*., 2017). With the purpose of further breeding and conservation, several *dura* palms from a short family in the second cycle NPM trial which was planted in Mamor Estate, Kluang, Johor in 1997 was selected. The selected palms of short stature were then hybridised by selfing, sibling and outcrossing with a breeding population of African origin of *dura* (Lobe) to produce the third cycle of NPM population. Amirul and Goh in 2019 presented the pre nursery stage vegetative measurement data of these crosses. This paper is the continuation of that study focussing on the vegetative measurement data and additional observation at the large polybag main nursery stage.

MATERIALS AND METHODS

The second cycle parents of the NPM have been crossed to produce the third cycle NPM populations. The list of the hybridisation programmes and the number of seedlings involved in the large polybag nursery study are given in *Table 1*.

The pedigree of the third cycle NPM population and the third cycle of LBP Deli population studied in this trial are given in *Figure 1*.

TABLE 1
LIST OF CROSSES AND NUMBER OF SEEDLINGS INVOLVED IN THE STUDY

Treatment	*Crosses*	*Number of seedlings per rep*	*Number of reps*	*Total seedlings per cross*
T1	Third cycle NPM[1] self mating from parent PKg877/4/12 (D)	12	6	72
T2	Third cycle NPM sibling cross between parents PKg877/4/12 (D) with PKg877/2/10 (D)	12	6	72
T3	Third cycle of NPM parent PKg 877/4/12 (D) outcross with Lobe material Pkg 1238/39/744 (D)	12	6	72
T4 (Control)	Third cycle LBP[2] Deli sibling cross PKg 493/227/2270(D) with Pkg 493/2/11 (D)	12	6	72

NPM = Nigerian Prospection Material
LBP = Limited Breeding Programme

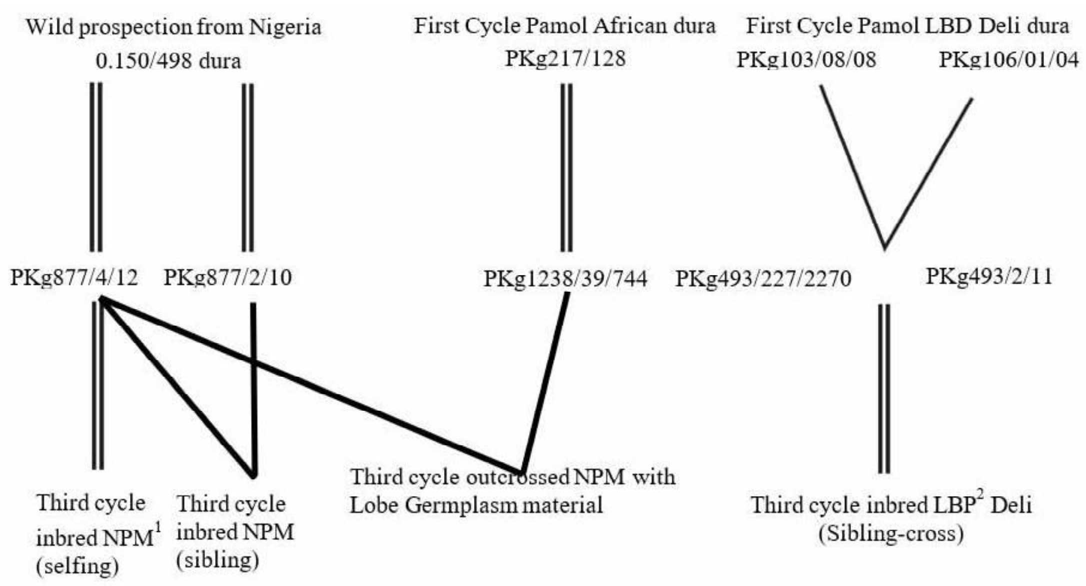

Figure 1 Pedigree of the third cycle NPM population and the third cycle of LBP Deli population.
Notes: [1]NPM - Nigerian Prospection Material, [2]LBP - Limited Breeding Programme

Additional information on the individual fruit characteristics of the second cycle parents are given in *Table 2*.

Although the second cycle of NPM palms were selected for short trunk stature, the oil content in the bunch of the second cycle parent material was low when compared to the LBP and Lobe *dura* materials.

Three-month-old seedlings from the pre-nursery were transplanted to soil filled large polybags (46 cm x 41 cm lay flat) and managed according to the standard operating procedure for large polybags in the main nursery (Mathews *et al.*, 2010). The soil used in the main nursery was highly weathered sedentary Bungor Series, Typic Kandiudult derived from

TABLE 2
FRUIT CHARACTERISTICS AND TRUNK INCREMENT OF THE SECOND CYCLE PARENTS USED FOR CROSSING IN THIS STUDY

Palm ID	*Bunch analysis*								*Average trunk increment*
	N	*F/B*	*WM/F*	*O/WM*	*K/F*	*S/F*	*O/B*	*F WT*	*(cm/year)*
PKg877/4/12	4	59.2	51.0	48.8	8.7	34.5	15.1	11.8	32
PKg877/2/10	4	61.7	47.0	43.3	11.0	34.3	12.7	10.7	39
PKg1238/39/744	5	67.9	62.7	52.9	7.6	23.9	22.7	10.9	100
PKg493/2/11	3	66.9	61.0	53.8	6.6	26.7	22.1	12.6	43
PKg493/227/2270	3	72.6	57.0	49.0	6.6	28.7	20.4	15.6	34

Notes: N - number of bunches analysed, F/B - % fruit/bunch, WM/F - % wet mesocarp/fruit, O/WM - % oil/wet mesocarp, K/F - % kernel/fruit, S/F - % shell/fruit, O/B - % oil/bunch, F WT - mean fruit weight (g).

shales. Seedling height and girth, and number of green leaves per plant were measured at 2.5 months, 4.5 months, 6.5 months and 8.5 months after transplanting. Additional census data on occurrence of chlorotic seedlings, sum of chlorotic leaves per seedling and scoring of frond maturity (i.e. lanceolate, bifurcate and pinnating) was conducted at 6.5 months and 8.5 months after transplanting. Frond maturity index calculation was calculated based on the formula created internally by company breeders as below:

$$\text{Frond maturity index/seedling} = \frac{\sum L*1 + \sum B*2 + \sum P*3}{\sum \text{leaves/seedling}}$$

Where: $\sum L$ = sum of lanceolate leaves
$\sum B$ = sum of bifurcate leaves
$\sum P$ = sum of pinnating leaves
$\sum$leaves = sum of total leaves of all types produced
Numbers indicating weightage of each leave type

At 8.5 months after transplanting, leaf sample from frond number 3 was taken for analysis (Mathews & Chong, 2007). Concurrently, soil sampling from the polybag medium representing each population was conducted. Both set of samples were sent to the IOI Research Centre laboratory, Batang Melaka for nutrient analysis.

RESULTS AND DISCUSSION

Vegetative measurement and growth of seedlings

The vegetative characteristics measured at 2.5 months, 4.5 months, 6.5 months and 8.5 months after transplanting are shown in *Table 3*. The third cycle inbred NPM *dura* self (T1) and sibling crossed (T2) seedlings consistently exhibited short stature. The measured height and girth were significantly lower as compared to both the third cycle of NPM (PKg877/4/12) out crossed with Lobe *dura* (PKg1238/39/744) (T3) and the sibling inbred cross of LBP (T4 Control). This finding is in line with the results of the pre-nursery measurements reported by Amirul and Goh (2019).

Depending on the intensity of inbreeding, the population derived from self (T1) significantly showed lower seedling height and girth throughout the study period as compared to the sibling-crossed population (T2). Only on one occasion, at 2.5 months after transplanting, did one of the third cycle inbred NPM *dura* population derived from sibling cross (T2) showed the highest number of green leaves per plant, slightly surpassing both the third cycle outcrossed NPM and Lobe *dura* (T3) and the control inbred LBP *dura* (T4). However, as the seedlings grew, the third cycle outcrossed NPM and Lobe *dura* (T4) performed well in growth compared to the rest of the treatments. The second cycle inbred NPM parents when outcrossed to a tall palm of Lobe origin produced hybrid vigour in progenies, with significantly taller seedlings, higher number of green leaves and better stem girthing. The effect of hybrid vigour when two distinctly different inbred lines of oil palm germplasm were outcrossed has been reported in progenies (Mathews *et al*., 2007). In general, the trend in height increment is somewhat similar to the parental generation in the order of T1<T2<T4<T3 (*Table 2*).

The fruit characteristics of the second cycle NPM parents used for crossing in this study was rather poor compared to other origins as shown in *Table 2*, as the selection was mainly based on height with the aim of further concentrating on dwarf character in the next cycle. The second cycle of NPM parents do not meet the minimum SIRIM (Standard and Industrial Research Institute of Malaysia) standard

TABLE 3
VEGETATIVE MEASUREMENTS OF SEEDLINGS IN LARGE POLYBAGS

Months after transplanting	*Parameters measured*	*Means of crosses (Refer to Table 1 for details)*				*Trial mean*	*LSD*[1]
		T1	*T2*	*T3*	*T4**		
2.5	Height (cm)	28.2	34.7	40.5	36.6	35.0	1.7
	Number of green leaves per seedling	6.6	7.6	7.4	7.2	7.2	0.3
	Stem girth (cm)	1.4	1.5	1.8	1.6	1.6	0.1
4.5	Height (cm)	40.3	49.9	64.4	53.1	51.9	3.0
	Number of green leaves per seedling	9.1	9.3	10.4	10.0	9.7	0.5
	Stem girth (cm)	2.8	3.6	4.4	3.4	3.6	0.2
6.5	Height (cm)	52.0	63.6	88.1	68.3	68.0	3.9
	Number of green leaves per seedling	11.7	12.5	12.9	13.2	12.6	0.6
	Stem girth (cm)	4.2	4.8	5.7	5.3	5.0	0.3
8.5	Height (cm)	80.5	96.2	136.3	102.8	103.9	5.7
	Number of green leaves per seedling	13.7	14.8	15.5	15.1	14.8	0.6
	Stem girth (cm)	6.5	7.2	8.8	8.7	7.8	0.4

[1]LSD - Least significance difference, P value at 95% confidence level
* Control

MS 157:2017 to produce commercial seeds. The mesocarp to fruit ratio, shell to fruit ratio, oil to dry mesocarp ratio and oil to bunch ratio do not meet the standard of SIRIM. Further introgression with palms of other origins for exceptional fruit characteristics in the subsequent cycle might give breeders a better chance to identify a potential *dura* parent with reasonable height and fruit characteristics. Such an approach is time consuming. At this stage, another possibility is to cross the NPM *dura* with a known *pisifera*, which has shown highest oil content through progeny testing programmes, although such progenies may not show high oil content as the LBP *dura* x *pisifera* progenies. The growth of the third cycle NPM outcrossed at the end of the study period (8.5 months after transplanting) was almost identical to the growth of the hybrid DxP grown in another main nursery study under the best management practices (BMP) in East Malaysia (Donough *et al.*, 2014).

Presence of leaf chlorosis (yellowing) symptom

Throughout the study, the seedlings exhibited symptoms of chlorosis (yellowing) in the leaflets of older fronds of T1 and T2 crosses and the percentage of incidence of chlorotic symptom is shown in *Table 4* and *Figure 2*. This occurred despite a standard fertiliser regime of magnesium (Mg) based Kieserite equivalent to 20 g per palm in the compound fertiliser (12:12:17:2) being applied equally to all the seedlings. Census on the occurrence of chlorosis was conducted to quantify the severity at 6.5 months and 8.5 months after transplanting and the results are given in *Table 4*.

In terms of the symptom progression within the affected populations, the percentage of seedlings affected was negligible between 6.5 months to 8.5 months after transplanting with only the third cycle NPM *dura* self (T1) showing a slight increase in percentage of affected seedlings from 34 per cent to 37 per cent while in the third cycle NPM *dura* sibling crossed (T2) the affected seedlings percentage remained unchanged at 44 per cent for both periods of measurement.

However, the progression of the symptom within the affected seedlings visibly increased over time. In the third cycle NPM *dura* self

TABLE 4
SUMMARY RESULTS FOR PRESENCE OF CHLOROSIS AT 6.5 AND 8.5 MONTHS AFTER TRANSPLANTING

Type of crosses (Refer to Table 1 for details)	*Months after transplanting*	*Percentage of seedlings (%)*		*Average chlorotic fronds in chlorotic seedlings (%)*
		with chlorosis	*without chlorosis*	
T1		34	66	61
T2	6.5	44	56	62
T3		0	100	0
T4 (control)		0	100	0
T1		37	63	67
T2	8.5	44	56	73
T3		0	100	0
T4 (control)		0	100	0

Figure 2 *Randomly selected seedlings of each population at 8.5 months after transplanting. Seedlings are arranged from tallest to shortest, starting from left to right: i) third cycle NPM x Lobe* dura *outcrossed (T3), ii) control Deli* dura *self (T4), iii) normal third cycle inbred NPM* dura *sibling cross (T2), iv) yellowing third cycle inbred NPM* dura *sibling cross (T2), v) normal third cycle inbred NPM* dura *self (T1) and vi) yellowing third cycle inbred NPM* dura *self (T1). The scale of each coloured box is equal to 10 cm. (Note:*NPM - Nigerian Prospection Material)

(T1), the average chlorotic fronds seedlings were 61 per cent at 6.5 months and increased to 67 per cent at 8.5 months after transplanting. Similarly, in the third cycle NPM *dura* sibling cross (T2), the average chlorotic fronds of affected seedlings increased from 62 per cent at 6.5 months to 73 per cent at 8.5 months after transplanting. However, in the current study there is no clear indication that the low height increment of the third cycle inbred NPM materials was attributed by the chlorosis symptoms. The mean height of seedlings with and without chlorosis of affected crosses were almost same at 6.5 months and 8.5 months after transplanting as shown in *Table 5*. The physical appearance of all the representative seedling crosses with or without chlorosis are given in *Figure 2*.

For main nursery seedlings, this symptom is usually associated with Mg deficiency (Corley & Tinker, 2016). Some palms may display abnormalities similar to the nutrient deficiency symptoms which are linked to the genetic origin of the palm. Faizah *et al*. (2016) identified Mg deficiency as one of the possible symptoms of inbreeding depression in a highly inbred oil palm population.

Foliar and soil analysis

Foliar (leaflets from frond number 3) and polybag soil samples representing each population were sent to IOI Research Centre Laboratory for nutrient analysis to study the chlorosis symptom. The samples were not separated based on seedlings of foliar yellowing symptom, but grouped together by treatment crosses.

The summary of results for foliar and soil exchangeable Mg analysis at the end of the study period (i.e. 8.5 months after transplanting) are shown in *Table 6*. The percentage of the level of nitrogen (N) and phosphorus (P) in

TABLE 5
MEAN HEIGHT OF SEEDLINGS WITH OR WITHOUT CHLOROSIS

Type of crosses (Refer to Table 1 for details)	*Months after transplanting*	*Height of seedlings (cm)*	
		with chlorosis	*without chlorosis*
T1		52.2	51.8
T2	6.5	63.5	63.7
T3		0	88.1
T4 (control)		0	68.3
T1		80.6	80.4
T2	8.5	96.1	96.3
T3		0	136.3
T4 (control)		0	102.8

TABLE 6
SUMMARY RESULTS FOR LEAF AND SOIL NUTRIENTS ANALYSIS AT 8.5 MONTHS AFTER TRANSPLANTING

Treatments (Details in Table 1)	*Nutrients in leaf 3 (%)*					*Exchangeable Mg (c mol/kg soil)*
	N	*P*	*K*	*Mg*	*Ca*	
T1	2.83	0.23	1.88	0.19	0.56	0.46
T2	2.80	0.24	2.00	0.19	0.58	0.50
T3	2.72	0.20	1.72	0.20	0.53	0.42
T4 (control)	2.85	0.21	1.44	0.33	0.46	0.43
Means	2.80	0.22	1.76	0.23	0.53	0.45
Standard deviation	0.06	0.02	0.24	0.07	0.05	0.04
Coefficient variation (%)	2	9	14	31	10	8

leaves for all four populations did not differ much, with the trial coefficient variation for the said two parameters being at 2 per cent and 9 per cent respectively. However, the third cycle NPM *dura* derivatives *viz*: self (T1), sibling cross (T2) and outcross (T3) had the lowest percentage of Mg level in leaves ranging from 0.19 per cent to 0.20 per cent, when compared to the control inbred LBP (T4), where the Mg level was the highest in the trial at 0.33 per cent. This gives an indication of poorer Mg uptake in the third cycle NPM *dura* derivatives compared to the control. At the current stage of the study, it remains unknown if it is due to physiological or genetic reasons that chlorosis is not shown in NPM *dura* outcross (T3), in spite of similar low Mg in leaves as compared to the self (T1) and sibling crosses (T2). An antagonistic effect was observed between Mg and particularly with potassium (K) followed by calcium (Ca) in this trial. Lower level of Ca and K was detected in the inbred Deli *dura* (T4) which had the higher level of Mg while the opposite antagonistic interaction was prevalent in the third cycle NPM *dura* derivatives. In spite of having nearly similar low levels of Mg in all three of the third cycle NPM *dura* derivatives, physical chlorotic symptoms occurred only in the self (T1) and sibling cross (T2) .

All phenotypical and anatomical traits are the product of physiological activity related to the complex product of the biochemical process (Acquaah, 2012). The foliar and soil analysis study in this trial indicated that the third cycle NPM *dura* derivatives had lower ability to uptake Mg. It could be due to the differences in root physiology or impairment in the root's lignocellulosic components *viz*: lignin, cellulose and hemicellulose which could have hindered the absorption of high exchangeable Mg from the soil medium. It is known that the Bungor Series derived from shale parents in general have high exchangeable Mg. This low Mg uptake in NPM materials could have some impact on the physiology of the palm itself, as Mg is the main constituent element of chlorophyll and chloroplast (Zelitech, 1971). In order to understand the leaf physiological effect on growth rate, further study on dry matter production of vegetative and reproductive components among seedlings with and without chlorosis symptoms are needed once the palms are planted in the field.

Frond maturity index

Results of frond maturity index scoring at the end of the study period (i.e. 8.5 months after transplanting) are as shown in *Table 7*.

A lower value of frond maturity index indicates slower period taken for seedlings to produce matured pinnating fronds (*Figure 3*). The third cycle inbred NPM *dura* self (T1) had significantly lower frond maturity index compared to the rest of the populations. The third cycle inbred NPM *dura* derived from sibling crossing (T2) ranked with the second lowest frond maturity index followed by the NPM *dura* derived from outcrossing (T3) albeit both are not statistically significant when compared with control seedlings.

CONCLUSIONS

At the end of this main nursery study, both third cycle inbred NPM *dura* [self (T1) and sibling cross (T2)] significantly exhibited short height and girth compared to the introgressed NPM

TABLE 7
SCORING OF FROND MATURITY INDEX AT 8.5 MONTHS AFTER TRANSPLANTING

Type of crosses (Refer to Table 1 for details)	*Fronds maturity index*	
	Means	*CV (%)*
T1	2.75	13
T2	2.77	9
T3	2.79	4
T4 (control)	2.82	3
Mean	2.78	
Standard deviation	0.24	
Coefficient variation (CV) (%)	9	
Least significance difference (LSD)[1]	0.06	

[1]P value at 95% confidence level

Figure 3 Example of leaf types recorded in this study

dura and inbred Deli *dura* (control). However, these populations are susceptible to Mg deficiency which is potentially due to inbreeding depression. Introgression of NPM *dura* population with other African origin palms not known for low height trait did not produce progenies of short and dwarf palms.

This study gives an early indication of the successful transfer of low height trait to the third cycle inbred NPM *dura* population from its predecessor. Breeders however might have to carry out additional selection at the main nursery stage to weed out seedlings with drawbacks such as Mg deficient appearance and lower frond maturity index. Similar phenotypic growth measurements after field planting will be required to confirm these early observations.

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