RESEARCH ARTICLE

Identification of oil palm fruit forms using SNP based CAPS marker among the *Tenera/ Tenera* (T X T) crossed progeny

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ABSTRACT

The oil palm *(Elaeis guineensis)* belongs to the family Arecaceae which is commonly known as African oil palm. In the present study we aimed at molecular identification of fruit forms among T X T progeny. In the present study, DNA is extracted from all 145 progenies of different T X T crosses by using CTAB Extraction Method. Generally fruit form identification is possible only after 4-5 years after ripe of the fruit. But by using CAPS marker EgSHP-Forward-TTGCTTTTAATTTTGCTTGAATACC, Reverse - TTTGGATCAGGGATAAAAGGGAAG which governs in the identification of fruit forms based on the thickness of the shell, which will identify the fruit form at seedling stage which saves a lot of time and space. The study identified 75 as dura, 44 as tenera, 6 progeny as pisifera lines. The results showed that the markers identified are able to clearly characterize the dura and pisifera genotypes.

Key words: oil palm, CAPS marker, *SHELL*, pisifera

Introduction

Elaeis guineensis is a species of palm commonly called as African oil palm (or) Macaw fat. It is the principle source of palm oil. It comes under kingdom plantae, Family Arecaeae, Genus *Elaesis*, species *E. guineensis*. It is now naturalized in Madagascar, Sri lanka, Malaysia, Indonesia, Central America, West indies and several islands in India and pacific ocean (Corley and Tinker 2003). The closely related American oil palm *Elaeis oleifera* and a more distantly related palm *Attalea maripa*, are also used to produce palm oil. Human use of palm oil may date as far back 5000 years in West Africa, in the late 1800s archaeologists discovered palm oil in a tomb at Abdyos dating back to 3000 BCE (Murphy 2014). It comprises of two species of Arecaceae, they are used in commercial agriculture in the production of palm oil. The palm oil tree is a tropical plant which grows commonly in warm climates at an altitude of less than 1600 feet above mean sea level. Mature palms are single stemmed and grow up to 20m tall. The leaves are pinnate and reach 3-5m long. A young palm produces about 20 leaves a year. The flowers are produced in dense clusters, each individual flower is small with three sepals and three petals. The palm fruit takes 5-6 months to mature from pollination to maturity. It is reddish, about the size of large plum and grows in large bunches. Each fruit is made up of an oily fleshy outer layer (pericarp) with a single seed (palm kernel) also rich in oil. When ripe, each bunch of fruit weighs between 5 and 30m kgs depending on age of palm trees.

The oil palm genotypes are divided in to dura, pisifera and tenera forms based on the shell thickness which is a monogenic and co-dominantly inherited trait. The dura (D) genotypes consists of thick shell (Sh/Sh, dominant homozygote) whereas pisifera (P) genotype has a shell less with recessive homozygous sh/sh allele. The tenera (T) genotype has a shell less which has 30% more mesocarp and oil production than dura and pisifera, which is generally produced as hybrid from the cross between dura and pisifera. The tenera hybrid yields

more oil and also is the basis for commercial palm oil production in all the oil palm growing parts of the world. Identification of these three fruit forms is a challenging task for oil palm breeders and growers. Molecular tolls aids the breeding programmes to a great extent which increases the specificity and reduces time (Babu et al. 2017; Babu and Mathur 2016; Kumar et al. 2018). However, the crude form determination can be possible only after 4-5 years by dissection of the fruit based on the thickness of the shell and fiber ring which requires a lot of time and space. Babu et al. (2017) identified one cleaved amplified polymorphic site (CAPS) marker for differentiation of oil palm fruit type which produced two alleles (280 and 250bp) in dura genotypes, three alleles in tenera genotypes (550, 280, and 250bp) and one allele in pisifera genotypes (550bp). The shell allele sequencing results showed that two SNPs were present, of which SNP2 contributed for variation of fruit forms. The nucleotide 'A' was present in only dura genotypes, where as 'T' was present only in pisifera genotypes, which in turn led to the change of amino acid lysine to aspargine. The objectives of the present study are 1) Isolation, purification and quantification of genomic DNA of selected T X T progeny lines of oil palm genotypes and 2) Identification of fruit form of T X T progeny seedling using CAPS marker.

MATERIALS AND METHODS

Fresh tender spear leaves samples from T X T progeny lines of oil palm genotypes were collected for extraction of DNA. Mid rib of each leaflet was removed and middle portion of the leaflet, which has fewer veins, without pigment was taken for DNA extraction. The DNA was extracted using modified protocol of Babu et al (2017). The list of the progeny used in the study is given in table 1.

SSR amplification using PCR

The forward and reverse sequences of the primers were obtained from Babu et al. (2017). Thermal reaction were carried out in a reaction mixture (20 ìl) consisting of 10 X buffer (Himedia), 2 ìl having 15 mm MgCl2, 0.2 mM of each forward and reverse primer, 2 ìl of 2 mMdNTPs, 0.2 ìl of 1 U of Taq DNA polymerase (Invitrogen, USA) and about 25-50 ng of template DNA. The PCR amplifications were performed in a Thermocycler (Biorad, USA) programmed for an initial denaturation of 3 min at 95^{0}Cfollowed by 35 cycles of 30s at 95^{0}C, 30s of 50^{0}C annealing temperature, extension of 1 min at 72^{0}C, with a final extension of 10 min at 72^{0}C, and hold at 4^{0}C. The PCR products were fractioned on 3 % Agarose gel.

Restriction site analysis

Ten ìL of the PCR product obtained in the amplification with SHELL gene specific primer were digested with 10 U of different restriction enzymes (Genetix, USA) along with given specific buffer. Digestion was performed overnight at 37^{0}C. Restriction fragments were visualized by electrophoresis as described above.

RESULTS AND DISCUSSION

Isolation of genomic DNA and quantification:

The genomic DNA is extracted from the spear leaf samples of already established oil palm garden. DNA 145 T X T progeny lines by using CTAB Extraction Method. Quality of genomic DNA is checked on 0.8% agarose gel, stained with ethidium bromide (EtBr) and documented using Alpha Imager gel documentation System. The quantity of DNA present in each sample is determined by comparing the intensity of sample DNA bands with the intensity of standard DNA bands *i.e.* of ë DNA marker.

Molecular identification of fruit form in T X T progeny:

In the present study, CAPS marker EgSHP-Forward-TTGCTTTTAATTTTGCTTGAATACC, Reverse –TTTGGATCAGGGATAAAAGGGAAG is used for the identification of fruit forms among T X T cross plants. Among 145 samples of T X T progeny, 92 are dura form, 47 are tenera form, 6 pisifera (Table 1). Dura, tenera and pisifera represented 64%, 32% and 4% of total progeny (Fig 1). The agarose gel pattern of the progeny using CAPS marker given in figure 2. Similarly Ritter *et al.* (2016) have used a molecular marker system composing of three primer pairs and two restriction enzymes that allowed in differentiation of three different *Sh* alleles. The developed marker system has been validated in *dura* and *pisifera* genotype from different origins which covered the standard gene pool that was currently used by the most of oil palm breeders. Recently, Babu et al (2017) also reported validation of the CAPS marker, on 80 DXP cross progeny lines, 60 lines of TxT cross progeny (Pisifera improvement block). All the results confirmed that the tenera genotypes had allele from both the dura and pisifera

genotypes as expected and could differentiate the dura and pisifera fruit forms.

Fig. 1: Pie chart representing fruit forms of T X T Series progeny.

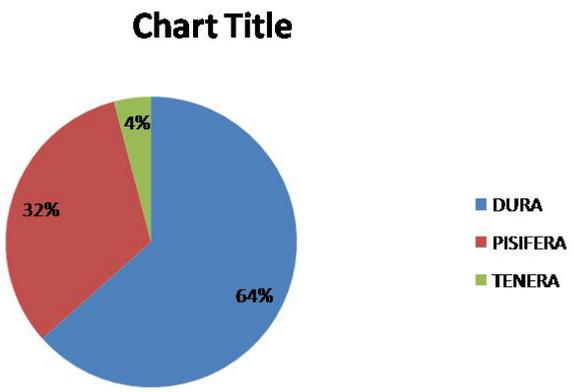

Table 1: The details of the TXT progeny along with fruit form.

Sl. No.	ID	Cross ID	Fruit form
1	L.99	16DX	Tenera
2	L.128	227DX	Dura
3	L.137	239VRX22VR	Dura
4	L.149	239VRX22VR	Dura
5	L.139	239VRX22VR	Tenera
6	L.149	239VRX22VR	Dura
7	L.134	227DX	Dura
8	L.150	239VRX22VR	Dura
9	L.138	239VRX22VR	Tenera
10	L.147	239VRX22VR	Dura
11	L.141	114VRX45VR	Tenera
12	L.130	227DX	Dura
13	L.148	239VRX22VR	Dura
14	L.106	228DX	Dura
15	L.145	239VRX22VR	Tenera
16	L.142	114VRX45VR	Dura
17	L.124	228DX	Tenera
18	L.100	16DX	Dura
19	L.89	6DX	Dura
20	L.84	16DX	Pisifera
21	L.82	16DX	Dura
22	L.83	16DX	Tenera
23	L.90	6DX	Dura
24	L.109	228DX	Dura
25	L.76	6DX	Dura
26	L.120	228DX	Tenera
27	L.72	6DX	Pisifera
28	L.108	228DX	Tenera
29	L.85	16DX	Dura
30	L.96	228DX	Dura
31	L.93	228DX	Tenera
32	L.20	NO NUMBER	Dura
33	L.97	228DX	Pisifera
34	L.55	6DX	Dura
35	L.54	6DX	Tenera
36	L.118	228DX	Tenera
37	L.35	448DPX429DP	Tenera
38	L.98	16DX	Dura
39	L.133	227DX	Tenera
40	L.116	228DX	Tenera
41	L.102	16DX	Tenera
42	L.80	16DX	Dura
43	L.87	6DX	Tenera
44	L.103	16DX	Tenera
45	L.125	228DEX	pisifera
46	L.57	6DX	Dura
47	L.61	6DX	Dura
48	L.119	228DX	Tenera
49	L.73	6DX	Dura
50	L.64	6DX	Dura
51	L.81	16DX	Dura
52	L.101	16DX	Tenera
53	L.94	228DX	Tenera
54	L.58	6DX	Dura
55	L.143	70VRX27VR	Dura
56	L.16	NO NUMBER	Dura
57	L.50	6DX	Dura
58	L.42	NO NUMBER	Tenera
59	L.27	NO NUMBER	Tenera
60	L.56	6DX	Dura
61	L.117	228DX	Tenera
62	L.26	448DPX429DP	Tenera
63	L.59	6DX	Dura
64	L.127	228DX	Dura
65	L.62	6DX	Dura
66	L.43	NO NUMBER	pisifera
67	L.40	NO NUMBER	pisifera
68	L.123	228DX	Dura
69	L.24	NO NUMBER	Dura
70	L.33	448DPX429DP	Dura
71	L.03	NO NUMBER	Dura
72	L.19	NO NUMBER	Dura
73	L.150	NO NUMBER	Dura
74	L.10	NO NUMBER	Dura
75	L.7	448DPX429DP	Dura
76	L.09	NO NUMBER	Dura
77	L.39	448DPX429DP	Dura
78	L.65	6DX	Tenera
79	L.49	448DPX429DP	Dura
80	L.29	448DPX429DP	Dura
81	L.31	NO NUMBER	Dura
82	L.08	NO NUMBER	Dura
83	L.30	NO NUMBER	Dura
84	L.23	NO NUMBER	Dura
85	L.13	NO NUMBER	Dura

86	L.41	NO NUMBER	Dura
87	L.231	139VRX202VR	Tenera
88	L.255	272VRX202VR	Tenera
89	L.226	139VRX202VR	Tenera
90	L.234	272VRX202VR	Dura
91	L.233	272VRX202VR	Dura
92	L.224	139VRX202VR	Tenera
93	L.279	239VRX202VR	Tenera
94	L.225	139VRX202VR	Tenera
95	L.257	272VRX202VR	Dura
96	L.262	272VRX202VR	Tenera
97	L.244	139VRX202VR	Tenera
98	L.271	239VRX202VR	Dura
99	L.264	272VRX202VR	Tenera
100	L.249	239VRX202VR	Dura
101	L.278	239VRX202VR	Tenera
102	L.248	138VRX202VR	Dura
103	L.223	138VRX202VR	Dura
104	L.246	138VRX202VR	Dura
105	L.166	256VRX45VR	Dura
106	L.218	139VRX202VR	Dura
107	L.216	139VRX202VR	Dura
108	L.253	239VRX202VR	Dura
109	L.193	430DX	Dura
110	L.221	139VRX202VR	Tenera
111	L.252	239VRX202VR	Dura
112	L.235	272VRX202VR	Dura
113	L.272	239VRX202VR	Dura
114	L.261	272VRX202VR	Dura
115	L.247	139VRX202VR	Tenera
116	L.259	239VRX202VR	Dura
117	L.241	272VRX202VR	Dura
118	L.269	239VRX202VR	Tenera
119	L.260	272VRX202VR	Dura
120	L.267	239VRX202VR	Tenera
121	L.263	272VRX202VR	Tenera
122	L.256	272VRX202VR	Tenera
123	L.250	239VRX202VR	Dura
124	L.210	139VRX202VR	Tenera
125	L.195	430DX	Tenera
126	L.165	257VRX45VR	Dura
127	L.238	272VRX202VR	Tenera
128	L.236	270VRX202VR	Dura
129	L.200	430DX	Dura
130	L.243	139VRX202VR	Dura
131	L.254	239VRX202VR	Dura
132	L.270	239VRX202VR	Dura
133	L.182	430DX	Tenera
134	L.177	430DX	Dura
135	L.163	430DX	Dura
136	L.152	239VRX202VR	Dura
137	L.211	139VRX202VR	Dura
138	L.265	239VRX202VR	Dura
139	L.258	272VRX202VR	Dura
140	L.251	239VRX202VR	Dura
141	L.277	239VRX202VR	Dura
142	L.266	239VRX202VR	Dura
143	L.127	228DX	Dura
144	L.220	139VRX202VR	Dura
145	L.273	239VRX202VR	Tenera

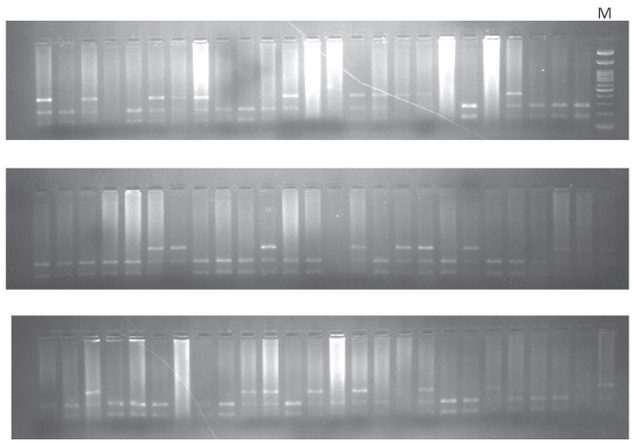

Fig. 2: Hind III digested TxT progeny showing different fruit forms (M-100bp Marker)

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