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## **Oil Palm in India with difference – in various agro-climatic conditions in the tropical and Sub-tropical conditions with inter/mixed cropping systems under irrigated conditions**

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### **ABSTRACT**

Oil Palm (*E. guineensis*) in India is more than 142 years old with the introduction of oil palm as ornamental plant in the botanical gardens of Kolkata. But the commercial oil palm was started during 1961 in the forest lands of Kerala, at the same time when Malaysia and Indonesia also took up commercial planting. The two corporate plantations of Kerala and Little Andaman did not make much progress as a forest crop due to poor management. The Government of India (GoI) also did not want to grow the crop at the cost of forest lands. However the concept of irrigated oil palm as small and marginal holders' crop put forth by the author took a different turn in the Indian oil palm which made India as the largest irrigated oil palm growing country in the different agro-climatic zones. The country has got a potential of about 2 million ha in 18 states under irrigated oil palm. The Government of India through state governments implementing the oil palm development project since 1990 with planting material, plantation maintenance. Later under ISOPOM (Integrated Scheme of Oilseeds, Pulses, Maize and Oil palm) the GoI provided subsidy for seedlings cultivation expenditure, pump sets, drip irrigation, Inter cropping, harvesting tools, etc. So for 20.3 lakh ha in 9 states has been brought under irrigated oil palm as small holders crop starting from less than 1.0 ha to 15 ha. Andhra Pradesh alone has got more than 16.1 lakh ha. The yield levels have gone up to maximum of 50 t fresh fruit bunch (FFB) / ha / year (10 t CPO / ha). Many farmers under average management conditions are getting 20-30 t FFB / ha. A few farmers who are not managing the gardens are getting lesser yield.

Many intercrops like cereals (Maize, Finger millet, even rice), pulses, Oil seeds, vegetables, flowers,

banana, sugarcane etc.), were grown economically during the juvenile period for their livelihood security. Inter crops like Cocoa, Heliconia etc. are grown in adult plantation for getting additional income. Cattle and sheep Rearing is also an attractive mixed farming in adult plantations. Micro irrigation (Drip irrigation) with one drip at seedling stage to 4 drippers at adult stage have been installed to get 40, 80, 120 and 180- 250 l water/palm per day respectively during 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> year onwards. Fertilizer application in two splits is the common recommendation, but the farmers have gone for 3, 6 and 12 splits of fertilizer application. Eighteen processing units spread all over the country processing the FFB with the capacity ranging from 5 t – 40 t /hour. The thousands of Oil Palm growers and the processors working in Hand in Hand are able to elevate their economic situations through Oil Palm. Indian Oil palm deviating the norms of environment conditions from rain forest to tropical plains; climatic condition of temperatures beyond 45 – 48 °C as maximum in summer, 7- 10 °C minimum for at least 2 months in winter. The rainfall ranging from 800 – 3500 mm, low humidity in drier region but a plenty of sunlight, proved to be successful. The pH of the soil ranges from 4.5 to 8.5. Thus the Indian Oil Palm is with difference and can through light of confidence to other countries.

**Key words:** oil palm, inter crops, potential areas, fresh fruit bunches

### **INTRODUCTION**

Oil Palm (*E. guineensis*) in India is also more than 142 years old with the introduction of oil palm as ornamental plant in the botanical gardens of Kolkata. But the commercial oil palm was started during 1961

in the forest lands of Kerala, the same time when Malaysia and Indonesia also took up commercial planting. The two corporate plantations of Kerala and Little Andaman raised under rain forest conditions did not make much progress as a forest crop due to inadequate care and management. The government of India also did not want to grow the crop at the cost of forest lands. However the concept of irrigated oil palm as small and marginal holders' crop put forth by the author took a positive turn in the Indian Oil palm which made India as the largest irrigated Oil palm growing country in the different agro climatic zones.

### Potential for growing irrigated oil palm in India

Irrigated oil palm in India is basically small holders' crop grown in the earlier identified nine states in the country as garden land replacing some low value crops. Presently it is being developed in all the 18 states (Fig 1) identified recently by the Government of India team. During the juvenile phase of the first 2 to 3 years, to overcome income loss, farmers have taken up large number of crops as inter mixed, multiple crops as well as mixed farming in the different agro climatic regions. The soil pH ranged from 5.5 in the West Coast to 8.5 in the East Coast, the temperature in the North Eastern India ranges from 7.0 °C to 20 °C minimum and maximum 15 to 30 °C to 18 to 22° C minimum and 35 to 45° C and in some days going up to 48° C (Prasad et al. 2000). The soil type ranges from river alluvium, peat, red loam, and red sandy to black soils. The performances

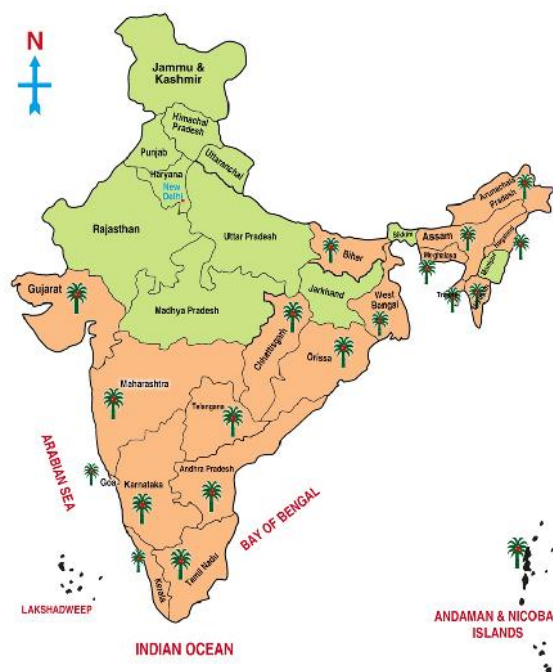


Fig. 1: Potential states for growing oil palm in India

were studied in the different agro climatic and environmental zones (Murugesan and Rethinam 2000).

For resource development, the country has been broadly divided into fifteen agricultural regions based on agro climatic features, particularly soil type, climate including temperature and rainfall and its variation and water resources availability as above. Oil Palm is grown in almost 11 agro climatic regions (Fig. 2) except five regions viz. Western Himalayan Region, Upper Gangetic Plains Region, Trans Gangetic Plains Region, Eastern Plateau and Hills Region, and Southern Plateau and Hills Region.

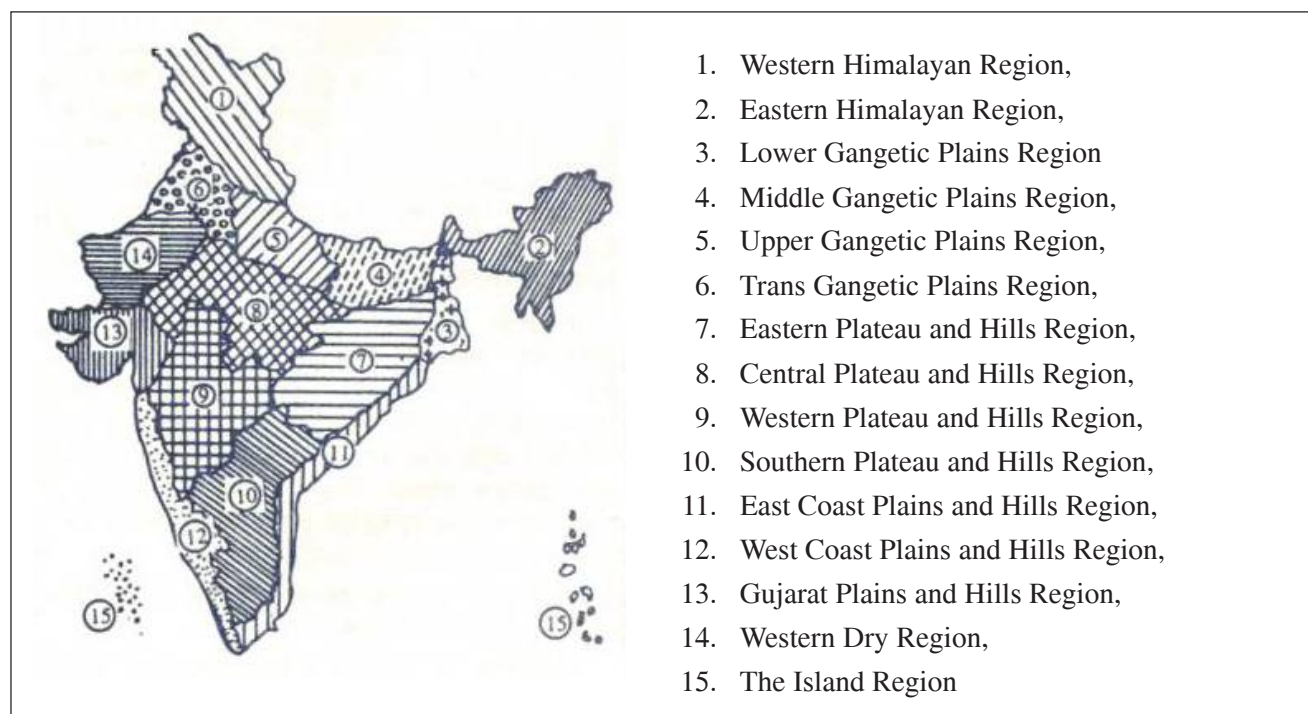


Fig. 2: Agro climatic zones of India

Western Dry Region and Central Plateau and Hills Region.

### Attempts by Government of India to increase vegetable oil production

In order to increase the vegetable oil production, GOI formed Technology Mission on Oil Seeds in the year 1986 along with four other Missions. Besides nine annual oil seeds, Coconut and Oil Palm were also included. Oil Palm is the highest yielding perennial crop which can yield 4 to 8 MT of Crude palm oil and 0.4 to 0.8 MTs of Palm Kernel Oil per hectare per year for more than 25 to 30 years. So this crop was included in the Mission. As part of TMOP a separate project by name Oil Palm Development Project (OPDP) was launched in the year 1990-91. The entire TMOP was implemented by a Special Secretary Agriculture supported by four Joint Secretaries. Over a period of time the scope of TMOP was widened by adding pulses and maize.

Presently only Additional Secretary with a Joint Secretary are managing the programme under the Secretary Agriculture. A new name was coined for the Project ISOPOM and RKVY came in with some additional financial facilities. This set up was fairly giving financial support for various aspects of oil palm

cultivation including intercrops, fencing, creating irrigation facilities etc. During the current Five Year Plan, it is again called as NMOOP where financial assistance has been reduced in many areas.

### Progress made so far in the Oil palm cultivation in India

Oil Palm being a new crop under irrigation, it had some initial starting problem and could reach 35000 ha during VIII FYP. In subsequent plan periods there were ups and downs due to price fall, change in subsidy pattern, power supply position etc., had delayed the area expansion. So far an area of 2.637 lakh ha (Table 1) has been brought under Oil Palm. However, today, it has been proved that Oil palm can be successfully grown as irrigated crop in all the identified states, it can be grown up to 8.0 soil pH, maximum temperatures up to 45°C and minimum up to 10-12°C.

Oil Palm growers of Andhra Pradesh are the fore runners and enjoying the benefit of growing Oil palm. Yield levels under average management is 20 MT FFB /ha / year, with better management farmers are getting 25 to 30 MT FFB/Ha/year and highest yield of 50 MT FFB/Ha (10 MT oil) also was obtained by a lady farmer). This shows the potentiality of this crop in our country. Molecular marker technologies also help in

**Table 1: Oil Palm Planted Area up to March' 2018**

| S. No | States                    | Area planted till 2013-14 (Ha.) | 2014-15      | 2015-16      | 2016-17      | 2017-18      | Total         |
|-------|---------------------------|---------------------------------|--------------|--------------|--------------|--------------|---------------|
| 1     | Andaman & Nicobar Islands | 1593                            |              |              |              | 0            | <b>1593</b>   |
| 2     | Andhra Pradesh            | 146373                          | 8194         | 5289         | 7019         | 5554         | <b>172429</b> |
| 3     | Arunachal Pradesh         |                                 |              |              | 504          | 767          | <b>1271</b>   |
| 4     | Assam                     | 10                              |              |              |              |              | <b>10</b>     |
| 5     | Bihar                     |                                 |              |              |              |              | <b>0</b>      |
| 6     | Chhattisgarh              | 345                             | 490          | 641          | 914          | 888          | <b>3278</b>   |
| 7     | Goa                       | 854                             |              |              |              |              | <b>854</b>    |
| 8     | Gujarat                   | 4916                            | 370          | 460          | 431          | 487          | <b>6664</b>   |
| 9     | Karnataka                 | 45045                           | 1952         | 1100         | 940          | 1119         | <b>50156</b>  |
| 10    | Kerala                    | 6000                            |              |              |              |              | <b>6000</b>   |
| 11    | Maharashtra               | 325                             |              |              |              |              | <b>325</b>    |
| 12    | Meghalaya                 |                                 |              |              |              |              | <b>0</b>      |
| 13    | Mizoram                   | 32977                           | 2790         | 2973         | 1607         | 979          | <b>41326</b>  |
| 14    | Nagaland                  | 21685                           |              |              |              |              | <b>21685</b>  |
| 15    | Orissa                    | 3361                            | 1686         | 886          | 1450         | 813          | <b>8196</b>   |
| 16    | Tamil Nadu                | 260                             | 762          | 664          | 758          | 930          | <b>3374</b>   |
| 17    | Tripura                   |                                 |              |              |              |              | <b>0</b>      |
| 18    | West Bengal               |                                 |              |              |              |              | <b>0</b>      |
|       | <b>Total</b>              | <b>263744</b>                   | <b>16244</b> | <b>12013</b> | <b>13623</b> | <b>11537</b> | <b>317161</b> |

improving the oil palm yield and germplasm (Babu and Mathur 2016; Kumar et al. 2018; Babu et al. 2017). This is the time that we have to encourage for larger area expansion with high target. The area covered under each state as on today and the potential area identified by experts in 18 states are given below:

### Processing facilities available

There are 16 Processing units to process around 420 MT of FFB / hour. Many units have been upgraded

to bigger units (Table 2). Palm kernel oil Processing units also have come. So far 5.733million tons of FFB have been processed and 1.001million tons of Crude Palm oil have been obtained. The cultivation is gaining momentum and will go fast if special mission mode approach by providing all infrastructure facilities for cultivation of Oilpalm like irrigation source, drip irrigation, electricity for lifting the irrigation, price fixing policies, minimum support price, price stabilization fund, crop insurance etc (Rethinam 2018).

**Table 2: Oil Palm FFB Processing units in India**

| S. No                 | Name of the Unit  | Location                                  | Capacity(tonnes/hr) |
|-----------------------|---|---|---------------------|
| <b>ANDHRA PRADESH</b> |   |   |                     |
| 1                     | A.P Cooperative Oil Seeds Growers Federation Ltd.,        | Pedavegi, West Godavari                   | 24                  |
| 2                     | Laxmibalaji Oils  | Takarakandi village Kurupum, Vizianagarm  | 10                  |
| 3                     | 3F Oil Palm Agro Tech.                                    | Yernagudem, West Godavari                 | 30                  |
| 4                     | 3F Oil Palm Agro Tech.                                    | Kottapet village Kaligiri Mandal, Nellore | 10                  |
| 5                     | Godrej Agrovet  | Pothepalli, West Godavari                 | 40                  |
| 6                     | Godrej Agrovet Ltd.,                                      | Chinthampalli                             | 60                  |
| 7                     | Navabharath Agro Products Ltd.,                           | Jangareddygudem, West Godavari            | 120                 |
| 8                     | Ruchi Soya Industries Ltd.,                               | Peddapuram, East Godavari                 | 75                  |
| 9                     | Ruchi Soya Industries Ltd.,                               | Ampapuram, Krishna                        | 50                  |
| 10                    | Radhika Vegetable Oils (PVT.) Ltd.,                       | Garividi, Vizianagaram                    | 15                  |
| 11                    | Sri Srinivasa Palm Oil Mills                              | Rajam, Srikakulam                         | 5                   |
| 12                    | Agro Cooperative Ltd.,                                    | Asilmetta, Visakhapatnam                  | 5                   |
| 13                    | Subrahmanyeswara Agro Products Pvt Ltd.,                  | Ambajipeta, East Godavari                 | 10                  |
|                       |   | <b>Sub Total</b>                          | <b>424</b>          |
| <b>GUJARAT</b>        |   |   |                     |
| 14                    | KalyanAgrl. Crop Sales and Processing Co-op. Society Ltd. | Maroli Bazaar, Navsari                    | 2.5                 |
|                       |   | <b>Sub Total</b>                          | <b>2.5</b>          |
| <b>KARNATAKA</b>      |   |   |                     |
| 15                    | BhadravathyBalaji Oil Palms Ltd.                          | Bhadravathy, Shimoga                      | 10                  |
| 16                    | Directorate of Horticulture, Government of Karnataka      | Kabini, Mysore                            | 2.5                 |
| 17                    | Simhapuri Agro Tech.                                      | Devangere                                 | 5                   |
| 18                    | 3F Oil Palm Agro Tech.                                    | Koppal                                    | 5                   |
|                       |   | <b>Sub Total</b>                          | <b>22.5</b>         |
| <b>KERALA</b>         |   |   |                     |
| 19                    | Oil Palm India Ltd.,                                      | Anchal, Kollam                            | 20                  |
| 20                    | United Oil Palm Planters & Extractors                     | Kuravilangad, Kottayam                    | 0.3                 |
|                       |   | <b>Sub Total</b>                          | <b>20.3</b>         |



| TAMILNADU                 |  |                                    |              |
|---------------------------|--|------------------------------------|--------------|
| 21                        | Cauvery Oil Palm Ltd., ' Godrej  | Varanavasi, Perambalur             | 5            |
|                           | <b>Sub Total</b>   |                                    | <b>5</b>     |
| GOA                       |  |                                    |              |
| 22                        | Godrej Agro vet Ltd.,  | Valpoi, Sattari                    | 5            |
|                           | <b>Sub Total</b>   |                                    | <b>5</b>     |
| ANDAMAN & NICOBAR ISLANDS |  |                                    |              |
| 23                        | Andaman & Nicobar Islands Forest<br>& Plantation Development Corporation | Little Andaman                     | 5            |
|                           | <b>Sub Total</b>   |                                    | <b>5</b>     |
| ODISHA                    |  |                                    |              |
| 24                        | Lakshmi Balaji Oils Pvt. Ltd.,   | Attada Village, Kerada Panchayat   | 5            |
|                           | <b>Sub Total</b>   |                                    | <b>5</b>     |
| MIZORAM                   |  |                                    |              |
| 25                        | Godrej Agrovet   | Bukvannei village Kolasib District | 5            |
|                           | <b>Sub Total</b>   |                                    | <b>5</b>     |
| TELANGANA                 |  |                                    |              |
| 26                        | T S Oil Fed  | Ashwarapet, Apparaopeta            | 3030         |
|                           | <b>Sub Total</b>   |                                    | <b>60</b>    |
| <b>Grand Total</b>        |  |                                    | <b>584.3</b> |

### Oil palm based cropping/farming systems

The review of available scientific information is quite encouraging. Many inter/mixed crops could profitably risen in both juvenile and adult phase.

#### Juvenile phase

A field experiment conducted at NRCOP to find out the most compatible and profitable crops in oil palm during 2001 and 2003. The yield of all inter crops (maize, tobacco, chillies, ridge gourd, bhendi, colocasia, banana, drum stick and guinea grass) except banana, drumstick and ridge gourd was comparable to yield obtained in a pure crop situation. And the benefit-cost ratio of different inter crops was varied from 1.02 for ridge gourd to 2.87 for maize (Reddy et al 2004). In another experiment at NRCOP, Pedavegi during 2003 and 2006, crops i.e., banana, papaya, drumstick, heliconia, bhendi, brinjal, radish, carrot, ridge gourd, bottle gourd, maize, curry leaf, sweet potato, maize, pumpkin and beans were evaluated (Table 3). Out of them, banana, maize, radish, carrot and heliconia were emerged as good yielders. All the crops have found profitable except drumstick, which gave only one good harvest during summer months. Therefore, drumstick may not be a good choice since it needs some amount of stress for fruiting but irrigation is must for oil palm (Rethinam. 2011).

#### Mature phase

A trial conducted at NRCOP RS, Palode, Kerala revealed that combination of cocoa and cinnamon in alternate inter rows, pepper trailed on palms and anthurium planted in the intra row spaces was found ideal for getting maximum net returns. Crops tried were cocoa, cinnamon, pepper, guinea grass, anthurium and kacholam. The amount of run off, soil and nutrient loss from a plantation under different cropping systems and management practices were also quantified from this experiment. In general, the soil and nutrient loss from the plantation was negligible under suitable cover crop mixtures and management practices such as mulching with EFB, organic matter application etc., (Varghese and Sunitha, 2005). Similarly, a study on mixed cropping has been initiated in 2007 at NRCOP, Pedavegi, A.P to observe the performance of various crops viz., cocoa, banana, heliconia, red ginger, betel vine, black pepper, bush pepper, anthurium and crossandra. Based on the results obtained so far, heliconia and red ginger have been found as most successful crops in oil palm.

Jessy Kuttyet al (2005) evaluated five types of medicinal plants viz., *Adhatodabeddomi*, *Alpinicalcarata*, *Kaempferiagalanga*, *Niliriantheshaenianus* and *Asparagus recemosus* in oil palm gardens of different age groups in Kerala. Among

**Table 3: Profitable inter and mixed crops suitable for different regions**

| Name of the state | Juvenile phase  | Mature phase  |
|-------------------|---|---|
| Andhra Pradesh    | Tobacco, maize, banana, oil seedslike ground nut, sunflower, sesamum vegetables-bhendi, chillies, brinjal, tomato, yam, tapioca, cucurbits, turmeric, pulses-black gram, green gram, horse gram, fodder crops, cotton, drumstick, flowers like Heliconia, Mango ginger                | Cocoa, banana, black pepper, long pepper, elephant foot yam, pine apple betel leaf etc. |
| Karnataka         | Cereals-ragi, maize and jowar, vegetables-onion, brinjal, cucurbits, chillies, tomato, cole crops, oil seeds-ground nut and sunflower, flowers-marigold and china aster, fruits-banana and fig, sugar cane, tobacco, cotton, red gram, turmeric, ginger, drumstick, fodder crops etc. | Banana, coffee, vanilla, medicinal and aromatic plants, arecanut, annato etc.           |
| Tamil Nadu        | Sugarcane, banana, maize, vegetables-bhendi, tomato, brinjal, chillies, cucurbits, ground nut and flowers-crossnadra, tuberose, marigold , jasmine  | Banana , Papaya   |
| Orissa            | Maize, sunflower, ground nut, banana, cotton, chillies, tomato, brinjal   | Banana, turmeric, arrow root and pine apple   |
| Gujarath          | Paddy, bajra, groundnut sugarcane, banana and brinjal, flowers like chrysanthemum, Lilies, rose etc.  | Banana  |
| Mizoram           | Paddy, banana, pine apple, ginger, chillies, cucurbits, cowpea, beans, mustard, maize, soya bean etc.   | —   |
| Goa               | Vegetables-bhendi, chillies, brinjal, vegetable cowpea, cluster beans, cucurbits, banana, ground nut, tapioca, cowpea, fruits-banana, papaya and pine apple, flowers like Heliconia   | Arecanut ,pepper  |

them, *Kaempferiagalanga* emerged as the most profitable crop in view of the highest cost benefit ratio.

In addition to the crops, milch animals and sheep rearing are also being done by the farmers for having continuous income and employment.

**Out come**

The progress made so far in terms of area expansion, production of FFB and extraction of oil id quite impressive and the project has proved the following:

1. India can grow Oil Palm successfully to get 15-20 tons FFB/ ha/ year. All the 18 states included in the Oil Palm Development Project (OPDP) are suitable for growing oil palm successfully with the existing varied soil,climatic conditions of very high summer temperature and very low winter temperature, very low rainfall quantity and

distribution. However assured irrigation throughout crop period of 25 to 30 years is must. Drip irrigation is very much suitable.

2. The oil palm presently grown in a wide pH 5.0 to 8.5, maximum temperature of 40o to 45o C, minimum temperature of below 16o C even up to 8o to 10o C for some days, as in North Eastern part.
3. Many of the progressive farmers have got 30 to 40 tons of FFB /ha/year from 6 year onwards which means 5 to 8 tons of CPO/ha/year. Highest yield of 54 t FFB / ha /year was recorded in Mysore State of Karnataka recently. Those farmers who are not taking care of oil palm in terms of optimum management are getting low yields. The farmers who are getting higher yield expanded the area further.
4. The economic development due to oil palm

cultivation is very well seen in the Coastal Andhra Pradesh. Farmers are getting 20 to 30 tons FF B / ha .In Tamil Nadu also now it is picking up. .It was possible to get economic yields in different agro climatic conditions.

5. The farmers could also raise as many intercrops as possible during juvenile phase of oil palm to make good of income loss. The setbacks of price fall for FFB during 1999-2000 and 2008-09 due to global vegetable oil price fall has reflected in low area.
6. Bulk of the waste lands and cultivable waste lands available in this country with adequate underground water facilities to be identified and go for irrigated Oil palm.
7. Oil palm is the best option for increasing the vegetable oil production in the country which can greatly help in building up lively hood, food. Nutritional and bio fuel security.

## CONCLUSION

Indian oil palm is grown in the East, West, North East and Maidan (plateau) regions of the country mostly in the plain arable land avoiding the rain forest, instead helps to build forest for about 30 years .Hence there is fear of deforestation and thereby causing damage to bio diversity. In the West Coast the soil is acidic and temperature ranges between 18 to 38° C, in the East coast the soils Ph ranging from 7.5 to 8.5 and temperature in summer goes beyond 40° C for some time .In the North East pH ranges from 5.5 to 7.0 and in some areas it is peat soil. So Indian Oil palm grown in tropical and sub tropical regions under irrigation is the largest example of growing Oil palm without touching forest lands but, creating forest for 25 to 30 years.Effective utilization of land committed to Oil palm for longer period with array of inter mixed multiple cropping/ farming systems have been demonstrated which are eco friendly and environmentally sustainable. It is always possible to grow the shade loving and shade tolerant crops like cocoa, banana, elephant foot yam, pepper, ginger, colocasia, some medicinal, aromatic and cut foliage plants and shade loving crops like anthurium, orchids, vanilla, betel vine, ginger, cut foliage, Heliconia, red ginger, some medicinal, aromatic and cut foliage plants in mature oil palm gardens. Integrating animals for milk and meat is also possible. All these definitely support that India, oil palm is with difference

and can provide lot of new information to oil palm industry as a whole.

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**RESEARCH ARTICLE**

**Physiological and biochemical responses of oil palm (*Elaeis guineensis* Jacq.) in relation to fresh fruit bunch yield under the influence of different methods and levels of irrigation at different crop factors**

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**ABSTRACT**

The present investigation was carried out on eighteen years old oil palm plantation at ICAR-Indian Institute of Oil Palm Research, Pedavegi, Andhra Pradesh with different methods and levels of irrigation water using crop factors to find out their influence on the physiological and biochemical responses in relation to yield of fresh fruit bunches. The result obtained has significantly indicated the highest relative water content (97.44%) and membrane stability index (25.26%) were observed with drip method of irrigation. Among the levels of irrigation, the highest relative water content (95.51%), membrane stability index (27.11%) were found significantly highest with crop factor 0.8. Among the interaction effects, the relative water content (98.00%), membrane stability index (28.85%) were found significantly highest with drip method of irrigation using crop factor 0.8. Significantly the highest proline content (2.37%) was recorded highest with drip method of irrigation. Among the levels of irrigation, proline (2.56%), lipid peroxidation (8.73n moles g<sup>-1</sup>) activities was found significantly highest with crop factor 0.8. Among the interaction effects, significantly highest proline (2.77%), and lipid peroxidation (9.03n moles g<sup>-1</sup>) activity were observed with drip method of irrigation using crop factor 0.6. The data pertaining to yield attributes has revealed that significantly the yield of fresh fruit bunches (19.84t/ha) were observed with micro-jet method of irrigation. Among the levels of irrigation, the number of fresh fruit bunches per palm per year (7.03), yield of fresh fruit bunches (19.83t/ha) were found significantly highest with crop factor 0.8.

**Key words:** Membrane stability index, oil palm, relative water content, proline, lipid peroxidation and fresh fruit bunch.

**INTRODUCTION**

Oil palm (*Elaeis guineensis* Jacq.) is an introduced crop into India for its valuable edible and industrial oil. The crop is strictly tropical in nature for its growth and development. The performance of oil palm is considered satisfactory in areas endowed with hot and humid tropical climate with optimal temperatures ranging between 80-90° F and average annual rainfall ranging between 2000- 3000 mm with well distribution for a larger part of the year. Zhu et al. (2008) reported that plants receiving direct sunlight for minimum of 5-7 hours per day have been found very much beneficial for optimal growth and development. So, availability of adequate moisture coupled with optimum temperature has been identified as the important factors in determining the yield of oil palm. Irrigation trials conducted on the performance of oil palm have shown positive response to irrigation in terms of growth and yield. Water availability in the soils of oil palm plantation plays an important role for its proper growth (Henson and Harun 2005) and functions as a signal for female sex representation (Jones 1997). In areas of water shortage, it is observed that a large number of male flowers are produced which is coupled with slow growth leading to poor yields Prasad et al. 2000). The basic information relating to water stress responses in oil palm is a prime topic of the day which should be investigated further for screening of tolerant lines for water deficit

coupled with their physiological efficiency (Murugesan and Rethinam 2000). Water deficit is a major abiotic stress, which is widely distributed worldwide over 1.2 billion hectares, especially in the rain-fed areas (Chaves and Oliveira 2004; Kijne 2006; Passioura, 2007). Application of irrigation water as supplemental dose has been reported by several workers to increase the yield of fresh fruit bunches (Gawankar et al. 2003; Rao, 2009; Gajbhiye et al. 2011; Sanjeevraddi et al. 2014). Keeping all these things in view the present investigation has been planned to investigate the influence of methods of irrigation water in conjunction with evapotranspiration based level of irrigation using crop factors with the intension of maximizing the yield of fresh fruit bunches and understanding the physiological responses of photosynthetic pigments in increasing the yield of fresh fruit bunches.

## MATERIAL AND METHODS

The present investigation was carried out on the existing eighteen years old oil palm plantation at ICAR-Indian Institute of Oil Palm Research, Pedavegi, Andhra Pradesh and was laid out in a split-plot design with four replications consisting of main plot treatments with two different methods of irrigation systems and three subplot treatments of irrigation levels using crop factors based on the rate of evapotranspiration. The level of irrigation water using crop factor was calculated as described below by Rao et al. (2016).

Water requirement of a crop is the quantity of water required by the crop in a given period of time for its optimum growth under field conditions. It is a function of rainfall, soil water reserves and evapotranspiration. Water requirement varies from place to place depending on climatic conditions like sunshine hours, temperature, relative humidity, wind velocity, etc. This is the best available method to estimate crop water requirement from direct measurement of evapo-transpiration. In this method, pan evaporation or panman's estimate of evaporation is multiplied by an appropriate crop factor. Water use of crop is very closely related to evaporation. In fact, crop water use is composed of evaporation of water from the soil surface and transpiration of water through the leaves, combined together these two factors are named as evapo-transpiration. While evaporation is easily measured, transpiration is not. Therefore, it is much simpler to relate the crop evapo transpiration to daily evaporation via a crop factor. A crop factor is related to the per cent of ground covered by the crop canopy and therefore will vary depending on the crop stage. For an adult oil palm, 0.7 is considered as crop

factor. The following simple method of calculation has been devised based on the evaporation rates prevailing in the area especially during summer months.

Evaporation from pan evaporimeter: 6.70 mm (for example)

Crop factor: 0.7

Potential evapo-transpiration (PE) = Pan evaporation × Crop factor

PE = 6.07 × 0.7 = 4.69 mm/day

46,900 L/day/ha as 1 mm of rainfall is equal to 1 L m<sup>2</sup>

Since 143 palms are accommodated in one hectare area, the quantity of water per palm per day works out to be 328 litres.

Water holding capacity at not less than 70% of the field capacity is acceptable and will not affect the FFB yield of oil palm significantly.

Therefore the minimum quantity of water to be applied will be:

4.69 mm × 70% = 3.283 mm/day or 32,830 L/ha/day or 220 L/palm/day.

The two methods of irrigation systems adopted were micro-jet and drip, while the three irrigation levels used were based on Crop Factors (CF) 0.6, 0.7 and 0.8. The treatments were: T<sub>1</sub>: Micro-jet method of irrigation system using irrigation level crop factor 0.6; T<sub>2</sub>: Micro-jet method of irrigation system using irrigation level crop factor 0.7; T<sub>3</sub>: Micro-jet method of irrigation system using irrigation level crop factor 0.8; T<sub>4</sub>: Drip method of irrigation system using irrigation level crop factor 0.6; T<sub>5</sub>: Drip method of irrigation system using irrigation level crop factor 0.7; T<sub>6</sub>: Drip method of irrigation system using irrigation level crop factor 0.8. Measurement of water content in a tissue is expressed either on fresh weight or dry weight basis, has been recently replaced by the measurement based on maximum amount of water a tissue can hold. These measurements were referred to as relative water content. The method used was as per the procedure outlined by Catsky (1974). Membrane Stability Index of plant tissue was measured as per the procedure explained by Sairam *et al.* (2002). Proline content of fresh leaves of each treatment was determined by using rapid colorimetric method as suggested by Bates *et al.* (1973). The proline concentration in the sample was determined based on the standard curve using analytical grade proline (SRL, Mumbai) and calculated on fresh weight basis. The level of lipid peroxidation in the leaf tissue was measured in

terms of malondialdehyde (MDA, a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) reaction with minor modification of the method of Heath and Packer (1968). Number of Fresh fruit Bunches per palm were recorded in every harvest and expressed on yearly basis as number of fresh fruit bunches per palm per year. Average yield of fresh fruit bunches per palm in each treatment was multiplied with number of palms planted per hectare (143 palms) and expressed in tonnes. The data thus arrived was subjected to statistical analysis as per the procedure outlined by Panse and Sukhatme (1985).

## RESULTS AND DISCUSSION

Significant differences were observed in the relative water content (Table 1) of leaves with different methods and levels of irrigation. Significantly highest relative water content (97.44 %) in the leaves was observed with drip method of irrigation than micro-jet method of (91.71 %) of irrigation. Among the irrigation levels, significantly highest relative water content (95.51 %) was observed by application of irrigation water using crop factor 0.8. Interaction effect of RWC between methods and levels of irrigation water using crop factor was found non-significant. However, highest relative water content (98.00 %) of leaves was observed with

drip method of irrigation using crop factor 0.8, whereas, lowest (90.82 %) RWC was observed with micro-jet method of irrigation using crop factor 0.7. Relative water content in the leaves of oil palm irrigated with micro-jet or drip method of irrigation has shown no definite trend with the amount of irrigation level increased. Maintenance of normal physiological processes under stress conditions require sufficient cell turgidity or relative water content with less injury to the cell membrane. A small quantity of water loss should therefore cause a shift in turgor so that the leaves tend to maintain high relative water content to retain a high symplast volume which indicates that under water deficit conditions the maintenance of high relative water content is more important in conferring the drought tolerance in the palms. Sun et al. (2011) reported that relative water content in the leaves is integrated with physiological traits regulated by water stress.

Significant differences were observed in the membrane stability index (Table 1) of oil palm leaves with different methods and levels of irrigation. Significantly highest membrane stability index (25.26%) of the leaves was observed with drip method of irrigation than micro-jet method of (19.75%) of irrigation. Among the levels of irrigation, significantly the highest membrane stability index (27.11%) was

Table 1 Effect of methods and levels of irrigation using crop factors on physiological and biochemical responses to yield of oil palm

| Treatments                        | Relative water content (%) | Membrane stability index (%) | Proline (%) | LOX activity (n moles g <sup>-1</sup> FW) | Number of fresh fruit bunches per palm | Yield of fresh fruit bunches (t/ha) |
|-----------------------------------|----------------------------|------------------------------|-------------|---|--|-------------------------------------|
| Irrigation methods (M)            |                            |                              |             |   |  |                                     |
| M <sub>1</sub> (Micro-jet)        | 91.71                      | 19.75                        | 2.01        | 7.20                                      | 6.43                                   | 19.84                               |
| M <sub>2</sub> (Drip)             | 97.44                      | 25.26                        | 2.37        | 7.45                                      | 6.45                                   | 18.17                               |
| LSD (p = 0.05)                    | 0.917                      | 1.222                        | NS          | NS  | NS                                     | NS                                  |
| Irrigation levels (L)             |                            |                              |             |   |  |                                     |
| (L <sub>1</sub> ) Crop factor 0.6 | 94.54                      | 17.25                        | 2.56        | 8.73                                      | 5.49                                   | 17.57                               |
| (L <sub>2</sub> ) Crop factor 0.7 | 93.67                      | 23.15                        | 1.93        | 6.51                                      | 7.03                                   | 19.83                               |
| (L <sub>3</sub> ) Crop factor 0.8 | 95.51                      | 27.11                        | 2.07        | 6.74                                      | 6.80                                   | 19.61                               |
| LSD (p = 0.05)                    | 0.956                      | 1.796                        | 0.514       | 1.262                                     | 1.010                                  | 1.944                               |
| Interaction of M x L              |                            |                              |             |   |  |                                     |
| M <sub>1</sub> L <sub>1</sub>     | 91.29                      | 13.01                        | 2.36        | 8.43                                      | 5.77                                   | 18.62                               |
| M <sub>1</sub> L <sub>2</sub>     | 90.82                      | 20.87                        | 1.73        | 5.81                                      | 7.09                                   | 19.55                               |
| M <sub>1</sub> L <sub>3</sub>     | 93.01                      | 25.38                        | 1.94        | 7.36                                      | 6.43                                   | 19.68                               |
| M <sub>2</sub> L <sub>1</sub>     | 97.78                      | 21.48                        | 2.77        | 9.03                                      | 5.22                                   | 16.51                               |
| M <sub>2</sub> L <sub>2</sub>     | 96.52                      | 25.44                        | 2.12        | 7.21                                      | 6.97                                   | 18.44                               |
| M <sub>2</sub> L <sub>3</sub>     | 98.00                      | 28.85                        | 2.21        | 6.11                                      | 7.16                                   | 21.23                               |
| LSD (p = 0.05)                    | NS                         | 2.709                        | NS          | NS  | NS                                     | NS                                  |

observed by application of irrigation water using crop factor 0.8, whereas, significantly the lowest membrane stability index (17.25%) was observed by application of irrigation water using crop factor 0.6. Interaction effect between methods of irrigation and levels of irrigation water using crop factors on membrane stability index was found significant. Significantly the highest membrane stability index (28.85%) was observed by application of irrigation water using crop factor 0.8 through drip method of irrigation, whereas, significantly the lowest membrane stability index (13.01%) was recorded by application of irrigation water using crop factor 0.6 through micro-jet method of irrigation. Abbas, 2012 reported that drought, salinity, high and low temperatures damages the structure of cell membrane thereby leading to an increase in the membrane permeability and thus resulting in the leakage of intracellular contents. Maintenance of membrane structure and integrity is the key factor in the water stress tolerance. Membrane integrity is usually determined by reducing the leakage of solutes (electrolytes, sugars, amino acids, organic acids and hormones) from cells. The capacity of stem for mobilization or translocation of reserves appears to be related to drought tolerance or resistance which could be due to accumulation of ABA in response to water stress.

The data were found significant with different levels of irrigation. Among the levels of irrigation, significantly the highest proline content (2.56 %) was observed with the application of water using crop factor 0.6, whereas, significantly the lowest proline content (1.93 %) was observed with the application of water using crop factor 0.7 and was found at par with the application of water using crop factor 0.8 (2.07 %). A decreasing trend in the proline content of oil palm leaves was noticed with an increase in the quantity of application of water. The interaction effect between the methods and levels of irrigation was found non-significant. The highest proline content (2.77 %) in the leaves was observed with the application of water using crop factor 0.6 through drip method of irrigation. Accumulation of proline content in oil palm leaves has been demonstrated as one of the most evident biochemical indices under severe water stress conditions (Cha-um et al. 2010). Accumulation of large quantities of proline in the leaves contributes to the osmotic adjustment and serves as a cytoplasmic osmotic balance for potassium accumulation as the main osmoticum in the vacuole.

Harun (1997) noticed accumulation of proline content in the leaves of oil palm seedlings under water stress conditions and also observed an increase in the stomatal resistance and a reduction in the leaf water potential due to increased water deficit in the leaves of oil palm. Heuer (1999) reported that accumulation of proline content was due to water stress resulted from a stimulated synthesis which inhibited the degradation or an impaired incorporation of proline into proteins. Nevertheless, it has been demonstrated that proline plays a more complex role in conferring the drought tolerance in the plants than enacting as a simple osmolyte (Szabados and Savoure 2009). It may protect protein structure by maintaining the structural stability (Rajendrakumar et al. 1994), act as a free radical scavenger (Reddy et al. 2004) as well as involved in the recycling of NADPH<sup>+</sup> via glutamate synthesis (Hare and Cress 1997). Proline synthesis may provide some protection against photoinhibition under adverse conditions by restoring the pool of the terminal electron acceptor of the photosynthetic electron transport chain (Szabados and Savoure 2009).

The lipoxigenase activity was found significant with different levels of irrigation. The highest LOX activity (7.45 n moles g<sup>-1</sup> FW) was recorded in the leaves of oil palm by the application of irrigation water through drip method of irrigation in comparison to micro-jet method of irrigation (7.20 n moles g<sup>-1</sup> FW).

Among the irrigation levels, application of water by using crop factor 0.6 has recorded significantly the highest LOX activity (8.73 n moles g<sup>-1</sup> FW), whereas, application of water by using crop factor 0.7 and 0.8 have recorded significantly lower activity of LOX (6.51 n moles g<sup>-1</sup> FW and 6.74 n moles g<sup>-1</sup> FW respectively) without any significant differences between the water levels.

The interaction effect between the methods and levels of irrigation was found non-significant. The highest LOX activity (9.03 n moles g<sup>-1</sup> FW) was recorded by the application of water using crop factor 0.6 through drip method of irrigation. The lowest LOX activity (5.81 n moles g<sup>-1</sup> FW) was observed by the application of water using crop factor 0.7 through micro-jet method of irrigation.

At cellular level, the impact of water stress is observed based on the integrity of cell membrane and the extent of solute leakage, which is regulated by the



cell membrane stability. Normal cell functions are affected due to changes in the peroxidation of cell wall lipids during water stress resulting increased cell membrane permeability and solute leakage (Rajagopal et al. 2005).

The data were found non-significant with regard to the number of fresh fruit bunches per palm by irrigating with different methods of irrigation. The highest number of fresh fruit bunches per palm per year (6.45) was observed with micro-jet method of irrigation than drip method (6.43) of irrigation.

Significant differences were observed among the levels of irrigation using crop factor. Among the irrigation levels, significantly the highest number of fresh fruit bunches per palm per year (7.03) was observed by the application of irrigation water using crop factor 0.7, whereas, significantly the lowest number of fresh fruit bunches per palm per year (5.49) was recorded by application of water using crop factor 0.6.

The interaction effect between the methods and levels of irrigation with regard to number of fresh fruit bunches per palm per year was observed non-significant. The highest number of fresh fruit bunches per palm per year (7.09) was observed with micro-jet method of irrigation using crop factor 0.7, whereas, lowest number of fresh fruit bunches per palm per year (5.22) was observed with drip method of irrigation using crop factor 0.6.

Occurrence of male and female inflorescences in oil palm was observed due to the process of differentiation of vegetative primordia to floral primordia that is known to occur between 27 to 35 months before anthesis and was concurrent with the process of leaf production (Hartley, 1988). Among the reproductive attributes production of female inflorescences appeared to be highly sensitive to water stress showing the reduction. The number of fresh fruit bunches per palm per year depends upon the number of productive female inflorescences.

In the present investigation, it was observed that quantity of irrigation water applied is the same through different methods of irrigation. Hence, it may be concluded that the method of irrigation has no significant impact on the vegetative growth of the plant as well as on the development of reproductive parts mainly the production of female inflorescences thereby influencing production of fresh fruit bunches per palm

per year. However, application of irrigation water based on the crop factor at 0.7 was found very influential in increasing the number of productive female inflorescences there by the number of fresh fruit bunches per palm per year. Gawankar *et al.* (2003), Krishna rao (2009), Gajbhiye *et al.* (2011) and Sanjeevreddi *et al.* (2014) reported similar kind of observations in their earlier reports on oil palm crop and were found in accordance with the present results.

The data pertaining to yield of fresh fruit bunches per palm per year (Table 1) has recorded non-significant differences between the methods of irrigation. However, application of different levels of irrigation water based on crop factors recorded significant differences with regard to yield of fresh fruit bunches per palm per year. Among the levels of irrigation, significantly the highest annual yield of fresh fruit bunches (19.83 t/ha) was observed by application of irrigation water using crop factor 0.7 and was found at par with the application of irrigation water using crop factor 0.8. Significantly the lowest annual yield of fresh fruit bunches per palm per year was observed by application of irrigation water using crop factor 0.6 (17.57 t/ha). Interaction effect between methods of irrigation and levels of irrigation using crop factors on the annual yield of fresh fruit bunches was found non-significant. However, the highest annual yield of fresh fruit bunches (21.23 t/ha) was recorded by drip method of irrigation using crop factor 0.8 followed by micro-jet method of irrigation using crop factor 0.8 (19.68 t/ha). The lowest annual yield of fresh fruit bunches (16.51 t/ha) was observed with drip method of irrigation using crop factor 0.6. the number of fresh fruit bunch production in oil palm depends upon the number of productive female inflorescences produced. A small reduction in the number of leaves produced due to shortage of water showed an amplification of inhibitory effect on the number of female inflorescences produced thereby a reduction was observed in the number of fresh fruit bunches per palm per year which ultimately led to a reduction in the annual yield of fresh fruit bunches. A small shortage in the application of irrigation water to the palms showed a reduction in the number of female inflorescences produced accordingly the number of fresh fruit bunches produced was influenced which led to a reduction in the annual yield of fresh fruit bunches per palm per year. Gawankar et al. (2003) and Rao (2009) also reported similar kind of observations on oil palm which supports the present investigation.

Based on the results obtained, it could be observed that several of the physiological and biochemical

parameters were influenced by supplemental irrigation water applied at different levels based on the crop factors rather than the method of irrigation during the critical periods of growth and development, which ultimately influenced the final output in terms of FFB yield. An increase in the level of irrigation water led to better maintenance of relative water content and membrane stability index in the plant, which favored opening of stomata thus taking gaseous exchange which ultimately favored accumulation of more photo-assimilates which contributed for better growth and development in terms of fresh fruit bunches.

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**RESEARCH ARTICLE**

**Identification of polymorphic microsatellite markers for genomic studies in oil palm (*Elaeis guineensis* Jacq.)**

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**ABSTRACT**

Oil palm with chromosome number  $2n=32$  and belongs to the family Arecaceae and having a major share of vegetable oil in India. To meet the edible oil requirement in the country, oil palm is the best options due to its high oil-yield (4-6 t/ha) potential compared to other annual oil-yielding crops. Polymorphic microsatellite markers play an important role in genetic diversity and mapping studies in a crop like oil palm. 30 SSR oil palm markers are selected for assessment of polymorphism and genetic diversity analysis among 24 Oil palm genotypes. The 30 Oilpalm SSR markers yielded 66 scorable alleles, all the loci are found to be polymorphic. The number of alleles ranged from 2 to 4 at an average of 2.2 alleles per locus. The SSR locus mEgCIR0195 was found to have maximum number of allele (4) followed by loci mEgCIR0874, mEgCIR087, mEgCIR0779, mEgCIR3328, (3 allele each). The dendrogram generated through UPGMA analysis grouped all the 24 oil palm genotypes into 2 major groups A and B.

**Key words:** oil palm, SSR marker, dendrogram, polymorphism

**INTRODUCTION**

Oil palm (*Elaeis guineensis*, also known as dende oil, from Portuguese) is an edible vegetable oil derived from the mesocarp (reddish pulp) of the fruit of the oil palm. Palm oil is naturally reddish in colour because of high beta-carotene content. It comprises two species of the Arecaceae, or palm family. They are used in

commercial agriculture in the production of palm oil. Palm oil formed the basis of soap products, such as Lever Brothers (now Unilever) "sunlight" soap and the American Palmolive Brand. By around 1870, palm oil constituted the primary export of some West African countries, such as Ghana and Nigeria, although this was overtaken by cocoa in the 1880.

Variation in growth and yield results from difference in the genetic makeup (genes) and its interaction with the environment (GxE Interaction). The markers are useful for confirmation of pedigree or legitimacy of parentage, for assessing genetic diversity or in selection of individual or parents for breeding (marker assisted selection, MAS) Selection based genes (DNA) is expected to be reliable, efficient and precise compared to conventional phenotypic selection which is based on morphological traits influenced. The variability in the population is the most important requirement for any breeding programme. Thottappilly et al. (2000), refer to molecular markers as naturally occurring polymorphism which include proteins and nucleic acids that are detectably different.

Initially, several genetic diversity works were based on using RAPD, RFLP and AFLP molecular markers. However, due to certain drawbacks these markers were replaced by SSR and SNP markers. Use of RAPD for genetic diversity study of oil palm was reported for the first time by Shah (1994). Oil palm germplasm accessions collected from Africa (Cameroon, Tanzania, Nigeria and Zaire) were studied using 20 primers and recorded high levels of genetic variation among the accessions. Rival et al. (1998)

studied the suitability of RAPD markers for detection of somatic clonal variants in oil palm. The results from the 387 arbitrary primers showed no intra clonal variability and no difference between mother and regenerated palms. The authors opined that RAPD approach is not suitable for the detection of the masked variant phenotype. Later Mayes et al. (2000) used RFLP markers (40 probes covering 60% oil palm genome) to assess genetic diversity within 54 palms of a specific oil palm breeding program. Molecular marker technologies help the breeding programmes to a larger extent which reduces time (Babu et al. 2017; Babu and Mathur 2016; Kumar et al. 2018).

Conventional breeding approaches take more time in identification of genetically diverse oil palm genotypes, since the breeding cycle is long. Hence, molecular marker technology plays an important role in genetic diversity and mapping studies. The objectives of the present study are, 1) Identification of polymorphic SSR Markers, 2) molecular and genetic diversity analysis of the selected oil palm genotypes.

## MATERIALS AND METHODS

Fresh tender spear leaves samples from each of 24 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). SSR amplification using PCR

A set of 30 SSR markers were used for amplification in the 24 selected genotypes of oil palm. The forward and reverse sequences of the primers were obtained from Billote et al. (2005). Thermal reactions were carried out in a reaction mixture (20 µl) consisting of 10 X buffer (HiMedia), 2 µl having 15 mM MgCl<sub>2</sub>, 0.2 mM of each forward and reverse primer, 2 µl of 2 mM dNTPs, 0.2 µl 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°C followed by 35 cycles of 30s at 95°C, 30s at 50°C annealing temperature, extension of 1 min at 72°C, with a final extension of 10 min at 72°C, and hold at 4°C. The PCR products were fractionated on 3% Agarose gel. The statistical analysis of polymorphism and UPGMA analysis for generating dendrogram was done by using PowerMarker v 3.0 (Liu and Muse, 2005).

## RESULTS AND DISCUSSION

In the present study, 30 SSR oil palm markers are selected for assessment of polymorphism and genetic diversity analysis among 24 Oil palm genotypes. DNA was extracted from 24 Oil Palm varieties by using CTAB Extraction Method. Then, quantity and quality of DNA was assessed by agarose gel electrophoresis (0.8%) respectively using λ DNA as marker to ensure the good quality of DNA for SSR assay (Figure 1).

In this present study, we used 30 Oil palm markers. The genomic DNA of the 24 Oil Palm accessions were amplified using 30 Oil palm SSR markers and yielded 66 scorable alleles, all the loci are found to be polymorphic. The SSR locus mEgCIR0195 was found to have maximum number of alleles (4) followed by loci mEgCIR0874, mEgCIR087, mEgCIR0779, mEgCIR3328, (3 alleles each). The number of alleles ranged from 2 to 4 at an average of 2.2 alleles per locus. Similar results were also obtained by Okoyo *et al.* (2016) where they found an extremely high mean percentage polymorphism (85.09%) and Arias *et al.* (2012) reported maximum PIC value with 0.822 in commercial oil palm material. The details of the markers along with allele number and polymorphism percentage are given in table 1. The gel banding pattern of mEgCIR0163 is given in figure 2.



Fig. 1: The banding pattern of genomic DNA of oil palm

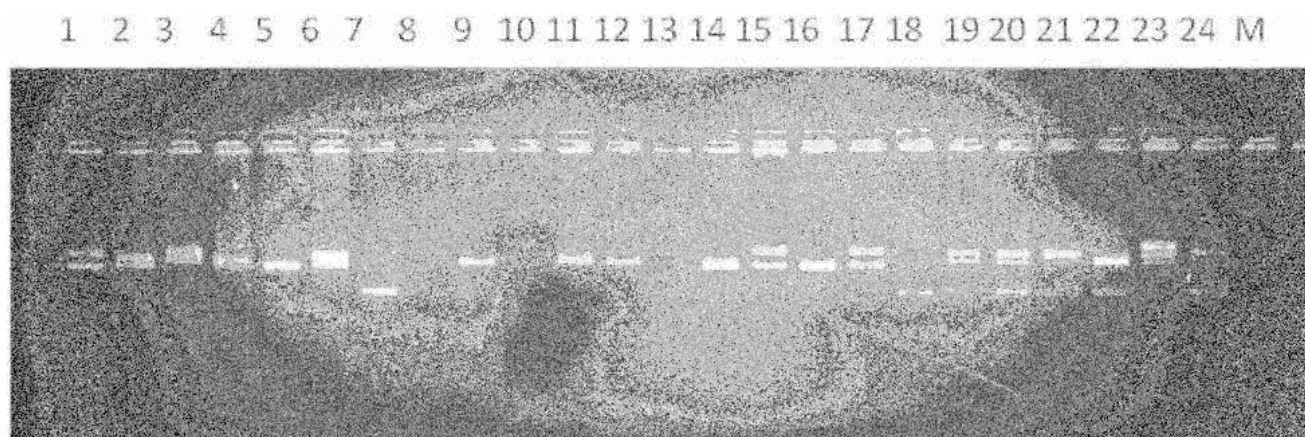


Fig. 2 The gel banding pattern of 24 oil palm genotypes using SSR marker mEgCIR0163

**Table 1: The details of the markers along with allele number and polymorphism percentage**

| S. No.  | Markers    | Allele | Percentage Of polymorphism |
|---------|------------|--------|----------------------------|
| 1.      | mEgCIR0195 | 2      | 100                        |
| 2.      | mEgCIR0163 | 4      | 100                        |
| 3.      | mEgCIR0243 | 2      | 100                        |
| 4.      | mEgCIR0037 | 2      | 100                        |
| 5.      | mEgCIR0177 | 2      | 100                        |
| 6.      | mEgCIR0802 | 2      | 100                        |
| 7.      | mEgCIR0874 | 3      | 100                        |
| 8.      | mEgCIR0793 | 2      | 100                        |
| 9.      | mEgCIR0800 | 2      | 100                        |
| 10.     | mEgCIR0894 | 2      | 100                        |
| 11.     | mEgCIR0555 | 2      | 100                        |
| 12.     | mEgCIR0774 | 2      | 100                        |
| 13.     | mEgCIR0775 | 2      | 100                        |
| 14.     | mEgCIR0825 | 2      | 100                        |
| 15.     | mEgCIR0878 | 3      | 100                        |
| 16.     | mEgCIR0779 | 3      | 100                        |
| 17.     | mEgCIR0773 | 2      | 100                        |
| 18.     | mEgCIR0782 | 2      | 100                        |
| 19.     | mEgCIR2575 | 2      | 100                        |
| 20.     | mEgCIR2518 | 2      | 100                        |
| 21.     | mEgCIR2595 | 2      | 100                        |
| 22.     | mEgCIR2813 | 2      | 100                        |
| 23.     | mEgCIR2387 | 2      | 100                        |
| 24.     | mEgCIR2291 | 2      | 100                        |
| 25.     | mEgCIR1773 | 2      | 100                        |
| 26.     | mEgCIR3282 | 2      | 100                        |
| 27.     | mEgCIR3232 | 2      | 100                        |
| 28.     | mEgCIR3286 | 2      | 100                        |
| 29.     | mEgCIR3310 | 2      | 100                        |
| 30.     | mEgCIR3328 | 3      | 100                        |
| Average |            | 2.2    |                            |

Genetic distances were estimated for pairs of varieties with their hybrids according to Jaccard's coefficient (Jaccard, 1908). High amount of similarity existed between P537 and P536 genotypes, followed 85% similarity between P45 and P43 genotypes. The list of highly similar genotypes given in table 2. Likewise highly dissimilar genotypes were also calculated using jaccard's similarity coefficient. High amount of dissimilarity existed between P42 and P143 genotypes followed by 30% dissimilarity between P143 and P72 genotypes. The list of highly dissimilar genotypes is given in table 3.

**Table 2: The list of highly similar genotypes**

| S. No. | Genotype name | Genotype name | % of Similarity |
|--------|---------------|---------------|-----------------|
| 1.     | P537          | P536          | 86%             |
| 2.     | P45           | P43           | 85%             |
| 3.     | P143          | P142          | 83%             |
| 4.     | P539          | P537          | 82%             |

**Table 3: The list of highly dissimilar genotypes**

| S. No. | Genotype name | Genotype name | % of Dissimilarity |
|--------|---------------|---------------|--------------------|
| 1.     | P42           | P143          | 28%                |
| 2.     | P143          | P72           | 30%                |
| 3.     | P539          | P143          | 32%                |
| 4.     | P60           | P143          | 33%                |
| 5.     | P535          | P173          | 35%                |

#### Genetic diversity

The dendrogram generated through UPGMA analysis grouped all the 24 Oil palm genotypes into 2 major groups A and B. The clustering of the Oil Palm genotypes was largely based on their geographical

origin. The following dendrogram contains two clusters A&B with different genotypes. Cluster A contains two clusters. The sub clusters A1 and A2 consisted of 19 genotypes. However, cluster B contains two sub clusters. The B cluster composed of 5 genotypes viz., P46, P142, P143, P173, and P174. The B cluster composed of all the tall genotypes and they all from Guinea Bissau origin. The genotypes under cluster A were belongs to different geographical origins like Cameroon, Zambia, and Tanzania.

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## RESEARCH ARTICLE

**Identification of oil palm fruit forms using SNP based CAPS marker among the *Tenera/ Tenera* (T X T) crossed progeny**G. Prasanna Kumar<sup>1,2\*</sup>, M. V. B. Venu<sup>1</sup>, B. Kalyana babu<sup>1</sup>, G. Ravichandran<sup>1</sup>, R. K. Mathur<sup>1</sup><sup>1</sup>ICAR-Indian Institute of Oil Palm Research, Pedavegi-534 450, West Godavari (Dt), Andhra Pradesh<sup>2</sup>Adikavi Nannaya University, Rajahmahendra varam, Andhra Pradesh

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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 Arecaceae, Genus *Elaeis*, 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 Abydos 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 into dura, pisifera and tenera forms based on the shell thickness which is a monogenic and co-dominantly inherited trait. The dura (D) genotypes consist 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 tools 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 asparagine. 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  $\mu$ l) consisting of 10 X buffer (Himedia), 2  $\mu$ l having 15 mM MgCl<sub>2</sub>, 0.2 mM of each forward and reverse primer, 2  $\mu$ l of 2 mM dNTPs, 0.2  $\mu$ l 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°C followed by 35 cycles of 30s at 95°C, 30s of 50°C annealing temperature, extension of 1 min at 72°C, with a final extension of 10

min at 72°C, and hold at 4°C. The PCR products were fractionated on 3 % Agarose gel.

### Restriction site analysis

Ten  $\mu$ l 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°C. Restriction fragments were visualized by electrophoresis as described above.

## RESULTS AND DISCUSSION

### Isolation of genomic DNA and quantification:

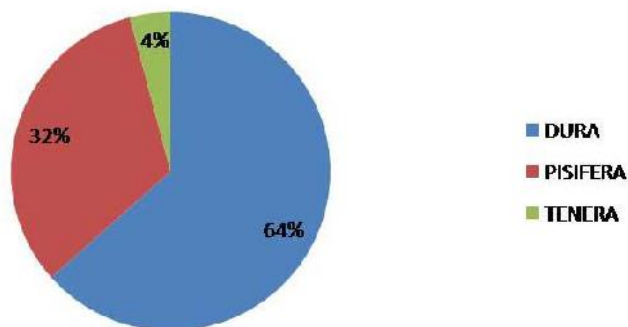
The genomic DNA is extracted from the spear leaf samples of already established oil palm garden. DNA 145 T X T progeny lines by using CTAB Extraction Method. Quality of genomic DNA is checked on 0.8% agarose gel, stained with ethidium bromide (EtBr) and documented using Alpha Imager gel documentation 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  $\lambda$  DNA marker.

### Molecular identification of fruit form in T X T progeny:

In the present study, CAPS marker EgSHP-Forward-TTGCTTTTAATTTTGCTTGAATACC, Reverse -TTTGGATCAGGGATAAAAGGGGAAG 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   | 138 | L.265 | 239VRX202VR | Dura   |
| 87  | L.231 | 139VRX202VR | Tenera | 139 | L.258 | 272VRX202VR | Dura   |
| 88  | L.255 | 272VRX202VR | Tenera | 140 | L.251 | 239VRX202VR | Dura   |
| 89  | L.226 | 139VRX202VR | Tenera | 141 | L.277 | 239VRX202VR | Dura   |
| 90  | L.234 | 272VRX202VR | Dura   | 142 | L.266 | 239VRX202VR | Dura   |
| 91  | L.233 | 272VRX202VR | Dura   | 143 | L.127 | 228DX       | Dura   |
| 92  | L.224 | 139VRX202VR | Tenera | 144 | L.220 | 139VRX202VR | Dura   |
| 93  | L.279 | 239VRX202VR | Tenera | 145 | L.273 | 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   |     |       |             |        |

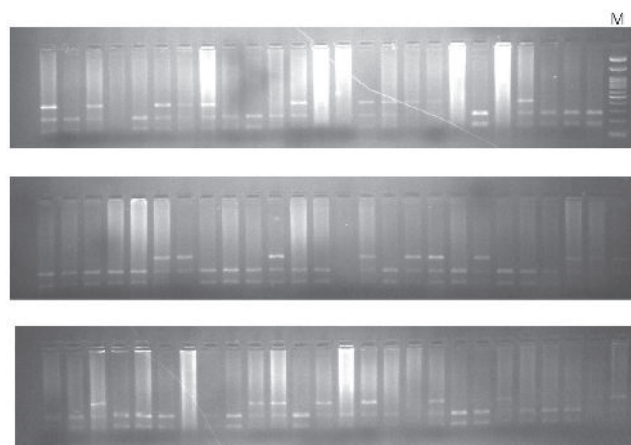


Fig. 2: Hind III digested TxT progeny showing different fruit forms (M-100bp Marker)

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## REVIEW ARTICLE

### Oil Palm breeding strategies through molecular and genomics technologies: status and way forward

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#### ABSTRACT

The collection and exploitation of germplasm material is considered a major factor in contributing towards improvement of oil palm industry. Conventional breeding can take several years, which greatly hampers rapid and efficient progress in the selection of individuals. Various molecular biology techniques are available today for detection of genetic variability and for establishing genetic similarity relationships among individuals and can be used in various applications in breeding programs. These modern genomics tools allow substantial knowledge to be attained on the biological traits of a species which can be exploited in improving productivity and obtain better product quality. The recent advances in genome sequencing through next generation sequencing (NGS) technologies provide opportunities to develop millions of novel markers, as well as the identification of agronomically important genes. The present review focused on molecular approaches for improving breeding capabilities in oil palm breeding programmes.

**Key words:** Oil palm, tenera, genomic selection, molecular breeding

#### INTRODUCTION

Improved oil palm varieties with higher yields, good oil quality, and compact in architecture, better adaptation to climate change and higher tolerance to diseases have been prioritized to meet future demands

of the oil palm industry. The collection and exploitation of germplasm material is considered a major factor in contributing towards improvement of oil palm industry. Oil palm breeding programs are characterized by using the reciprocal recurrent selection scheme which uses two *dura* and *pisifera*-type starting populations to make the crossings and progeny testing of which the best parents are chosen and *tenera*-type seeds are produced. Subsequently, new populations are generated and the cycle is repeated (Corley and Tinker, 2003). Same female parental stock, *Deli dura*, with some introgressions and are combined with three male parental stocks (*pisiferas*), the *Avros*, *La Me* and *Yagambi* (Bakoume 2007 and Cochard, 2009). This narrow genetic base has driven oil palm breeders to play greater importance on genetic resources of the species to increase the genetic variability in breeding programs.

The breeding scheme primarily involves a reciprocal recurrent selection scheme (RRS) which has been adapted by a number of oil palm breeders. In this scheme, the *dura* and *pisifera* genotypes are kept as distinctly separate base populations. In the form of hybrids (*tenera*), the heterosis effect is obtained by crossing origins with complementary characteristics. The performance of inter-origin crosses is attributed to the additive effect of favorable genes combination from the parents. It was reported that the RRS scheme has increased oil yield by almost 18% per cycle compared to the base population (Rajanaidu et al. 2000). Introgression of current materials with selected

materials could be a helpful way to broaden their genetic diversity. Combining ability is essential to identify superior parents for hybrid seeds production. There are two types of combining abilities, general combining ability (GCA) and specific combining ability (SCA). GCA plays an important role in the identification of parents for the development of superior genotypes while SCA provides information about the performance of hybrids (Cruz and Regazzi 1994). The AVROS *pisifera*s were known to have high general combining ability. In oil palm, efforts have been made to exploit the GCA and SCA among parents to increase fresh fruit bunch (FFB) yield, oil-to-bunch ratio (O/B) and kernel-to-bunch ratio (K/B) by 42%, 18% and 29% (Breure and Konimor 1992; Dumortier and Konimor 1999 and Rafi et al. 2001). Okwuagwu et al. (2008) reported that cross of Deli *dura* x *tenera* breeding in Nigeria indicated that high estimates of genotypic coefficient of variation, heritability and genetic advance recorded for the bunch yield traits. Noh et al. (2012) evaluated the performance of 11 oil palm AVROS *pisifera*s and observed low genetic variability among *pisifera* parents for most of the characters indicating uniformity of the *pisifera* population and suggested that the low variability is due to the small population size of AVROS *pisifera* from which they have been derived.

Conventional breeding can take several years, which greatly hampers rapid and efficient progress in the selection of individuals. Various molecular biology techniques are available today for detection of genetic variability and for establishing genetic similarity relationships among individuals and can be used in various applications in breeding programs. These modern genomics tools allow substantial knowledge to be attained on the biological traits of a species which can be exploited in improving productivity and obtain better product quality. Complementation of conventional breeding technique with novel approaches from biotechnology will accelerate the progress in oil palm improvement (Ramli et al. 2016 and Murphy 2014). Though there are some reviews available (Babu and Mathur 2016; Kumar et al. 2018), the present review focused on recent trends in molecular breeding and next generation technologies in oil palm.

### **Marker-Assisted Selection and QTL mapping**

Marker assisted selection has been carried out in the progeny, which allows the early selection of desired progeny. DNA markers such as restriction fragment length polymorphism (RFLP), random amplification of polymorphic DNA (RAPD), amplified fragment length

polymorphism (AFLP), single sequence repeat (SSR), and single nucleotide polymorphisms (SNPs) have been identified and applied to improve breeding of oil palm. Simple Genetic linkage information is potentially a very powerful tool for accelerating oil palm breeding through marker-assisted selection. Ritter et al. (2004) used three primers for differentiation of *dura*, *pisifera* and *tenera* forms. Moretzsohn et al. (2000) constructed a linkage map with RAPD markers and identified two RAPD markers to be linked on both sides of the *sh+* locus on linkage group 4. Sequence Repeats (SSR) have been efficiently used to study the genetic structure of oil palm (Billotte et al. 2007 and Singh et al. 2008), for varietal identification (Norziha et al. 2008), genome mapping and QTL detection for molecular marker assisted selection (Billotte et al. 2010). Fatty acid composition is an important agronomic trait which is associated with oil quality and QTL mapping for this trait using molecular markers will speed up the production of new and improved oil palm planting materials. Singh et al. (2009) described the first successful detection of QTLs for fatty acid composition in oil palm. The genetic linkage maps enriched with simple sequence repeat (SSR) markers were developed for *dura* (ENL48) and *pisifera* (ML161), the two fruit forms of oil palm to study the tissue culturability and identified two quantitative trait loci (QTLs) associated with callusing rate and embryogenesis rate (Ting et al. 2013). Lee et al. (2014) constructed a linkage map of oil palm using 2795 co-dominant DNA markers and mapped a major QTL for stem height on the linkage group 5 and stated that the markers flanking the QTL could be used in the selection of dwarf trees at the seedling stage, thus accelerating the breeding for shorter trees. Babu et al. (2017) developed *Sh* gene specific primer pairs using 300 genomic, 8 genic SSR markers and identified one cleaved amplified polymorphic site (CAPS) marker for differentiation of oil palm fruit type and suggested that selection and distribution of desirable high yielding *tenera* sprouts to the farmers could be possible at seedling stage instead of waiting for 4–5 years and saves a lot of land, time and money which will be a major breakthrough to the oil palm community. Bai et al. (2017) constructed a high-density linkage map with 1357 SNPs and 123 microsatellite markers to provide a basis for fine mapping of QTL and improve the assembly of the genome of oil palm and found four QTLs for oil to bunch (O/B) and oil to dry mesocarp (O/DM) on LG1, LG8, and LG10 in a F1 breeding population and also identified DNA markers associated with these traits. They have suggested to pyramid the identified QTL with beneficial genotypes associated with oil content traits using DNA markers has the

potential to accelerate genetic improvement for oil yield in the breeding population of oil palm.

### Genomic selection

Genomic selection (GS) uses genome-wide markers as an attempt to accelerate genetic gain in breeding programs for perennial crops such as oil palm, which have long breeding cycles; Genomic selection (GS) is an efficient method of marker-assisted selection to improve quantitative traits using markers distributed across the entire genome (Meuwissen et al. 2001).

In traditional breeding schemes, the progeny tests enable highly accurate selection, but the low rate of genetic gain is the main drawback. The difficulty and costs associated with long term evaluations of progenies limit the number of individuals evaluated, resulting in low selection intensity. The ultimate goal of GS is to expedite the breeding progress by maximizing the genetic gains per generation. In this context, the potential of GS for palm oil yield is high, and several previous researchers also report the potential application of GS in oil palm (Wong et al., 2008 and kwong et al. 2017). Pootakham et al. (2015) reported the efficiency of this approach for quantitative trait loci (QTL) detection in oil palm. Cros et al. (2017) reported the possible application of GS in oil palm by conducting genomic pre selection in the parental populations prior to progeny tests, which increased selection intensity for yield components thus improving the performance of commercial hybrids using GBS and suggested further research to increase the benefits from GS, which should revolutionize oil palm breeding.

### Genome-wide association studies (GWAS)

The CK et al. (2016) performed GWAS for oil-to-dry-mesocarp content on 2045 genotyped tenera palms using 200K SNPs and found that 80 loci were significantly associated with oil-to-dry mesocarp yield ( $P < 10^{-4}$ ), and three key signals were found. Ithnin et al. (2017) conducted the multi-locus Genome-Wide Association Studies (GWAS) and identified 19 quantitative trait loci (QTLs) for 8 traits and further reported the potential application of GWAS for introgression of desirable genes to advanced breeding populations for improvement of bunch and oil yield traits.

### Next generation sequencing for oil palm improvement

The recent advances in genome sequencing through next generation sequencing (NGS) technologies

provide opportunities to develop millions of novel markers, as well as the identification of agronomically important genes (Edwards and Batley, 2010). SNPs now dominate over other molecular marker applications, with the advancement in sequencing technology. Advancements in NGS enabled the development of high-density genetic maps. Genetic mapping places the markers in linkage groups based on their co-segregation. With the advancement of genomics technology, the generation of ESTs, genetic mapping and application of DNA chip technology have been employed in oil palm (Sambanthamurthi et al. 2009). A linkage map was constructed comprising 17 linkage groups with 117 RFLP loci, 384 AFLP markers and 23 SSR markers (Singh, 2005). Several QTLs for economic traits and the fruit colour genes (*vir*) have been successfully tagged in the linkage map. The markers associated with shell thickness have been identified. The ESTs also provided a platform for large-scale functional analysis of the genes using microarrays.

With the recent surge in next generation sequencing, the 1.8 Gb *E. guineensis* genome was sequenced with a combination of Roche/454 and Sanger Bacterial Artificial Chromosome (BAC) end sequencing (Singh et al. 2013b). In addition, transcriptome data from 30 tissues and a draft sequence of the South American oil palm, *Elaeis oleifera* were reported. A total of 34,802 genes were predicted, including oil biosynthesis genes, homologues of WRINKLED1 (WRI1), and other transcriptional regulators, which are highly expressed in the kernel (Singh et al. 2013b). In the subsequent studies, the gene responsible for the shell thickness (*SHELL*) was identified and mapped (Singh et al. 2013a), delivering the opportunity for further exploitation in breeding programmes. Recently, an SNP based high density linkage map was constructed using genotyping by sequencing approach, and 3 QTL affecting trunk height and a single QTL associated with fruit bunch weight were identified (Pootakham et al. 2015). The sequence information provides the opportunity to mine other key genes responsible for higher productivity and resistance to biotic and abiotic stress.

### Omics technology

Omics technology promises consistency and predictability in plant breeding towards better yield and quality food crops. Several emerging omics technologies have been introduced to oil palm research for unravelling the molecular mechanisms of oil palm system biology under various conditions.

Multidisciplinary approaches such as genomics, transcriptomics, proteomics and metabolomics are being developed and adopted to pave this endeavour forward. The advancement of omics platforms has provided valuable resources for the discovery, assessment and establishment of molecular markers and precise gene modification through genetic engineering. The omics methodologies applied in oil palm research have facilitated extensive discoveries of indicative transcripts, proteins and metabolites associated with yield traits such as fruit ripeness, fruit quality, fruit form and lipid formation (Wong *et al.*, 2017; Hassan *et al.*, 2016; Ooi *et al.*, 2015; Teh *et al.*, 2014; 2013; Loei *et al.*, 2013; Neoh *et al.*, 2013; Dussert *et al.*, 2013 and Hassan *et al.*, 2014). The application of transcriptomics, proteomics and metabolomics has been employed to investigate the interactions between oil palm and *G. boninense* fungus (Nusaibah *et al.* 2016; Dzulkafli *et al.* 2016; Tee *et al.* 2013; Nurazah *et al.* 2013; Alizadeh *et al.* 2011 and Aswad *et al.* 2011).

### Genome editing

In essence, the advances in genome sequences and genetic engineering techniques have also laid a foundation towards genome editing approach. Genome editing is a new technology that could be applied to allow the modification of oil palm genome in a precise and predictable manner without introducing foreign DNA. The use of genome editing in plant for improvement of various traits was reviewed by Malzahn *et al.* (2017). Genome editing involves the introduction of targeted DNA double-strand breaks (DSB) using engineered nuclease and stimulating DNA repair mechanisms. Zinc finger nuclease (ZFN) and transcription activator-like effector-based nucleases (TALEN) are sequence specific nucleases with DNA binding domain commonly used in genome editing for targeting DNA mutagenesis. A simpler genome editing approach known as clustered regularly interspaced palindromic repeats (CRISPR) has been developed, which allows more effective regulation of targeted gene. The CRISPR/Cas 9 system is an adaptive of bacterial II immune system which requires Cas 9 nuclease to degrade DNA that matches to a single guided RNA (sgRNA) (Malzahn *et al.* 2017 and Song *et al.* 2016).

### CONCLUSION

Advancement in sequencing technologies has had a great impact on crop genetics, enabling the sequencing of genomes and transcriptomes of several crops. A massive re-sequencing and gene expression studies are

essential to identify the key genes responsible for a desired trait and to find its allele variability. Utilization of this knowledge in crop breeding would empower the development of better crop varieties and may lead to a second green revolution. This would reduce the hunger of billions and revolutionize the economies of developing tropical countries. Through these post-genomic researches, the oil palm can be manipulated towards the production of highest possible yield with sustainable practices to fulfill the mission for advancing the palm oil industry.

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Brown B, Aaron M (2001) *The politics of nature*. In: Smith J (ed) *The rise of modern genomics*, 3rd edn. Wiley, New York, pp 230-257

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