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# Effect of triacontanol on the lipid composition of cotton (*Gossypium hirsutum* L.) leaves and its interaction with indole-3-acetic acid and benzyladenine

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**Abstract**

Application of triacontanol (TRIA), a long chain aliphatic alcohol (C-30), to cotton (*Gossypium hirsutum* L.) leaves resulted in an increase in dry weight and an alteration in lipid composition. A significant increase in monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) was attained 24 h after TRIA treatment. However, no significant change in any of the individual phospholipids was observed. Benzyladenine (BA) treatment increased only phosphatidylcholine (PC) levels without having any effect on either glycolipids or other phospholipids. Indole-3-acetic acid (IAA) initiated no significant change in the lipid composition. Combined treatment with TRIA and BA resulted in an increase of MGDG, DGDG and PC, indicating that the individual effects of these two growth regulators were not altered.

The combined treatment of IAA and TRIA did not bring about any change in the levels of MGDG and DGDG indicating that the effect of TRIA was nullified by IAA. MDGD is known to be involved in the packaging of photosystem I proteins. Whether TRIA-induced increase in dry weight which is due to the enhanced photosynthetic rate, is related to increased MGDG levels is not yet discernible.

*Abbreviations:* BA = benzyladenine; DGDG = digalactosyldiacylglycerol; IAA = indole-3-acetic acid; MGDG = monogalactosyldiacylglycerol; PC = phosphatidylcholine; PE = phosphatidylethanolamine; PG = phosphatidylglycerol; PI = phosphatidylinositol; PS = phosphatidylserine; SQDG = sulfoquinovosyldiacylglycerol; TRIA = triacontanol

## 1. Introduction

Recently, there have been some additions to the existing membership of the family of plant growth regulators. Long chain aliphatic alcohols (C20–C34), which have been identified as natural constituents of plant wax in the cuticle, have been currently identified as one of the new groups of plant growth regulators [14, 21, 24]. Growth-stimulating long-chain aliphatic alcohols in nanomolar quantities increase dry weight, $CO_2$-fixation, reducing sugars, soluble proteins, and free amino acids in addition to increasing crop yield in a large number of crop plants [14, 15, 21, 24]. It has also been shown that an aliphatic alcohol, triacontanol (TRIA) stimulates membrane-bound ATPase activity [10, 11, 21], and enhances $^{86}Rb^{+}$ and $^{32}PO_4^{2-}$ uptake [20].

Long chain aliphatic alcohols have been marketed for agricultural use by various multinational and local companies in different formulations and trade names [14, 15, 21]. There have been claims of increased crop yield induced by these compounds [14], and they are increasingly becoming popular among farmers in India. Of late, some interesting investigations have been made to understand their mechanism of action [10, 11, 22, 23, 24]. A signal transduction mechanism has been envisaged for the action of TRIA which acts by eliciting a second messenger, 9-$\beta$-L(+)-adenosine [24], and therefore, aliphatic alcohols appear to be well entrenched as natural growth regulators.

Cell membranes are considered to be the first target of hormone action in plants, followed by a signal transduction involving a cascade of chemical events leading to the cellular expression of hormone action [27]. It has also been shown that in the course of action these growth regulators bring about changes in the composition of membrane constituents, mainly the alteration in the lipid composition [3, 7, 25, 26]. However, no information is available on the effect of these aliphatic alcohols on lipids in plants. Further, even though the long chain aliphatic alcohols are considered to be natural growth regulators, no attention has been given to their interaction with other growth regulators. In this regard, TRIA, being one of the long chain aliphatic alcohols that are strongly lipophilic has attracted our attention, and the effect of TRIA on lipid composition, and its interaction with IAA and BA in its action has been investigated.

## 2. Materials and methods

### *2.1 Chemicals*

Technical grade TRIA was a gift from a local agrochemical dealer (Jagadish Enterprises) and the remaining chemicals were of analytical grade. The organic solvents were purified and freshly distilled before use following standard procedures [29].

### *2.2 Plant material and treatment*

Cotton (*Gossypium hirsutum* L., CV, DCH 32; AP State certified, India) seeds were purchased from the local dealer. The seeds were sown on moist vermiculite and the seedlings were grown in a glasshouse under natural conditions without environmental control. The vermiculite was kept moist throughout the course of the plant growth. Eight-day-old plants were sprayed with 50 $\mu$M IAA, 5 $\mu$M BA and 120 nM TRIA individually or in combination. Cotyledonary leaves were harvested 24 h after spraying of the growth regulators and the lipids were extracted soon after harvesting.

### *2.3 Extraction, chromatography, identification and estimation of lipids*

Freshly harvested cotyledonary leaves were boiled in 2-propanol for 2 min to inhibit lipase activity and subsequently homogenized. Total lipids were extracted according to Turnham and Northcote [28], purified by washing as described by Folch et al. [5], concentrated under vacuum, and taken up in a small volume of chloroform. The total lipid thus obtained was chromatographed [8] on a column of silicic acid (Mallinckrodt, CC-4). Neutral lipids were eluted with 10 column volumes of chloroform; glycolipids with 40 column volumes of acetone, and phospholipids with 10 column volumes of methanol. Further separation of individual glycolipids and phospholipids was carried out by thin-layer chromatography (TLC) on Silica gel H (BDH, Glaxo Laboratories, India), using the solvent system, chloroform-methanol-acetic acid-water (85:15:10:3.5, by volume), according to Liljenberg and Arnold [13]. Lipids were visualized as bands with iodine vapor, identified by their Rf values [12], and further confirmed with specific spray reagents [8]; $\alpha$-naphthol for glycolipids, molybdate stain for phospholipids in general, and Dragendorff's reagent for PC. PE and PS were further verified with ninhydrin spray reagent for the amino group. The silica gel containing the lipids were scraped-off the plates after visualizing with iodine vapor and packed into small columns. Lipids were recovered from the silica gel by repeated elution of the columns (4–5 times) with chloroform-methanol (1:2 v/v) while centrifuging the columns at 200×g. The individual glycolipids were quantified according to Kushwaha and Kates [9] and the phospholipids by a modified Bartlett's method [1].

### *2.4 Statistical treatment*

The results were subjected to 'paired comparison' of non-independent variables in which each observation of the treatments was compared to that of corresponding control for proportionate change and significant difference (t) [2].

## 3. Results

Various concentrations of IAA, BA and TRIA were tested (results not shown) and optimal concentrations of these growth regulators were found to be 50 $\mu$M, 5 $\mu$M and 120 nM respectively. TRIA treatment of the plants resulted in an increase in the dry weight of the leaf (Fig. 1) within

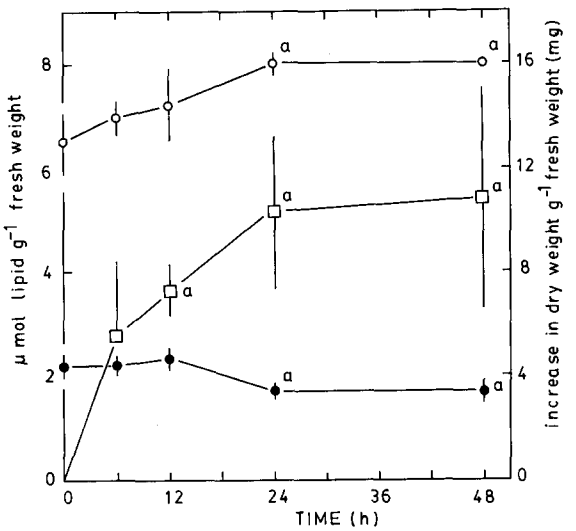


*Fig. 1.* Changes in dry weight (□) and in the levels of total glycolipids (○) and total phospholipids (●) in the silicic acid column fractions of the total lipid extracted from cotyledonary leaves of cotton (*Gossypium hirsutum* L.), treated with 120 nM triacontanol. Each point represents the mean value and the vertical bars indicate ± SE, (n = 5); a = significant at t = 0.05. Total glycolipids and phospholipids at 'O' time represent control. The dry weight of the tissue without treatment (control) is considered as 'O', and each point (□) represents the increase in dry weight over the control.

6 h of foliar application. However, statistically significant increases in dry weight were observed after 12 h of treatment. In addition, there was a considerable increase in the level of total glycolipids in the silicic acid column fractions in the TRIA-treated plants 24 h after treatment (Fig. 1). On the other hand, there was a slight but statistically significant decrease in the total phospholipid levels after 24 h of treatment with TRIA (Fig. 1). Individual glycolipids that were quantified were MGDG, DGDG and SQDG (Table 1), while the phospholipids were PC, PE, PG and PS + PI (Table 2). It was not possible to separate PS and PI with the solvent system used, and therefore, PS and PI were estimated together in terms of phosphate.

There was an increased level of MGDG (10 ± 3%) and DGDG (19 ± 4%) in the leaves of the plants treated with TRIA alone (Table 1). However, in the combined treatment of TRIA and BA, there was about 12% increase in MGDG and 24% increase in DGDG (Table 1). No significant change in the levels of these glycolipids was noticed in the leaves of the plants treated with TRIA + IAA, IAA or BA alone (Table 1). BA enhanced the level of only PC to about 15% when used alone and there was about 28% increase when used in combination with TRIA (Table 2). TRIA and IAA did not significantly affect any of the phospholipids (Table 2). In addition, treatment of plants with TRIA, BA and IAA individually or in combination did not alter significantly the levels of SQDG, PG, PE and PS + PI (Tables 1, 2).

## 4. Discussion

Since cotyledonary leaves have been used for lipid extraction (see materials and methods) investigations on the effect of ontogeny and age of the plant organ on lipid composition and plant response have been avoided. Further, in each experiment, individual glycolipids (MGDG, DGDG and SQDG) and phospholipids (PC, PE, PG and PS + PI) were obtained by the separation on TLC of the same aliquots of acetone (for glycolipids) and methanol (for phospholipids) fractions. Therefore, the change in the proportional level of different lipids (Tables 1, 2) seems to be due to the changed lipid composition following treatment with growth regulators. The large variations observed in the basal levels of lipids in different experiments (Tables 1, 2) appears to be due to changes in the natural environmental conditions, as the plants were grown throughout the year during the course of this investigation in a glasshouse without environmental control; this growth condition is similar to the field conditions. Such variations in lipids are not uncommon [12]. However, changes in the lipid levels on treatment with growth regulators were proportional to that of corresponding controls (Tables 1, 2). Wherever there is a statistically significant increase in the level of any lipid species (Tables 1, 2), there has been a consistent increase in all the experiments throughout the course of this investigation irrespective of change in the environmental conditions. Therefore the 't' test for 'paired comparison' with reference to respective control [2] has been found to be the most appropriate statistical treatment for these experiments.

TRIA is known to increase the dry weight in many crop plants [21]. A similar effect is also observed in cotton (Fig. 1). Further, a significant increase in the total glycolipids (Fig. 1) also occurs, which is reflected in the elevated levels of MGDG and DGDG (Table 1). Even though there

*Table 1.* Effect of triacontanol, IAA and BA, individually and in combination on the glycolipid composition of cotyledonary leaves of cotton (*Gossypium hirsutum* L.)

| Glycolipid | Experiment No. | $\mu$mol g$^{-1}$ dry weight | | | | | |
|---|---|---|---|---|---|---|---|
| | | Control | TRIA | IAA | BA | TRIA + IAA | TRIA + BA |
| MGDG | 1 | 45.03 | 46.87[a] | 46.01 | 41.34 | 46.83 | 46.68[a] |
| | 2 | 41.94 | 44.98[a] | 42.85 | 38.40 | 39.97 | 43.48[a] |
| | 3 | 41.71 | 44.85[a] | 42.62 | 38.03 | 42.62 | 46.28[a] |
| | 4 | 24.06 | 24.65[a] | 23.93 | 22.71 | 22.97 | 25.62[a] |
| | 5 | 27.43 | 27.78[a] | 27.28 | 26.82 | 26.19 | – |
| | 6 | 25.75 | 27.00[a] | 26.03 | 24.48 | 24.58 | 29.68[a] |
| | 7 | 24.12 | 30.38[a] | – | 24.52 | – | 30.56[a] |
| | 8 | 33.49 | 41.47[a] | – | 34.69 | – | 38.46[a] |
| | | | 10 ± 3%* | | | | 12 ± 3%* |
| DGDG | 1 | 32.10 | 33.88[a] | 32.79 | 29.92 | 27.84 | 31.60[a] |
| | 2 | 16.53 | 19.45[a] | 19.78 | 16.23 | 21.77 | 22.99[a] |
| | 3 | 21.07 | 24.11[a] | 24.51 | 23.55 | 24.51 | 26.41[a] |
| | 4 | 12.27 | 12.94[a] | 12.21 | 12.00 | 11.72 | 15.07[a] |
| | 5 | 13.96 | 16.06[a] | 13.88 | 15.56 | 13.35 | – |
| | 6 | 17.32 | 19.19[a] | 18.07 | 17.77 | 16.54 | 19.13[a] |
| | 7 | 10.30 | 15.84[a] | – | 9.32 | – | 15.06[a] |
| | 8 | 18.15 | 23.31[a] | – | 16.37 | – | 22.97[a] |
| | | | 19 ± 4%* | | | | 24 ± 4%* |
| SQDG | 1 | 10.30 | 10.34 | 10.52 | 10.11 | 10.52 | 10.67 |
| | 2 | 7.26 | 8.42 | 8.57 | 8.23 | 8.57 | 8.69 |
| | 3 | 5.39 | 4.23 | 5.50 | 5.29 | 5.50 | 5.59 |
| | 4 | 2.45 | 2.28 | 2.61 | 2.40 | 2.34 | 2.37 |
| | 5 | 2.79 | 2.74 | 2.78 | 2.40 | 2.67 | – |
| | 6 | 3.46 | 3.53 | 3.45 | 3.39 | 3.31 | 3.50 |
| | 7 | 2.89 | 3.42 | – | 3.13 | – | 2.91 |
| | 8 | 2.82 | 3.33 | – | 3.06 | – | 2.84 |

A = Values are significant at $< 0.01$ (paired comparison).
* Mean percent change (±SEM) over control (given only for the values which are significantly different from that of control).
In each experiment 5 g of tissue was taken for lipid extraction from each treatment.

is a significant decrease in the total phospholipid levels in silicic acid chromatographic fractions in TRIA-treated plants (Fig. 1), there is no significant decrease in any of the individual phospholipids estimated after separation on TLC (Table 2). This may be due to changes in other phosphate-containing lipid components. It is known in animal system that CTP: cholinephosphate cytidyltransferase is rate limiting in PC synthesis [16]. Price-Jones and Harwood [17] have shown that IAA inhibits CTP: cholinephosphate cytidyltransferase in plants, but this enzyme in pea stem appears to be regulated differently from that in mammals [18, 19]. Furthermore, whether auxin regulates PC synthesis in plants is not well established [6]. In this context, it is interesting to note that IAA does not have any significant effect on PC levels in cotton leaves (Table 2). TRIA enhances MGDG and DGDG levels (Table 1) having no effect on PC levels, while BA exhibits stimulatory effect on PC (Table 2) without having any effect on MGDG and DGDG levels (Table 1). The fact that the combination of TRIA and BA does not alter these individual effects of the compounds (Tables 1, 2) indicates that these two growth regulators do not interact with each other and thus they have independent action in regulating the levels of these lipids. On the contrary, the effect of TRIA in enhancing MGDG and DGDG levels is nullified in the presence of IAA (Table 1) which shows that IAA suppresses TRIA action.

Increase in dry weight by TRIA are attributed to enhancement of photosynthesis and accumulation of photosynthates [21, 24]. However, it is not understood exactly how these aliphatic alcohols could enhance the rate of photosynthesis. It is worth mentioning here that MGDG appears to be involved in the packing of photosystem I proteins

*Table 2.* Effect of triacontanol, IAA and BA, individually and in combination on the phospholipid composition of cotyledonary leaves of cotton (*Gossypium hirsutum* L.). Other details are the same as in Table 1

| Phospholipid | Experiment No. | $\mu$mol g$^{-1}$ dry weight | | | | | |
|---|---|---|---|---|---|---|---|
| | | Control | TRIA | IAA | BA | TRIA + IAA | TRIA + BA |
| PC | 1 | 7.14 | 5.93 | 7.29 | 10.03[a] | 6.03 | 11.24[a] |
| | 2 | 7.60 | 7.63 | 7.76 | 8.29[a] | 7.76 | 10.50[a] |
| | 3 | 12.70 | 12.76 | 8.65 | 13.29[a] | 8.65 | 13.16[a] |
| | 4 | 5.38 | 4.99 | 5.61 | 6.05[a] | 5.39 | 6.22[a] |
| | 5 | 5.91 | 5.23 | 5.88 | 6.31[a] | 5.65 | – |
| | 6 | 5.38 | 4.61 | 5.35 | 6.31[a] | 5.26 | 6.09[a] |
| | 7 | 6.71 | 6.81 | – | 7.70[a] | – | 9.20[a] |
| | 8 | 7.48 | 7.78 | – | 8.53[a] | – | 9.90[a] |
| | | | | | 15 ± 3%* | | 28 ± 4%* |
| PE | 1 | 1.92 | 2.17 | 2.59 | 2.85 | 1.96 | 3.01 |
| | 2 | 1.35 | 1.35 | 1.38 | 1.49 | 1.20 | 1.75 |
| | 3 | 3.06 | 3.28 | 2.85 | 2.21 | 2.01 | 2.04 |
| | 4 | 0.75 | 0.70 | 0.75 | 0.95 | 0.82 | 0.93 |
| | 5 | 0.86 | 0.80 | 0.91 | 1.00 | 0.86 | – |
| | 6 | 0.65 | 0.60 | 0.64 | 0.81 | 0.64 | 0.83 |
| | 7 | 1.72 | 1.47 | – | 1.97 | – | 1.74 |
| | 8 | 1.59 | 1.66 | – | 1.93 | – | 1.99 |
| PS + PI | 1 | 2.29 | 2.17 | 2.21 | 2.49 | 2.97 | 3.27 |
| | 2 | 2.02 | 2.03 | 2.07 | 1.98 | 2.07 | 2.10 |
| | 3 | 3.60 | 2.80 | 2.85 | 2.74 | 2.85 | 2.89 |
| | 4 | 1.18 | 1.10 | 1.07 | 1.16 | 1.13 | 1.09 |
| | 5 | 1.08 | 1.00 | 1.28 | 1.16 | 1.03 | – |
| | 6 | 0.54 | 0.50 | 0.53 | 0.59 | 0.62 | 0.67 |
| | 7 | 1.05 | 1.70 | – | 1.56 | – | 1.25 |
| | 8 | 2.35 | 3.13 | – | 2.86 | – | 2.57 |
| PG | 1 | 1.67 | 1.43 | 1.20 | 1.40 | 1.20 | 1.35 |
| | 2 | 1.18 | 1.52 | 1.20 | 0.82 | 1.20 | 1.05 |
| | 3 | 3.06 | 3.62 | 4.23 | 4.52 | 4.32 | 3.41 |
| | 4 | 1.08 | 1.10 | 1.07 | 1.05 | 1.13 | 0.93 |
| | 5 | 0.97 | 0.90 | 0.96 | 0.89 | 0.87 | – |
| | 6 | 0.86 | 0.85 | 0.83 | 0.81 | 0.90 | 0.75 |

[4]. In this regard, the relationship between TRIA-enhanced MGDG level (Table 1) and elevated photosynthetic rate by TRIA [21, 24] warrants further investigations.

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