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# An Evaluation of the Role of Cytokinins in the Development of Abnormal Inflorescences in Oil Palms (*Elaeis guineensis* Jacq.) Regenerated from Tissue Culture

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**Abstract.** Tissue cultures and regenerant plants from cell lines producing palms with normal and abnormal flowers were analyzed for cytokinin content and compared with zygotic embryos and seedlings. Immature inflorescences at the critical stage of flower development dissected from normal and abnormal palms were also analyzed. High performance liquid chromatography (HPLC)/radioimmunoassay and HPLC/enzyme-linked immunosorbent assay methods were used over a period of several years to measure the isoprenoid cytokinins. The results of analyses of endogenous aromatic cytokinins, present at comparable levels, will be reported separately. Oil palm cultures and regenerant plants contained relatively high concentrations of the 9-glucosides of isopentenyladenine ([9G]iP) and zeatin ([9G]Z). The predominant biologically active isoprenoid cytokinin present was zeatin riboside ([9R]Z), with lesser amounts of isopentenyladenine (iP) and isopentenyladenosine ([9R]iP). There was evidence of small amounts of dihydrozeatin compounds, but high concentrations (mainly as dihydrozeatin-9-glucoside ([9G]DHZ)) were confined to the haustorium of the zygotic embryo. Callus tissue contained very low concentrations of cytokinin. Frequently only [9G]iP could be detected, at about 1 pmol $\cdot$ g$^{-1}$ fresh weight, with [9R]Z at less than 0.05 pmol $\cdot$ g$^{-1}$. In comparison, nodular embryogenic tissues in vitro contained between 30 and 1,500 pmol $\cdot$ g$^{-1}$ of [9G]iP, 5–50 pmol $\cdot$ g$^{-1}$ of [9G]Z, and up to 12 pmol $\cdot$ g$^{-1}$ of [9R]Z. Shoots of regenerant plantlets and seedlings contained lower concentrations of [9G]iP (3–30 pmol $\cdot$ g$^{-1}$), although this was still the predominant cytokinin. [9R]Z and [9G]Z were present at between 2 and 15 pmol $\cdot$ g$^{-1}$, with iP at 1–5 pmol $\cdot$ g$^{-1}$ and [9R]iP at between 1 and 12 pmol $\cdot$ g$^{-1}$. Seedlings contained similar amounts with the exception of a lower [9G]iP content (5–10 pmol $\cdot$ g$^{-1}$) and more [9R]iP (10–20 pmol $\cdot$ g$^{-1}$). Root tissues of ramets contained significantly higher concentrations of [9G]iP than shoots. Comparison of two isogenic lines of one clone giving rise to normal and abnormal palms showed significantly higher concentrations of [9R]Z and [9G]Z in the normal than in the abnormal line and, in embryoids only, higher [9G]iP in the normal line. In all other cases the between-clone differences were greater than any normal/abnormal differences. There was a general tendency for increased concentrations of [9G]iP in abnormal lines and for this compound to be in a higher concentration in embryoids and plants derived from culture than in zygotic embryos and seedlings. Analysis of cytokinins in immature female inflorescences of normal and abnormal palms of a single clone showed the abnormal inflorescences to have higher concentrations of [9R]Z and [9R]DHZ and less [9G]Z than the normal inflorescences at comparable stages of development.

**Abbreviations:** HPLC, high performance liquid chromatography; [9G]iP, 9-glucoside of isopentenyladenine; [9G]Z, 9-glucoside of zeatin; [9R]Z, zeatin riboside; iP, isopentenyladenine; [9R]iP, isopentenyladenosine; [9G]DHZ, dihydrozeation-9-glucoside; ELISA, enzyme-linked radioimmunosorbentassay; ANOVAR, analysis of variance.

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In recent years efforts to develop a commercial process for the large scale in vitro propagation of elite oil palm hybrids have been held back by the development, in some clones, of abnormal flowers (Corley et al. 1986). The abnormality consists of the

conversion of the stamen primordia into carpel-like tissues. In male inflorescences no pollen is formed, whereas in female inflorescences there is a ring of supplementary carpels surrounding the gynoecium, giving rise to a mantled fruit. Frequently the fusion of the three carpels of the gynoecium is incomplete, and no functional stigma is formed, leading to pollination failure and either formation of parthenocarpic fruit or in severe cases to flower abortion. Expression of the abnormality is quite variable, from the production of a few mantled fruit in a bunch, with variable numbers of supplementary carpels, to complete failure of all florets in the bunch to set fruit. It has been suggested that the abnormality is caused by disturbed cytokinin metabolism, resulting from specific conditions in the tissue culture stage (Besse et al. 1992, 1994).

Evidence was presented (Besse et al. 1992, 1994) that somatic embryos obtained from friable fast growing calluses resulted in abnormal palms, whereas those from nodular calluses had low frequencies of abnormality. They reported that nodular calluses contained higher levels of cytokinin than friable callus, and at the mature flower stage abnormal inflorescences contained less cytokinin than normal inflorescences.

In our experience the correlation between callus type and expression of abnormal flowering in regenerant palms is not at all clear cut. The original observations of abnormality (Corley et al. 1986) involved clones that originated from either friable or nodular callus types and were subsequently multiplied by proliferation of nodular proembryogenic tissues to produce palms for field evaluation. Only after clonal selection in the field, when no floral abnormalities were seen, were these clones further multiplied for commercial production. In this process the plants were produced by further multiplication of the nodular proembryogenic material without a callus phase. In addition, some clones showing the least tendency to produce abnormal flowers came from friable callus.

There is some evidence that the presence of cytokinins in the culture media is associated with an increased probability of floral abnormality in regenerated palms (unpublished data). It is important to distinguish between an effect of exogenous cytokinin (or other growth regulators) at the tissue culture stage as responsible for a fault in reproductive development some years afterward, and an imbalance in the endogenous metabolism of that particular growth regulator. It does not follow that if cytokinin in the medium increases the likelihood of the abnormality, it is the metabolism of cytokinin some years later, in tissues that did not exist at the time of cytokinin treatment, which is impaired.

The affected palms show no vegetative symptoms. In the case of the $SL_2$ gene in tomato (Sawhney and Greyson 1973) a global change in growth regulator concentration (in this case gibberellin) affected only developing flower primordia. It is therefore possible that analysis of vegetative tissues might reveal unusual concentrations of plant growth regulators in affected palms. Alternatively it is possible that the abnormality is due to the anomalous local expression of regulatory genes involved in a specific stage of floral development rather than a global change in concentrations of some metabolite or growth regulator. In such a case, biochemical differences may only be detectable in flower primordia at the specific stage of differentiation of the stamen primordia. We think this probably occurs at about leaf +5 to +8 (relative to the unexpanded spear leaf conventionally designed leaf 0) while the inflorescence is still very small, but after the formation of morphologically distinct spikelets of recognizable sex (Corley 1976).

From our studies of the cytokinin content of undifferentiated oil palm callus it is clear that endogenous cytokinin concentrations are very low (Jones 1990). Oil palm callus consists predominantly of large vacuolate cells, many of which are nondividing and senescent. Growth is maintained by the presence of relatively few centers of active meristematic activity (Jones 1983, Schwendiman et al. 1988). The embryoids and nodular calluses, on the other hand, consist mainly of small densely cytoplasmic cells, many of which are actively dividing. It is therefore not surprising that the cytokinin content of these tissues is different. Zygotic embryos, nodular calluses, and embryogenic tissues consistently contain higher concentrations of cytokinins, particularly zeatin riboside, than the friable open calluses (Jones 1990). These findings are in broad agreement with those of Besse et al. (1992). In attempting to evaluate the role of cytokinins in the expression of the abnormality it is important to compare the endogenous cytokinin contents of nodular embryogenic tissues and young regenerant plantlets of clones giving rise to normal palms with the same tissue types from those clones known to produce abnormal palms and with normal zygotic embryos and seedlings.

We have evidence that some clones consistently produce normal or abnormal palms and in some cases that different cell lines of the same genotype differ in their subsequent expression of abnormality. In addition, some cultures produce a small number of inflorescences in vitro at the apices of young regenerant plants. Frequently these inflorescences are abnormal, consisting only of clusters of floral bracts, but sometimes recognizable female flowers

are formed. These flowers can be scored for the presence of extra carpels produced from the coronet ring of vestigial stamen primordia.

We have used these two criteria (known susceptibility of certain cell lines and genotypes, and supplementary carpel formation on in vitro flowers) to distinguish culture lines with a high risk of abnormality from those of low risk. The results of cytokinin analysis from high and low risk lines were grouped separately for statistical analysis. We have also carried out analyses of the cytokinin content of immature inflorescences from normal and abnormal palms of the same clone at the critical stage of flower bud development when spikelet sex was recognizable but development of the flower primordia was only just beginning.

## Materials and Methods

### *Plants*

All tissue-cultured material was obtained from Unifield TC Ltd. Cultures were grown on media based on the formulation of Murashige and Skoog (1962) (M&S) with Unifield additives. Embryogenic cultures were cultured on a medium containing naphthalene-1-acetic acid at 0.1 mg · $L^{-1}$ (0.53 μM); in the first experiment kinetin was also supplied at 0.05 mg · $L^{-1}$ (0.12 μM). Rooted ramets were grown on half-strength M&S basal medium without hormone additives. Cultures were maintained under continuous fluorescent tube illumination (75 W · $m^2$) and maintained at a temperature of 30°C.

Commercial heat-treated seeds were supplied by Dr. R. H. V. Corley of Unilever Plantations. The seeds were germinated in polythene bags at 28°C. After emergence of the embryos the nuts were cracked in a vise and the embryos separated into the haustorium (contained within the endosperm) and the tigellum protruding through the emergence pore. One hundred embryos were used for extraction and analysis.

Inflorescences were provided by Pamol Plantations Sdn. Bhd. Kluang, Malaysia. Two palms of clone 90a, one with normal and the other with abnormal inflorescences, were felled and dissected in the field. Young inflorescences at an early stage of flower development (leaf axils 6, 7, and 8) were wrapped in aluminum foil, labeled, and dropped into liquid nitrogen. They were subsequently transported to the UK in dry ice and stored frozen at −70°C.

### *Cytokinin Nomenclature and Abbreviations*

The abbreviations used in this paper follow the convention proposed by Crouch et al. (1993).

### *Cytokinin Extraction, Purification, HPLC Separation, and Radioimmunoassay*

Extraction methods were as described in Turnbull and Hanke (1985). At the initial ethanol extraction stage, 50 μl of a solution of the tritiated diol of isopentenyladenosine (approximately 10,000 dpm of $^3H$ at 17 μM) was added to the extraction mixture as internal recovery marker. This compound was routinely used as the ligand in the radioimmunoassays for isopentenyladenine cytokinins. The diol has a retention time in HPLC about 2 min later than [9G]iP and does not interfere with other cytokinins. The counts recovered in the appropriate HPLC fractions were used to calculate cytokinin recoveries and assumed to be representative of recoveries of the natural cytokinins. Recoveries obtained varied between 60 and 85% with occasional lower values. HPLC separation and radioimmunoassay followed the method of Jones (1990) and Kraigher et al. (1991). The latter paper also describes the source and preparation of the antisera to [9R]Z, [9R]DHZ, and [9R]iP. Kinetin compounds were detected using antiserum to [9R]iP as described by Jones and Hanke (1992).

### *ELISAs*

The use of radioimmunoassay was superseded by the biotin-avidin-enhanced ELISA of Maldiney et al. (1986) and Sotta et al. (1987) using the same antibodies as used in the radioimmunoassay. Later, this method began to give erratic results and high backgrounds. Subsequently we employed the slightly less sensitive but more robust ELISA described by Strnad et al. (1992). The IgG fraction was prepared from the existing antisera as in Strnad et al. (1992). All ELISAs were on 96-well NUNC Immunosorb plates. The first 24 wells were used for blanks and replicated cytokinin standards in a logarithmic concentration series from 12.5 to 6,250 pmol in 50 μl. The remaining 72 wells were used for replicated analyses of 36 HPLC fractions. A four-parameter logistic curve was fitted to the optical densities of the standards on each plate using DeltaSoft software on an Apple Mackintosh SE30 computer. The cytokinin content in each experimental well on the ELISA plate was then automatically calculated by interpolation on the standard curve. The concentrations of individual cytokinins were estimated from the peaks coincident with the retention times of known standards run on the HPLC immediately before or after the experimental sample. Estimates were corrected for background by subtraction of the baseline values on either side of the known peaks. Peak identities were confirmed by comparing the cross-reactivities in assays with three different antisera specific for the isopentenyladenine, zeatin, and dihydrozeatin groups of cytokinins. Nonspecific interference with antibody binding was detected by the presence of a similar response in all three assays. Results are expressed in terms of cytokinin riboside equivalents, corrected for recovery losses but, with the exception of kinetin compounds, without correction for differences in cross-reactivity.

## Results

Table 1 shows the results of analysis of embryoids of six clones, three of which gave abnormal flowering plants and three normal clones. Cultures were sampled 6 weeks after transfer to media containing 0.23 μM kinetin. The presence of kinetin-9-glucoside in the samples precluded the estimation of zeatin, [9R]Z, dihydrozeatin, and [9R]DHZ. Two separate extracts were made from five pooled cultures of each clone, and radioimmunoassay analysis was done in duplicate on each sample. The estimates of

**Table 1.** Cytokinins in oil palm embryoids from normal and abnormal flowering clones (three normal, three abnormal). Cultures were sampled 6 weeks after transfer to medium containing 0.23 μM kinetin and analyzed by HPLC/radioimmunoassay (Turnbull and Hanke 1985). Shown are the means for six clones (two assays) with main effect means for clones and flower types. Significance level was from ANOVAR (see the Materials and Methods section for experimental design). NS, not significant.

| | | Cytokinin content (pmol riboside equivalent · $g^{-1}$ fresh weight) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Normal | | | | Abnormal | | | |
| Cytokinin | | 1 | 2 | 3 | | 4 | 5 | 6 | |
| [9G]KIN | | 1.3 | 6.3 | 8.0 | | 2.6 | 12.8 | 2.0 | |
| | Flower type mean | | 5.2 | | | | 5.8 | | NS |
| | All clone mean | | | | 5.5 | | | | $P = 0.03$ |
| KIN | | 0.6 | 1.9 | 0.5 | | 0.9 | 0.5 | 0.3 | |
| | Flower type mean | | 1.0 | | | | 0.6 | | NS |
| | All clone mean | | | | 0.8 | | | | $P = 0.1$ |
| [9R]KIN | | 0.4 | 2.9 | 3.4 | | 1.3 | 0.6 | 0.2 | |
| | Flower type mean | | 2.2 | | | | 0.7 | | NS |
| | All clone mean | | | | 1.5 | | | | NS |
| [9G]Z | | 0.7 | 9.4 | 3.4 | | 0.3 | 4.5 | 0.4 | |
| | Flower type mean | | 4.5 | | | | 1.7 | | NS |
| | All clone mean | | | | 3.1 | | | | $P = 0.01$ |
| [9G]DHZ | | 0.2 | 2.8 | 2.6 | | 2.4 | 2.4 | 0.6 | |
| | Flower type mean | | 1.8 | | | | 1.8 | | NS |
| | All clone mean | | | | 1.8 | | | | $P = 0.1$ |
| [9G]iP | | 11.2 | 9.1 | 40.7 | | 60.7 | 29.9 | 19.5 | |
| | Flower type mean | | 20.3 | | | | 36.7 | | NS |
| | All clone mean | | | | 28.5 | | | | $P = 0.003$ |
| iP | | 0.2 | 0.4 | 0.2 | | 0.4 | 0.7 | 0.1 | |
| | Flower type mean | | 0.3 | | | | 0.4 | | NS |
| | All clone mean | | | | 0.3 | | | | NS |
| [9R]iP | | 0.1 | 0.3 | 0.2 | | 0.3 | 0.2 | 0.1 | |
| | Flower type mean | | 0.2 | | | | 0.2 | | NS |
| | All clone mean | | | | 0.2 | | | | NS |

*Note.* The presence of kinetin-9-glucoside and kinetin interferes with the estimation of Z, [9R]Z, DHZ, and [9R]DHZ. Kinetin compounds were detected using antiserum to [9R]iP and estimated using cross-reactivities (50% inhibition of binding) relative to [9R]iP: KIN 3.6%, [9G]KIN 5.0%, [9R]KIN 10.3%.

concentration of the kinetin compounds were corrected for differences in cross-reactivity with the [9R]iP antibody. Otherwise, all data are given as cytokinin riboside equivalents. Since clone and flower type are not independent, the estimates of concentration of eight cytokinins were subjected to one-way ANOVARs, first comparing clones and then using clones as replicates for comparison of flower type. The main effect means for clone and normality status are given in Table 1, together with the significance levels where appropriate. It can be seen that there were large between-clone differences for most compounds detected but no significant differences between normal and abnormal lines.

In a subsequent experiment embryoids and ramets of 12 different clonal lines were made available (Table 2). Six were normal flowering and six abnormal (determined from the flower type of in vitro inflorescences). In this case we also analyzed control samples of early germinating embryos (equivalent to the embryoid stage in tissue culture) and young seedlings for comparison with ramets from tissue culture. Analysis of the HPLC fractions was by the ELISA method of Sotta et al. (1987). Although replication was not sufficient for full statistical analysis the results are given as means and standard deviations of duplicate analyses averaged over all clones within flower type. An ANOVAR was performed using clones as replicates for normal/abnormal comparison. The standard deviations were of the same order as the means, and it was clear that the differences between clones were far greater than any differences between normal and abnormal. It was reported (Jones 1990) that zygotic embryos were rich in [9G]DHZ. In this experiment it was found that the [9G]DHZ was predominantly located within the haustorium. Although no definitive conclusions could be reached there were consistently higher amounts of 9-glucosides in the tissue-cultured material (both embryoids and ramets) than in the embryos and seedlings, confirming our observations over several years.

It was clear that clonal differences in cytokinin

**Table 2.** Cytokinin analysis of 12 clones (six normal, six abnormal) plus zygotic embryos and seedlings. Samples were analyzed by HPLC followed by ELISA using the method of Sotta et al. (1987). Each assay was done in duplicate. Data are for means of values for clones and for flower type (normal or abnormal), shown with standard deviations (in parentheses). No significant differences were detected in ANOVAR. ND, not detected; —, not measured.

| | Cytokinin content (pmol riboside equivalent · $g^{-1}$ fresh weight) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Embryoids | | | Ramet shoots | | |
| Cytokinin | Embryos | | Seedling shoots | Clone mean (SD) | Normal mean (SD) | Abnormal mean (SD) | Clone mean (SD) | Normal mean (SD) | Abnormal mean (SD) |
| | Tigellum | Haustorium | | | | | | | |
| [9R-MP]Z | ND | ND | ND | 0.9 (1.1) | 0.4 (0.4) | 1.5 (1.3) | 0.7 (0.8) | 0.8 (0.6) | 0.7 (2.5) |
| [9G]Z | 0.2 | ND | 2.5 | 19.4 (23.9) | 13.6 (13.9) | 25.3 (31.4) | 6.6 (4.7) | 3.7 (1.9) | 8.3 (4.9) |
| Z | 0.2 | 0.2 | 0.1 | 0.4 (0.6) | 0.1 (0.2) | 0.7 (0.8) | 0.5 (0.5) | 0.3 (0.3) | 0.6 (0.6) |
| [9R]Z | 1.8 | 0.6 | 4.4 | 4.9 (4.3) | 2.9 (2.5) | 6.9 (5.0) | 6.0 (3.0) | 4.7 (2.8) | 5.5 (4.2) |
| [9R-MP]DHZ | ND | ND | ND | 0.4 (0.5) | 0.6 (0.6) | 0.2 (0.1) | 0.8 (1.3) | 0.9 (0.8) | 0.7 (1.6) |
| [9G]DHZ | 1.7 | 12.6 | 0.6 | 1.4 (1.0) | 1.9 (2.2) | 0.6 (0.3) | 0.8 (0.7) | 0.6 (0.7) | 1.0 (0.7) |
| DHZ | 0.1 | 0.8 | ND | 0.4 (0.5) | 0.3 (0.4) | 0.5 (0.6) | 0.2 (0.3) | 0.4 (0.5) | 0.04 (0.09) |
| [9R]DZ | 0.2 | 0.9 | 1.1 | 0.6 (0.9) | 0.9 (1.3) | 0.3 (0.2) | 3.2 (2.1) | 2.5 (1.5) | 3.7 (2.5) |
| [9RMP]iP | — | — | — | 0.6 (0.7) | 0.5 (0.6) | 0.8 (0.9) | — | — | — |
| [9G]iP | 1.4 | 1.8 | 6.6 | 588 (1192) | 308 (395) | 869 (1668) | 14.2 (18.8) | 5.1 (2.8) | 17.1 (23.8) |
| iP | 0.3 | 0.8 | ND | 2.3 (2.3) | 2.7 (3.1) | 1.9 (1.3) | 2.8 (2.5) | 2.6 (3.7) | 2.1 (1.9) |
| [9R]iP | ND | 0.2 | 13.3 | 2.7 (3.3) | 1.2 (1.0) | 4.2 (4.1) | 6.4 (4.7) | 4.7 (4.4) | 5.6 (5.3) |

concentration were large in comparison with any putative differences between normal and abnormal flowering lines. Eventually two cell lines of a single genotype became available, one of which continued to produce normal palms, and the other plants flowering abnormally. In this case we used the ELISA method of Strnad et al. (1992) to analyze HPLC fractions of extracts of embryoids, young shoots still in culture, and young ramets. The plantlets were separated into root and shoot tissues, which were analyzed separately. The results were subjected to ANOVAR and are given in Table 3 with significant differences between normal and abnormal lines marked * ($P < 0.05$). For the first time we were able to find a difference between normal and abnormal lines in the cytokinin concentrations. Abnormal lines contained significantly less [9G]Z and [9R]Z in embryoids and shoots and roots of ramets. As most of the active cytokinin was in the form of [9R]Z and there was approximately eight times more of it in the line flowering normally, this difference is likely to be of physiologic importance. The level of [9G]iP was significantly lower in abnormal than in normal embryoids, but this difference disappeared during development. There was a highly significant difference in the [9G]iP content of roots and shoots, irrespective of flowering type, with much greater accumulation in the roots.

Finally we obtained young inflorescences from normal and abnormal flowering palms of clone 90a growing in a clone trial in Pamol Plantations Sdn. Bhd. The isoprenoid cytokinin contents of female inflorescences at an early stage of flower development (leaf axils 6, 7, and 8) are shown in Table 4, together with the total inflorescence fresh weight, including the inner spathe (mature oil palm inflorescences weigh in excess of 1 kg).

The fresh weight was beginning to increase rapidly at this stage of development. Cytokinin concentrations increased markedly between the inflorescences in leaf axils 6 and 7, and there was evidence of relatively high levels of ribotides in inflores-

**Table 3.** Comparison of isoprenoid cytokinins in normal and abnormal lines of one genotype. Samples were analyzed in duplicate by HPLC followed by ELISA using the method of Strnad et al. (1992). Data shown are for embryoids, green shoots in culture and rooted ramets, divided into shoots and roots analyzed separately. Ramets were analyzed at three stages of development. Since no significant stage differences were detected the data were combined to provide more replication of these samples. ND, not detected. ANOVAR was done on each set of analyses.

| | Cytokinin content (pmol riboside equivalent · $g^{-1}$ fresh weight) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Embryoids | | Shoots in vitro | | Ramet shoots | | Ramet roots | |
| Cytokinin | Normal | Abnormal | Normal | Abnormal | Normal | Abnormal | Normal | Abnormal |
| [9R-MP]Z | 1.0 | ND | 0.3 | 0.1 | 0.1 | 0.1 | 0.4 | 0.4 |
| [9G]Z | 5.4[a] | 1.6[a] | 5.1[a] | 2.7[a] | 4.3[a] | 1.5[a] | 10.8[a] | 1.8[a] |
| Z | 1.9 | 0.2 | ND | 0.1 | 1.8 | 0.1 | 0.3 | 0.1 |
| [9R]Z | 7.9[a] | 0.9[a] | 152[a] | 10.6[a] | 1.3 | 1.1 | 3.0[a] | 0.8[a] |
| [9R-MP]DHZ | 0.2 | 0.2 | 0.8 | 0.8 | 0.1 | 0.3 | 0.1 | 0.3 |
| [9G]DHZ | 1.9 | 3.3 | 0.5 | 0.7 | 0.4 | 0.7 | 4.0 | 1.7 |
| DHZ | 0.8 | ND | 0.9 | 0.6 | 0.1 | ND | 0.2 | 0.1 |
| [9R]DHZ | 2.0 | 2.2 | 3.3 | 0.2 | .04 | 0.5 | 1.1 | 0.1 |
| [9G]iP | 41.0[a] | 6.6[a] | 34.0 | 21.0 | 4.9[b] | 2.9[b] | 29.9[b] | 31.0[b] |
| iP | 0.7 | 0.2 | 6.3 | 0.2 | 1.0 | 0.7 | 1.2 | 0.9 |
| [9R]iP | 1.5 | ND | 0.3 | 0.3 | 0.9 | 0.1 | 1.2 | 1.4 |

[a] Significant difference ($P < 0.05$) between normal and abnormal.
[b] Significant difference ($P < 0.05$) between roots and shoots.

**Table 4.** Isoprenoid cytokinins in immatue female inflorescences of normal and abnormal oil palms of Clone 90a. Inflorescences from leaf axils 6, 7, and 8 were analyzed by HPLC followed by ELISA using the method of Strnad et al. (1992). Data are means of duplicate ELISAs on single inflorescences. ND, not detected; —, not measured.

| | Cytokinin content (pmol riboside equivalent · $g^{-1}$ fresh weight) | | | | | |
|---|---|---|---|---|---|---|
| Cytokinin | 6 Normal | 6 Abnormal | 7 Normal | 7 Abnormal | 8 Normal | 8 Abnormal |
| [9R-MP]Z | — | — | 19 | 8 | 12 | 57 |
| [9G]Z | 3 | 1 | 161 | 44 | 78 | 58 |
| Z | 0.1 | 1 | ND | ND | 0.5 | 5.8 |
| [9R]Z | ND | 2 | 30 | 48 | 20 | >80 |
| [9R-MP]DHZ | — | — | 6 | 2 | 1 | 2 |
| [9G]DHZ | 2 | 2 | 6 | 3 | 2 | 2 |
| DHZ | 1 | 1 | ND | ND | ND | ND |
| [9R]DHZ | 2 | 7 | 3 | 7 | 2 | 6 |
| [9R-MP]iP | — | — | 10 | 2 | — | — |
| [9G]iP | 0.2 | 1.9 | 4 | 4 | 2 | 4 |
| iP | 0.3 | 0.2 | 3 | 1 | 3 | 2 |
| [9R]iP | 0.1 | 0.1 | 0.4 | 0.5 | 4 | 16 |
| | Inflorescence fresh weight (g) (outer spathe removed) | | | | | |
| | 1.54 | 1.18 | 2.38 | 2.76 | 2.45 | 4.57 |

cences 7 and 8. There was no clear indication of real differences between the cytokinin content of normal and abnormal types, although the cytokinin ribosides, [9R]Z, [9R]DHZ, and [9R]iP, were consistently higher in abnormal than in normal inflorescences, whereas the inactive [9G]Z was higher in the normal inflorescences. Without replication on other palms no significance testing was possible.

## Discussion

Although the definitive method for quantitative analysis would be gas chromatography/mass spectrometry with heavy isotope internal standards, this technology is not available to most laboratories, and the internal standards are not available for most of the cytokinins. The technique is not suitable for

the rapid analysis of multiple samples required in physiologic work. The ELISA method coupled with HPLC is a practical substitute, provided its limitations are fully appreciated in the interpretation of the data obtained. The use of antisera with different specificities in the assay of each sample provides a safeguard against false positives resulting from nonspecific interference with antibody binding.

### *Cytokinins in Oil Palm Tissues and Their Role in Flower Development*

As reported by Jones (1990) and confirmed by Besse et al. (1994), the cytokinin content of oil palm callus is very low, close to the limits of detection. The detection of differences in cytokinin concentration between friable callus and nodular embryogenic callus is not a priori evidence of the involvement of cytokinins in floral abnormalities. Rather it demonstrates the expected difference between a tissue containing large, vacuolated nondividing cells and one with predominantly small, highly cytoplasmic meristematic cells. In the current study a comparison of morphologically equivalent tissues of normal and abnormal lines was made. A difference in cytokinin content was found only in an otherwise isogenic line, when the abnormal line had significantly lower concentrations of [9R]Z and [9G]Z than the normal line. Although this finding supports that of Besse et al. (1992, 1994), differences between clones were much greater than any difference between normal and abnormal lines. Besse et al. (1992, 1994) also reported large between-clone differences in cytokinin content. The concentrations detected in oil palm tissues are in broad agreement between the two studies, although we consistently found relatively high concentrations of [9G]iP, especially in material from tissue culture, which was not reported by the French group. A rough comparison can be made with the results of Besse et al. (1992, 1994) who found 0.1–0.2 pmol $\cdot$ mg$^{-1}$ dry weight of [9G]Z in compact nodular callus, equivalent to approximately 10–20 pmol $\cdot$ g$^{-1}$ fresh weight, assuming 90% moisture (a mean of 20 pmol $\cdot$ g$^{-1}$ fresh weight in our assays). They reported 0.05 pmol $\cdot$ mg$^{-1}$ dry weight of [9R]Z, roughly equivalent to 5 pmol $\cdot$ g$^{-1}$ fresh weight (a mean of 5 pmol $\cdot$ g$^{-1}$ fresh weight in our assays). We thus have very similar results in quantitative terms but differ in our interpretation of the significance of cytokinin metabolism as a factor in induction of abnormal flowering. Differences between comparable tissues from normal and abnormal flowering cell lines were small and in most cases nonsignificant in comparison with the between-clone differences. The only case in which a significantly higher cytokinin content was found in normal than abnormal tissues was in a comparison within genotype. Finally, Besse et al. (1992) found large well replicated differences in the cytokinin contents of mature female flowers from mantled and normal inflorescences. We were unable to find similar differences at earlier stages of development when any differences should also be expressed. Although it is not possible to draw firm conclusions from unreplicated observations, there were higher levels of zeatin and dihydrozeatin and iP ribosides in the abnormal inflorescences than in the normal. This trend is in a direction opposite to the reported data for mature flowers. One possible explanation of the results of Besse et al. (1992) is that carpel tissues are low in cytokinins relative to the other flower parts, and this tissue forms a larger proportion of the total in the samples of mantled flowers.

Jones and Hanke (1992) demonstrated the ability of oil palm cultures to 9-glucosylate exogenously applied kinetin rapidly, and the presence of an active 9-glucosyltransferase can be inferred. The 9-glucosides are formed by glucosylation of the free bases following cleavage of the 9-ribosyl group. These compounds are thought to be nonmetabolizable end products sequestered in the cell vacuoles (Chen 1982, Palmer et al. 1981). In material from tissue culture there were consistently high concentrations of cytokinin 9-glucosides compared with noncultured tissues even in cultures not treated with exogenous cytokinins. The 9-glucosides can be regarded as providing an integrated record of cytokinin metabolic activity in the tissues, which is apparently high in vitro.

From the large differences in cytokinin concentrations between different clones with normal flowering it appears that concentration per se does not by itself cause the flowering abnormality, although this does not preclude the possibility that high cytokinin applied at the tissue culture stage might result in disruption of later gene expression. It is also clear from this work that oil palm tissues contain a very complex spectrum of cytokinins (including aromatic cytokinins to be reported later).

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