BIOLOGIA PLANTARUM 35 (4): 577-581, 1993

Physiological responses of mangrove seedling to triacontanol

P. MOORTHY and K. KATHIRESAN

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai 608502, Tamil Nadu, India

Abstract

In viviparous hypocotyls of *Rhizophora apiculata* Blume the triacontanol treatment enhanced the growth of root and shoot (number of primary and secondary roots, length of roots, shoot height and biomass) protein and energy contents of leaves and roots, *in vivo* nitrate reductase activity, contents of chlorophylls and carotenoids in leaves as well as the amount of chlorophylls present in photosystems 1 and 2 and in the light harvesting complex of chloroplasts. These promotory effects were recorded at 40 and 80 mg m^{-3} triacontanol, but they decreased with increasing growth regulator concentration.

Introduction

Long chain aliphatic alcohols increase production potential of crop plants (Menon and Srivastava 1984) by increasing the rate of photosynthesis and reducing photorespiration (Ries *et al.* 1977, Jones *et al.* 1979). Earlier reports have shown the growth regulating properties of a long chain aliphatic alcohol, *viz.* triacontanol (Ries *et al.* 1977). Ries and Wert (1977) have reported increases in leaf area and dry matter of maize and paddy treated with triacontanol. Ries *et al.* (1977) have also reported yield increases, resulting from foliar application of triacontanol. Jones *et al.* (1979) found growth stimulation when analogues of triacontanol were tested. Triacontanol has been studied as soil or seed or foliar treatment in some crop plants (Ries *et al.* 1978). Nevertheless, its application on a viviparous hypocotyl or on a mangrove halophyte has not yet been reported. Hence, the present study has been made on the triacontanol-induced changes in seedlings of a mangrove species *R. apiculata* Blume.

Received 21 October 1992, *accepted* 16 February 1993.

Abbreviations: Car - carotenoids; Chl - chlorophyll; PS1 - photosystem 1; PS2 - photosystem 2; LHC - light harvesting complex; NRA - nitrate reductase activity.

Acknowledgements: Authors are thankful to the Director of this Centre, Prof. T. Govindarajulu, Dean, Faculty of Science, and to the authorities of Annamalai University for providing facilities and to Tamil Nadu State Council for Science and Technology, Madras for providing financial assistence.

Material and methods

Healthy, 27 ± 2 cm long hypocotyls of *R. apiculata* Blume were collected from individual plants in May 1992 from the Pichavaram mangroves (lat. 11° 27' N; long 79° 47' E), southeast coast of India. The hypocotyls were placed separately in 500 cm^3 beakers containing 250 cm^3 of the test medium (distilled water with triacontanol at 40, 80, 120, 160, 200 and 240 mg m^{-3}) for 24 h. The triacontanol was obtained under a trade mark "*Miraculan*" from *National Organic Chemical Industries*, Bombay, India. After soaking the hypocotyls were transferred into estuarine water and incubated at room temparature (28 ± 2 °C). Five replicates of ten hypocotyls each were maintained for each treatment. After 20 d, the seedlings were analysed for physiological characteristics of root and shoots.

Dry matter production was measured by drying the tissue in an oven at 70 °C for 72 h. Total soluble protein content and the corresponding energy value in root and shoot were estimated according to Lowry *et al.* (1951) and Brody (1945), respectively. *In vivo* nitrate reductase activity (NRA) in the shoot tissue was assayed using the method of Jaworski (1971). Chlorophylls (Chl) and carotenoids (Car) were extracted in 80 % acetone and measured spectrophotometrically following the methods of Arnon (1949) and Ridley (1977). Amounts of Chl in PS1, PS2 and LHC were quantified according to Krivosheeva *et al.* (1991).

Results and discussion

Root growth characteristics: In general, triacontanol stimulated root growth especially at lower concentrations (Table 1). Triacontanol increased the number of roots (significant increase of 84 % at 40 and 80 mg m^{-3}). There was a decline in number of roots at 240 mg m^{-3}. Triacontanol (40 to 160 mg m^{-3}) stimulated significantly the length of roots (maximum at 40 mg m^{-3}), but reduced it at 240 mg m^{-3}. The number of secondary roots was also increased, but, it was not statistically significant. Thus the triacontanol stimulated cell elongation rather than cell initiation. This effect may be attributed to hormone-like activities; similarly gibberellin and cytokinin activate cell elongation and auxin activates cell initiation (see Raghava and Nisha Raghava 1990). This is supported by an earlier report that mixtalol, analogue of triacontanol, shows positive reaction when tested by standard bioassays for plant hormones like the coleoptile straight growth test (auxin), α-amylase induction in the seeds (gibberellin) and cucumber cotyledon expansion test (cytokinin) (Treharne 1982, Venkataramani *et al.* 1987). In the present study, triacontanol acted similarly to gibberellin and cytokinins rather than the auxin.

Triacontanol increased root biomass (maximum of 133 % at 40 mg m^{-3}), but reduced it at high concentrations (200 and 240 mg m^{-3}). Similar increase in root biomass has been reported in various crops (Menon and Srivastava 1984).

Root protein and root energy increased with increasing concentrations of triacontanol up to 240 mg m^{-3}, when it might require to overcoming the stress effects of triacontanol on root growth.

Shoot growth and leaf responses: Triacontanol increased shoot length significantly at 40 mg m^{-3}. At the same triacontanol concentration, root characteristics were improved significantly (Table 1). However, at 160 and 200 mg m^{-3} it reduced the shoot length.

Table 1. Changes in root growth characteristics of *Rhizophora apiculata* as influenced by triacontanol (n = 10). Values in the parentheses are % increase or decrease over control.

Treatment [mg m^{-3}]	Number of roots per hypocotyl	Total length of roots per hypocotyl [cm]	Maximum length of roots per hypocotyl [cm]	Number of secondary roots per hypocotyl	Dry matter accumulation per hypocotyl [g]	Root protein in fresh matter [%]	Root energy [kJ kg^{-1}]
Control	6.5	12.4	4.8	69.2	0.480	2.3	544
40	12.0* (84)	79.7* (542)	10.2* (112)	106.2 (53)	1.123* (133)	2.8 (22)	661 (22)
80	12.0* (84)	79.6* (541)	9.7* (102)	138.0 (99)	0.885* (84)	3.6* (57)	854* (60)
120	10.0 (53)	63.5* (412)	9.7* (102)	86.5 (25)	0.834* (73)	3.5* (52)	829* (52)
160	9.5 (46)	54.7* (341)	9.5* (97)	78.2 (13)	0.701* (46)	3.7* (61)	875* (61)
200	8.0 (23)	34.1 (175)	8.5* (77)	55.7 (-20)	0.375 (-22)	4.0* (74)	942* (74)
240	1.7 (-74)	5.6 (-55)	2.0 (-98)	1.2 (-98)	0.350 (-27)	4.2* (83)	996* (83)
L.S.D. (0.05)	5.3	22.3	0.3	NS	0.128	0.8	134

* - significant change at P = 0.05.

Triacontanol treatment caused also a significant increase in leaf protein and leaf energy (up to 53 %). Similar increase in leaf protein of enzyme extracts has already been reported in maize treated with mixtalol and has been attributed to the synthesis of photosynthetic carboxylases, ribulose-1,5-bisphosphate carboxylase in paddy and phosphoenolpyruvate carboxylase in maize, which resulted in high rate of photosynthesis due to mixtalol (Menon and Srivastava 1984).

In the present study, *in vivo* NRA was enhanced by 13 % due to triacontanol treatment. A good correlation was found between the increase in NRA and leaf protein content as found earlier in a grass *Pennisetum* in response to long chain aliphatic alcohol (Muthuchelian *et al.* 1990).

40 and 80 mg m^{-3} triacontanol increased dry matter production of shoot (Table 2). Promotory effect of triacontanol on biomass formation has already been reported in *Pennisetum* (Muthuchelian *et al.* 1990) and in maize and paddy (Ries and Wert 1977). However, the dry matter production decreased with further increasing triacontanol concentration, and at 240 mg m^{-3} no shoot growth was observed.

Changes of pigment levels in protein complexes of chloroplast: The contents of photosynthetic pigments increased in seedlings treated with 40 to 120 mg m^{-3} triacontanol. Similar increase in pigments has also been demonstrated in paddy, maize and tomato (Venkataramani *et al.* 1987) and attributed to inhibition of senescence or iron uptake which regulates Chl biosynthesis (Menon and Srivastava 1984). Triacontanol increased the contents of Car and Chl (a + b) to the magnitude of 208 to 120 %, respectively, in seedlings of *R. apiculata* (Table 3). Venkataramani *et*

Table 2. Changes in leaf and shoot growth characters of *Rhizophora apiculata* as influenced by triacontanol (n = 10). Values in parentheses are % increase or decrease over control.

Treatment [mg m^{-3}]	Shoot length [cm]	Dry matter accumulation per hypocotyl [g]	Leaf protein in fresh matter [%]	Leaf energy [kJ kg^{-1}]	NRA *in vivo* [μmol(NO_2) $kg^{-1}s^{-1}$]
Control	9.2	0.690	3.4	804	2.50
40	13.5* (46)	0.900 (30)	4.2* (24)	996* (24)	2.72 (8)
80	12.2 (31)	0.850 (23)	5.2* (53)	1231* (53)	2.83* (13)
120	10.7 (16)	0.688 (-1)	4.1* (21)	971* (29)	2.81* (12)
160	6.5 (-30)	0.598 (-23)	3.8 (11)	900 (20)	2.75 (10)
200	6.5 (-30)	0.505 (-27)	3.3 (-3)	783 (-3)	2.58 (3)
L.S.D. (0.05)	3.2		0.6	29	0.27

* - significant increase over control (P = 0.05)

Table 3. Variation in contents of chlorophyll and carotenoids in *Rhizophora apiculata* as influenced by triacontanol (n = 10). The values in parentheses are % increase or decrease over control.

Treatment [mg m^{-3}]	Chlorophyll (a + b) [g kg^{-1}(f.m.)]	Carotenoids [mg kg^{-1}(f.m.)]	Content of chlorophyll (a + b) [g kg^{-1}(f.m.)] PS1 + PS2	LHC
Control	10.4	0.206	2.58	3.93
40	22.5* (116)	0.473* (130)	7.11*(176)	7.69* (96)
80	22.9* (120)	0.634* (208)	5.63*(118)	8.67* (121)
120	18.9* (82)	0.257* (25)	4.80* (86)	7.00* (78)
160	5.65 (-46)	0.037 (-82)	1.28 (-50)	2.56 (-35)
200	4.01 (-61)	0.006 (-97)	0.54 (-79)	1.36 (-65)
L.S.D. (0.05)	6.29	0.041	2.08	2.41

* - significant increase over control (P = 0.05)

al. (1987) found no significant increase in Chl content in the leaves of paddy, maize and tomato; but they found a significant increase in the Car content. The high accumulation of Car reveals a rich LHC in the treated plants (Dahlin 1988). The Chl *b* is also associated with the LHC of the PS1 and PS2 (Thornber 1975). High amount of Chl in the LHC, PS1 and PS2 was recorded in seedlings treated with 40 - 120 mg m^{-3} triacontanol, but higher triacontanol concentrations were inhibitory. LHC plays an important role in the organization of the chloroplast membrane and the light-harvesting efficiency of PS2 (Lam *et al.* 1983), and hence an increase in photochemical activity in the triacontanol-treated seedlings may be assumed. Thus the treatments may be beneficial in raising vigorous seedlings in nurseries for conservation and management of mangroves.

References

Arnon, D.I.: Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-15, 1949.

Brody, S.: Bioenergetics and Growth. - Reinhold, New York 1945.

Dahlin, C.: Correlation between pigment composition and apoproteins of the light harvesting complex II (LHC II) in wheat (*Triticum aestivum*). - Physiol. Plant. **74**: 342-348, 1988.

Jaworski, J.G.: Nitrate reductase assay in intact plant tissue. - Biochem. biophys. Res. Commun. **43**: 1274-1279, 1971.

Jones, J., Wert, V.F., Ries, S.K.: Specificity of 1-triacontanol as a plant growth stimulator and inhibition of its effect by other long chain compounds. - Planta **144**: 277-282, 1979.

Krivosheeva, A.A., Shavnin, S.A., Kalinin, V.A., Venedikov, P.S.: [Effect of industrial pollutants on seasonal changes of chlorophyll content of Scots pine seedlings.) - Fiziol. Rast. **38**: 163-168, 1991. [In Russ.]

Lam, B., Baltimore, B., Ortiz, N., Chollars, W., Melis, A., Malkin, R.: Characterization of a resolved oxygen evolving photosystem II. Preparation from spinach thylakoids. - Biochim. biophys. Acta **724**: 201-211, 1983.

Lowry, O.H., Roseborough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with folin phenol reagent. - J. biol. Chem. **193**: 265-275, 1951.

Menon, K.K.G., Srivastava, H.C.: Increasing plant productivity through improved photosynthesis. - Proc. indian Acad. Sci., Plant Sci. **93**: 359-378, 1984.

Muthuchelian, K., Paliwal, K., Gnanam, A., Mitchell, R.K.: The effect of three long chain aliphatic alcohols on photosynthesis and growth of *Pennisetum polystachyeon* Schult. - Photosynthetica **24**: 257-259, 1990.

Raghava, R.P., Raghava, N.: Use of bioregulators in orchards and nurseries. - In: Prakash, R. (ed.): Advance in Forestry Research in India. Vol. VI. Pp. 19-54. International Book Distributors, Dehradun 1990.

Ridley, S.M.: Interaction of chloroplast with inhibitor induction of chlorosis by diuron during prolonged illumination *in vitro*. - Plant Physiol. **59**: 724-732, 1977.

Ries, S.K., Bittenbender, H., Hangarter, R., Kolker, L., Morris, G., Wert, V.: Improved growth and yield of crops from organic supplements. - In: Lokeretz, W. (ed.): Energy and Agriculture. Pp. 377-394. Academic Press, New York 1977.

Ries, S.K., Richman, T.L., Wert, V.: Growth and yield of crops treated with triacontanol. - J. amer. Soc. hort. Sci. **103**: 361-364, 1978.

Ries, S.K., Wert, V.: Growth responses of rice seedlings to triacontanol in light and dark. - Planta **135**: 77-82, 1977.

Thornber, J.P.: Chlorophyll protein-light harvesting and reaction centre components of plants. - Annu. Rev. Plant Physiol. **26**: 127-158, 1975.

Treharne, K.J.: Chemical manipulation of crop growth and development. - In: McLaren, J.S. (ed.): Hormonal Control of Photosynthesis and Assimilate Distribution. Pp. 27-32. Butterworths, London 1982.

Venkataramani, S., Srivastava, H.C., Menon, K.K.G.: Effect of mixtalol on physiological aspects of plant growth. - Plant Physiol. Biochem. **14**: 28-35, 1987.

Communicated by Z. ŠESTÁK