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Chlorophylls and Nitrate Reductase Activity in Relation to Heterosis in Coconut seedlings

S. SHIVASHANKAR, K. RAJGOPAL* and A. RAMADASAN

Division of Plant Physiology, Central Plantation Crops Research Institute, Kasaragod 670 124, India

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ABSTRACT

The F_1 progeny of Chowghat Dwarf Orange (CDO) and West Coast Tall (WCT) cross tend to be highly heterozygous with respect to the colour of petiole and seedling vigour. The study indicated that the greenish brown coloured vigorous plants had higher chlorophyll ($a+b$) content and nitrate reductase (NR) activity compared to the orange coloured vigorous plants and the dwarfs. The greenish brown seedlings were also characterized by a larger leaf area and shoot d. wt. The results provide biochemical evidence in support of the morphological criteria being used at present in the selection of hybrid coconut seedlings.

Key words: *Cocos nucifera* L. (Coconut), Chowghat Dwarf Orange, West Coast Tall, heterosis, chlorophyll, nitrate reductase, leaf area, shoot dry weight.

Chowghat Dwarf Orange (CDO) and West Coast Tall (WCT) are two distinctive forms of coconut palms that are grown on the West Coast of India. Of these, the WCT is predominantly out-crossed and therefore heterozygous, whereas CDO is predominantly self-pollinating due to overlapping male and female phases and therefore the more homozygous of the two forms. Owing to their inherent genetic diversity, the CDO, on crossing with WCT (male) gives F_1 hybrids varying in both petiole colour and seedling vigour. The F_1 seedling progeny of CDO × WCT cross can be categorized mainly into three groups, namely, the orange coloured dwarfs, greenish brown and orange coloured vigorous plants. In the nursery, the greenish brown seedlings (the heterotic hybrids) produce high yield with good copra characteristics, while both the dwarf and vigorous orange coloured segregants (dwarf and intermediate hybrids respectively) are rejected as they are believed to resemble the dwarf parent in copra content and quality. However, the biochemical basis for such a classification has not been understood. In this paper, we report the relationship of chlorophyll content and NR activity with leaf area and dry matter production in the one-year-old F_1 hybrid coconut seedlings of CDO × WCT cross obtained after hand-pollination.

The seedlings were classified as dwarf, intermediate and heterotic hybrids based on colour of the petiole, seedling vigour in terms of collar girth, leaf number and the time taken for seed germination. All biochemical estimations were conducted in duplicate on fifteen 12-month-old seedlings of each hybrid type. Chlorophylls and carotenoids were extracted by macerating 25 leaf discs (total area 9·62 cm²) in 10 ml of 80 per cent aqueous acetone. The extracts were vacuum filtered and absorbance measured at 663, 645 and 429 nm to estimate chlorophylls and carotenoids (Mackinney, 1941; Jensen and Jensen, 1971).

Nitrate reductase (NR) activity was induced in excised leaflets by keeping their cut ends dipped in nitrate medium in diffuse daylight for 4 h. For whole plant induction, the seedlings in the nursery were treated individually with one litre of 2·5 per cent

* Present address: Central Horticulture Experimental Station, P. O. Rajaulatu, Ranchi, 834 010, India.

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potassium nitrate solution containing 10 mg l^{-1} molybdenum [as $(NH_4)_7 MoO_4$]. They were irrigated daily with one litre of water. The NR activity was measured by a modified method of Jaworski (1971) as described by Shivashankar and Rajgopal (1983). Both excised leaf and whole plant induction methods were adopted and compared. Determination of NR activity as a function of time after nitrate application was carried out under uniformly sunny induction conditions. Measurements in cloudy conditions were performed on the sixth day after nitrate application.

The total nitrogen content of nitrate fed seedlings was determined by the micro-kjeldahl procedure (AOAC, 1970). Leaf area and shoot dry weight were estimated following the method described by Ramadasan *et al.* (1980).

Chlorophylls

The content of chlorophylls *a*, *b* and carotenoids in the leaves of heterotic hybrids were significantly higher than that in the intermediate and dwarf hybrids (Table 1). Mathew and Ramadasan (1972) reported significantly higher chlorophyll content in the hybrids of Tall × Dwarf palms, and also showed that the chlorophyll content was positively correlated with photosynthetic rate in WCT (Mathew and Ramadasan, 1975). A higher chlorophyll content in hybrids than the mean of the respective two inbred parents have been reported in other crops also (Fleming and Palmer, 1975; Balasubramanian *et al.*, 1977; Bhatt *et al.*, 1981). Thus, it is clear that the chlorophyll content in leaves could serve as a biochemical marker for the identification of heterotic hybrids of CDO × WCT in the nursery.

TABLE 1. *Chlorophylls and carotenoids in the leaf tissues of F_1 hybrid coconut seedlings of various types*

Type	Chlorophyll *a* (mg dm^{-2})	Chlorophyll *b* (mg dm^{-2})	Chlorophyll (*a*+*b*) (mg dm^{-2})	Carotenoids (mg dm^{-2})
Heterotic hybrid	2·26[a]	1·31[a]	3·57[a]	1·59[a]
Intermediate hybrid	1·98[b]	1·11[b]	3·09[b]	1·37[b]*
Dwarf hybrid	1·74[c]	0·96[c]	2·70[c]	1·23[b]*

[a, b, c] For comparison between the genotypes, values bearing different letters are significantly different ($P = 0·01$). Where values bearing the same letter are significantly different at $P = 0·05$ this is indicated by *.

NR activity

In the whole plant induction method, the NR activity in all the three groups of seedlings increased exponentially up to 2 d after nitrate application and thereafter remained steady up to 5 d under irrigated conditions (Fig. 1). Although the conditions were uniformly sunny up to day 5 the weather became cloudy on day 6 and values of NR activity were significantly lower than on the previous day. These values were comparable to those measured in the excised leaves, in which induction was carried out in diffuse light throughout (Table 2). A paired *t*-test showed no significant differences between the values obtained by the two methods. It is thus clear from the results presented that the excised leaf induction method provides an excellent means of measuring the nitrate reduction potential in coconut. Furthermore, since the method is quicker and easier than the whole plant induction method, it can be used with advantage for comparison of activities in large populations. The NR activity measured by both the methods showed significant differences among the groups, the heterotic hybrids recording higher values than the intermediate and dwarf hybrids.

The total reduced N content was significantly higher in heterotic hybrids and the

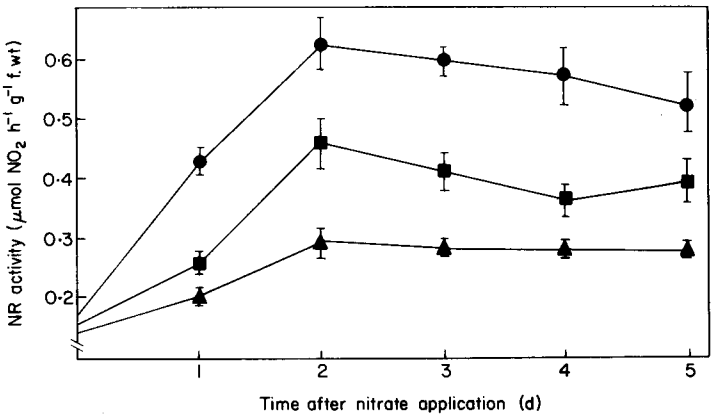

FIG. 1. Changes in nitrate reductase activity of CDO × WCT seedling progenies after nitrate application to the intact seedlings. Vertical bars represent s.e. of the means of 15 seedling replicates. ●, Heterotic hybrid; ■, intermediate hybrid; ▲, dwarf hybrid.

TABLE 2. *Nitrate reductase activity and related growth parameters in F_1 hybrid coconut seedlings of various types*

Type	Nitrate reductase activity (nmol NO_2 h^{-1} g^{-1} f. wt)			Reduced N (mg g^{-1} d. wt)	Shoot d. wt (g)	Leaf area (m^2)
	Excised leaf induction	Whole plant induction				
	subdued light*	Cloudy day*	Sunny day			
Heterotic hybrid	188·7[a]	184·6	457·4[a]	18·52	221·3[a]	0·84[a]
Intermediate hybrid	109·6[b]	104·0	183·4[b]	18·56	148·1[b]	0·53[b]
Dwarf hybrid	69·3[c]	64·7	119·0[c]	15·34	47·6[c]	0·30[c]

[a, b, c] For comparison between genotypes, values bearing different letters are significantly different at $P = 0·01$.

* Paired t test analysis showed no significant ($P = 0·01$) difference between the NR values determined by excised leaf and whole plant induction in the same hybrid type.

intermediate hybrids than the dwarf hybrids (Table 2). The differences between heterotic and intermediate hybrids were not significant.

Growth parameters

The heterotic hybrids showed significantly higher rates of dry matter accumulation as seen by their shoot dry weights (Table 2). The shoot d. wt, which is considered as an index of vigour, was also significantly and positively correlated with NR activity ($r = 0·5467$). The NR activity was earlier shown to be positively correlated with the annual yield of nuts in adult WCT palms (Shivashankar and Ramadasan, 1983). The total leaf area of heterotic hybrids was also the highest followed by the intermediate and dwarf hybrids. These results indicate a close relationship among NR activity, leaf area development and dry matter accumulation. These findings are in agreement with the observations of Hageman *et al.* (1967) that the level of NR plays a crucial role in the

utilization of nitrate and consequently dry matter accumulation. Although the levels of reduced N/unit weight of leaf in heterotic and intermediate hybrids were similar, the total plant N would be much higher in the heterotic hybrids due to their higher shoot d. wt and larger leaf area. Thus, the higher level of chlorophylls and nitrate reductase activity in heterotic hybrids may enable them to develop a larger leaf area and consequently produce more dry matter. The results, therefore, are in support of the methods presently being adopted to select hybrid seedlings based on colour and vigour.

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