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Research Article

Auxin (Indole-3-acetic acid), Gibberellic acid (GA_3), Abscisic Acid (ABA) and Cytokinin (Zeatin) Production by Some Species of Mosses and Lichens

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Abstract: In this study, the levels of endogenous free, bound and total auxin (Indole-3-acetic acid, IAA), gibberellic acid (GA_3), abscisic acid (ABA) and cytokinin (zeatin) were examined in some species of mosses and lichens. For determination of the levels of these plant growth regulators, spectrophotometry was used. Our findings show that the mosses and lichens used in this study produce the plant growth regulators IAA, GA_3, ABA and zeatin.

Key Words: Moss, Lichen, Indole-3-acetic acid, Gibberellic acid, Abscisic acid, Zeatin.

Bazı Yosun ve Liken Türlerinde Oksin (Indol-3-asetile asit), Gibberellik asit (GA_3), Absisik Asit (ABA) ve Sitokinin (Zeatin) Üretimi

Özet: Bu çalışmada, bazı liken ve yosun türlerinde içsel serbest, bağlı ve toplam oksin, (İndol-3 asetik asit, IAA) gibberellik asit (GA_3), absisik asit (ABA) ve sitokinin (zeatin) miktarları incelenecektir.

Bu bitki büyüme düzenleyicilerinin miktarlarının tayininde spektrofotometre tekniği kullanıldı. Bulgularımız, bu araştırmada kullanılan yosun ve likenlerin bitki büyüme düzenleyicileri IAA, GA_3, ABA ve zeatin ürettiğini göstermektedir.

Anahtar Sözcükler: Yosun, Liken, İndol-3 asetik asit, Gibberellik asit, Absisik asit, Zeatin.

Introduction

Due to the recent discovery of plant growth regulators, it has been possible to control plant growth and many activities concerned with growth. In addition to higher plants, fungi and bacteria, there is evidence that mosses also synthesize the plant growth regulators auxin, gibberellic acid, abscisic acid and cytokinin. For example, auxin is produced by sea algae (Weij, 1933; Mowat, 1965; Abe et al., 1974), *Fucus* L. sp. (Weij, 1933; Du Buy & Olson, 1937; Van Overbeek, 1940), *Bryopsis* J.V.Lamour. sp., *Elodea* Michx. sp. and *Macrocystis* C.Agardh sp. (Van Overbeek, 1940). The production of IAA was also detected in the mosses of *Phycomitrella patens* (Hedw.) B., S. & G. (Ashton et al., 1985) and *Funaria hygrometrica* Hedw. (Jayaswall & Johri, 1985; Atzorn et al., 1989a; Atzorn et al., 1989b; Bhatla, 1992). Gibberellic acid and ABA are produced by algae (Mowat, 1965; Radley, 1961; Mowat, 1963; Jennings & Mclomb, 1967; Jennings, 1968; Moss, 1965; Jennings, 1969; Hussain & Boney, 1973; Kingman & Moore, 1982; Tietz & Kasprik, 1986; Sabbatini et al., 1987; Boyer & Dougherty, 1988; Tietz et al., 1989; Hirch et al., 1989). Cytokinins are produced by brown algae [*Laminaria digitata* (Hudson) J.V.Lamour. and *Ecklonia* Hornem. sp.] (Jennings, 1969) and red algae (*Hypnea* J.V.Lamour.) (Hussain & Boney, 1969). The production of isopentenyl adenine and zeatine from cytokinins was also detected in the mosses of *Funaria hygrometrica* (Beutelmann &

Bauer, 1977) and *Physcomitrella patens* (Wang et al., 1980; Wang et al., 1981a; Wang et al., 1981b).

There are a limited number of reports on the level, metabolism and physiological effects of all of these plant growth regulators in mosses. However, almost no study has been directed toward the production, metabolism and physiological effects of all of these plant growth regulators in lichens.

For this reason, we studied the level of IAA, GA_3, ABA and zeatin in some species of mosses and lichens.

Materials and Methods

Ten species of mosses (*Homalothecium sericeum* (Hedw.) B., S. & G., *H. lustescens* (Hedw.) Robins., *Rhynchostegium riparioides* (Hedw.) C.Jens., *Brachythecium starkei* (Brid.) B., S. & G., *Tortella flavovirens* (Bruch) Broth., *Grimmia pulvinata* (Hedw.) Sm., *Antitrichia californica* Sull., *Pellia epiphylla* (L.) Corda, *Reboulia hemisphaerica* (L.) Raddi, *Ulva lactuca* (L.) Le Jollis) and nine species of lichens (*Cladonia* Hill ex Browne sp., *Cladonia foliacea* (Huds.) Willd., *Xanthoria parietina* (L.) Th.Fr., *X. polycarpa* (Hoffm.) Rieber, *Pseudevernia furfuracea* (L.) Zopf, *Cetraria islandica* (L.) Ach., *Usnea* Hill sp., *Letharia vulpina* (L.) Hue, *Squamarina cartilaginea* (With.) P.James) were used as experimental materials.

Extraction, purification and quantitative determination of free and bound IAA, GA_3, ABA and zeatin in both the moss and the lichen samples were done, with minor modifications, according to the methods of Ünyayar et al. (1996). The extraction and purification procedures are shown in Fig. 1. Spectrophotometric techniques were used to determine the amounts of IAA, GA_3, ABA and zeatin.

Either one gram fresh weight of each moss sample or one gram dry weight of each lichen sample was taken and combined with 60 ml of methanol: chloroform: 2N ammonium hydroxide (12:5:3 v/v/v). Each combined extract (60 ml) was kept in a bottle at –20°C in deep freeze for further analysis.

IAA, GA_3, ABA and zeatin extraction assays were done according to the schematic diagram (Fig. 1). Combined extract was treated with 25 ml of distilled water. The chloroform phase was discarded. The water-methanol phase was evaporated. The water phase was adjusted to

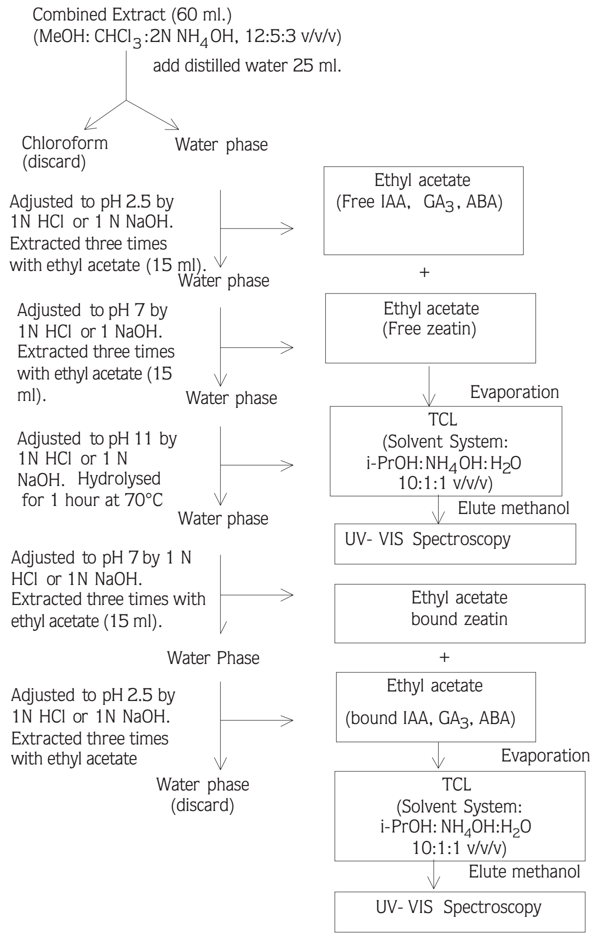

Figure 1. Flow diagram outlining the extracts used in purification of IAA, GA_3, ABA and zeatin for UV-spectrophotometer.

the extract pH value of 2.5 or 7 or 11 with 1 N HCl or 1 N NaOH respectively and 15 ml ethyl acetate was added at each of three steps. This procedure provided the isolation of free-form IAA, GA_3, ABA and zeatin from the extraction solvent. After an incubation period of 1 hour at 70°C, the same procedure was used for the isolation of bound-form IAA, GA_3, ABA and zeatin from the extraction solvent. Evaporation of ethyl acetate was performed at 45°C using a rote-evaporator system (Büchi Instruments). Thin-layer chromatography (TLC) was done using silica gel GF254 (Merck Chemicals, Germany) according to the method of Ünyayar et al. (1996). TLC-separated IAA, GA_3, ABA and zeatin were isolated from the glass plaques according to the standard synthetic IAA, GA_3, ABA and zeatin Rf values. IAA, GA_3, ABA and zeatin were dissolved with 2 ml of methanol for filtration and separation from silica using cotton-glass filled transferring pipettes.

Spectrophotometric assay was done using 222 nm and 280 nm wave lengths for IAA, 254 nm for GA_3, 263 nm for ABA, and 269 nm for zeatin and for all standard synthetic IAA, GA_3, ABA and zeatin and isolated samples.

All experiments were repeated three times. Total IAA, GA_3, ABA and zeatin was then obtained as the sum of free and bound IAA, GA_3, ABA and zeatin. The amounts of IAA, GA_3, ABA and zeatin in the mosses and lichens samples were expressed as standard synthetic IAA, GA_3, ABA and zeatin equivalent.

Statictical analysis were performed using SPSS for windows statistical software (SPSS Inc., USA) for ± standard error and mean of each value.

Results and Discussion

The amounts of IAA, GA_3, ABA and zeatin in the moss samples are given in Table 1.

The highest total IAA level was determined in *H. sericeum* (33.68µg/ml., 119.28µg/ml), while the lowest was recorded in *H. lustescens* (8.24µg/ml, 22.83µg/ml) at 222 nm and 280 nm wavelengths respectively.

Many researchers have established that auxin is produced by mosses. For example, Thomas et al. (1983) demonstrated that the level of free IAA in *Pellia epiphylla* was 2.5-2.9 µg/g fresh weight. While they determined the free form of IAA, they did not find the bound form of IAA in this species. However, we have demonstrated that IAA occurs in either free or bound forms in the all mosses used in the study. In our study, the level of free IAA in *Pellia epiphylla* was found to be 10.10 µg/ml and 15.36 µg/ml at 222 nm and 280 nm wave lengths respectively (Table 1).

IAA levels in various species have been investigated by different researchers. For example, the level was found to be 2-5 µg/g fw in *Funaria hygrometrica* cultures (Jasaywal & Johri, 1985), about 0.5 nmol/g dw in *Physcomitrella patens* (Ashton et al., 1985), about 80 gamma/kg fw in *Bryopsis* sp. (Van Overbeek, 1940), and 0.5 gamma/kg fresh weight in *Macrocystis pyrifera* (Linnaeus) C. Agardh (Van Overbeek, 1940). These results are in accordance with our results on auxin production by mosses.

In our experiment, the highest total GA_3 and zeatin levels were detected in *H. sericeum* (58977.07µg/ml. and 68.89µg/ml, respectively) and the lowest total GA_3 and zeatin levels were found in *H. lustescens* (10581.11µg/ml) and in *U. lactuca* (15.95µg/ml) respectively. As for ABA, it was highest in *B. starkei* (227.05µg/ml), and lowest in *A. californica* (60.05µg/ml).

Although there is evidence that gibberellins are synthesized by sea algae (Radley, 1961; Mowat, 1963; Jennings & Mclomb, 1967; Jennings, 1968), there are no reports on their levels. However, there is evidence concerning ABA and zeatin levels. For example, 0.014-0.044 µg/g dry weight ABA for *Stigeoclonium* sp. Kütz. (Tietz & Kasprik, 1986), 0.10-0.46 µg/g dry weight ABA or 0.03-0.14 µg/ml ABA for *Ascophyllum nodosum* (L.) Le Jolis (Boyer & Dougherty, 1988), 34 nmol/kg fresh weight ABA for *Halimeda tuna* (J.Ellis & Sol.) J.V.Lamour., 26 nmol/kg fresh weight ABA for *Caulerpa mexicana* Sonder ex Kütz., 3 nmol/kg fresh weight ABA for *Caulerpa prolifera* (Forssk.) J.V.Lamour., 7 nmol/kg fresh weight ABA for *Caulerpa racemosa* (Forssk.) J.Agardh (Hirch et al., 1989), 0.004 µg/g fresh weight zeatin for the wild type of *Physcomitrella patens*, 0.300 µg/g fresh weight zeatin for the mutant OVEA 200 of *Physcomitrella patens*, 0.270 µg/g fresh weight zeatin for the mutant OVEB 100 of *Physcomitrella patens* (Wang et al., 1981b).

The amounts of IAA, GA_3, ABA and zeatin in the lichen samples are given in Table 2.

While the highest total IAA level was determined in *Usnea* sp. (216.01µg/ml, 494.23µg/ml, at 222nm and 280nm wavelengths respectively), the highest GA_3, ABA and zeatin levels were determined in *Pseudevernia furfuracea* (102678.16µg/ml), in *Squamarina cartilaginea* (562.56µg/ml) and in *Xanthoria parietina* (93.78µg/ml). The lowest levels of the same hormones, IAA, GA_3, ABA and zeatin, were detected in *C. foliacea* at a wavelenth of 222nm (38.10µg/ml) and *Xanthoria parietina* at a wavelength of 280nm (77µg/ml); *X. polycarpa* (41941.40µg/ml); *Pseudevernia furfuracea* (42.52µg/ml); and *Cetraria islandica* (13.66µg/ml) respectively.

In conclusion, our result, for the first time, showed that growth regulators studied, IAA, GA_3, ABA and zeatin, are produced in lichens. However, further studies are needed for an integrative understanding of hormone metabolism in lichens.

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Table 1. The amount of free, bound and the total IAA, GA3, ABA and zeatin equivalent assayed from the lichen species. Results are shown as ± standard error mean for each value.

	THE FORMS OF	EQUIVALENT AMOUNTS (µg/mL) SPECIES									
HORMONES	HORMONES	*H. sericeum*	*H. lustescens*	*R. riparioides*	*B. starkei*	*T. flavovirens*	*G. pulvinata*	*A. californica*	*P. epiphylla*	*R. hemisphaerica*	*U. lactuca*
	Free IAA	33.68 ± 2.09	8.24 ± 0.05	16.44 ± 1.29	35.53 ± 6.93	10.49 ± 0.81	32.32 ±3.03	20.14 ± 1.21	10.10 ± 0.20	17.59 ± 2.01	10.71 ± 0.93
IAA 222 nm	Bound IAA	29.32 ± 0.83	8.00 ± 0.05	7.48 ± 0.32	23.28 ± 0.46	15.76 ±0.42	17.97 ±0.72	15.80 ± 0.62	14.01 ± 0.47	9.92 ± 0.72	12.44 ± 0.00
	Total IAA	63.20 ± 2.92	16.24 ± 0.05	23.92 ± 1.46	58.81 ± 6.52	26.25 ± 1.10	50.29 ± 3.47	35.94 ± 1.24	24.11 ± 0.63	27.50 ± 2.65	23.15 ± 0.93
	Free IAA	71.57 ± 7.95	11.44 ± 0.09	17.08 ± 0.16	81.65 ± 25.33	13.92 ± 0.32	41.04 ± 7.04	36.62 ± 0.20	15.36 ± 0.40	32.78 ± 10.10	14.29 ± 3.73
IAA 280 nm	Bound IAA	47.71 ± 5.18	11.39 ± 0.84	9.78 .± 0.06	37.24 ± 1.59	22.34 ± 0.50	19.32 ± 2.89	31.34 ± 0.27	22.45 ± 4.42	14.64 ± 0.08	13.80 ± 0.21
	Total IAA	119.28 ± 5.86	22.83 ± 0.90	36.86 .± 0.20	118.89 .± 26.89	36.26 ± 0.82	60.96 ± 6.71	67.96 ± 0.40	37.81 ± 4.24	47.42 ± 1.10	28.09 ± 3.73
	Free GA_3	46456.00 ± 1265.55	5440.41 ± 445.27	7335.19 ± 889.20	24665.12± 356.53	8469.03 ± 381.52	13086.00±936.38	13338.16±1457.82	11671.01 ± 424.04	7970.18 ± 287.94	7236.84 ± 50.78
GA_3	Bound GA_3	12521.07 ± 1686.74	5140.69 ± 351.51	3566.20 ± 993.47	9262.04 ± 67.50	4338.82 ± 33.73	6392.80 ± 241.60	4222.01 ± 73.50	5889.00 ± 16.90	32291.12 ± 278.18	4641.65 ± 734.39
	Total GA_3	58977.07 ± 1289.63	10581.11 ± 379.28	10901.39 ± 980.95	33927.16 ± 394.63	12807.85 ± 415.22	19478.80± 756.19	17560.17± 528.44	17560.01± 436.38	40261.30± 455.48	11878.49 ± 728.43
	Free ABA	91.95 ± 14.50	43.64 ± 8.57	43.23 ± 4.91	110.97 ± 0.79	38.99 ± 9.86	136.29 ± 7.35	37.27 ± 0.63	35.20 ± 0.02	54.18 ± 1.61	46.12 ± 2.33
ABA	Bound ABA	76.48 ± 2.54	63.28 ± 15.40	43.16 ± 3.75	116.08 ± 5.13	40.12 ± 5.12	83.49 ± 2.70	22.78 ± 1.33	66.44 ± 0.23	6.78 ± 0.10	18.28 ± 1.10
	Total ABA	168.43 ± 13.30	106.92 ± 22.29	86.39 ± 5.16	227.05 ± 5.56	79.11 ± 3.97	219.78 ± 10.04	60.05 ± 0.97	101.64 ± 0.22	60.96 ± 1.71	64.40 ± 3.61
	Free Zeatin	54.52 ± 1.92	13.15 ± 0.63	15.63 ± 2.90	15.22 ± 3.19	38.77 ± 3.78	20.73 ± 1.21	32.88 ± 0.94	25.12 ± 0.12	15.57 ± 1.76	13.02 ± 1.77
ZEATİN	Bound Zeatin	14.37 ± 23.28	4.84 ± 0.07	2.74 .± 0.23	28.83 ± 6.26	5.38 ± 0.18	16.07 ± 1.51	6.00 ± 0.94	9.97 ± 1.07	6.34 ± 0.15	2.93 ± 0.52
	Total Zeatin	68.89 ± 5.19	17.99 ± 0.66	18.37 ± 2.80	44.05 ± 9.45	44.15 ± 3.71	36.80 ± 0.92	38.88 ± 1.48	35.09 ± 1.03	21.91 ± 1.69	15.95 ± 2.19

Table 2. The amount of free, bound and the total IAA, GA_3, ABA and zeatin equivalent assayed from the lichen species. Results are shown as ± standard error mean for each value.

	THE FORMS OF	EQUIVALENT AMOUNTS (µg/mL) SPECIES								
HORMONES	HORMONES	*C. foliacea*	*Cladonia* sp.	*Xantoria parietina*	*X. polycarpa*	*P. furfuracea*	*Cetraria islandica*	*Usnea* sp.	*Letharia vulpina*	*S. cartilaginea*
	Free IAA	24.82 ± 0.1	28.43 ± 0.56	31.04 ± 0.23	29.50 ± 0.17	44.28 ± 0.14	136.72 ± 12.39	179.29 ± 6.47	54.96 ± 2.17	67.08 ± 1.02
IAA 222 nm	Bound IAA	13.28 ± 1.68	11.61 ± 0.27	16.56 ± 0.12	23.33 ± 1.27	16.72 ± 0.70	31.35 ± 3.61	36.72 ± 0.88	26.72 ± 0.42	55.32 ± 2.01
	Total IAA	38.10 ± 1.78	40.04 ± 0.69	47.60 ± 0.31	52.83 ± 1.36	61.00 ± 0.65	168.07 ± 13.07	216.01 ± 6.05	81.68 ± 1.76	122.40 ± 2.26
	Free IAA	73.79 ± 9.13	155.32 ± 1.22	53.12 ± 0.58	195.43 ± 71.43	242.04 ± 0.00	174.21 ± 33..21	416.96 ± 42.86	96.96 ± 9.84	132.13 ± 3.27
IAA 280 nm	Bound IAA	21.30 ± 2.48	25.25 ± 0.74	23.88 ± 0.00	30.05 ± 1.59	17.46 ± 0.13	68.05 ± 9.48	77.27 ± 9.78	54.68 ± 1.45	134.50 ± 26.43
	Total IAA	95.09 ± 11.59	180.57 ± 1.64	77.00 ± 0.58	225.48 ± 72.19	259.50 ± 0.13	242.26 ± 37.88	494.23 ± 52.20	151.64 ± 11.30	266.63 ± 28.42
	Free GA_3	38590,80 ± 421.23	42302.25 ± 748.89	47740.80 ± 9893.52	33283.20 ± 5943.54	94409.28 ± 2237.30	44168.00 ± 4186.28	47580.48 ± 0.00	58512.96 ± 903.99	50256.00 ± 928.38
GA_3	Bound GA_3	6465.27 ± 482.81	6478.13 ± 50.85	23800.80 ±9560.00	8658.20 ± 42.09	8268.88 ± 303.46	45870.00 ± 590.05	14886.76 ± 1997.70	12627.40 ± 253.69	25370.80 ± 594.21
	Total GA_3	45056.07 ± 891.84	48780.38 ± 698.16	71541.60 ± 5741.92	41941.40 ± 5985.32	102678.16 ± 2201.31	90038.00 ± 4621.11	62467.24 ± 1997.70	71140.36 ± 1053.99	75626.80 ± 814.45
	Free ABA	273.60 ± 17.21	48.48 ± 2.01	65.26 ± 5.45	66.25 ± 0.72	25.08 ± 0.67	36.00 ± 1.22	427.47 ± 41.65	190.80 ± 2.26	446.80 ± 23.80
ABA	Bound ABA	34.16 ± 0.69	30.09 ± 1.88	14.05 ± 0.49	26.80 ± 1.78	17.44 ± 0.55	37.48 ± 1.80	25.73 ± 2.32	27.91 ± 0.49	115.76 ± 0.18
	Total ABA	307.76 ± 16.51	78.57 ± 1.80	79.31 ± 5.32	93.05 ± 2.51	42.52 ± 1.06	73.48 ± 3.03	453.20 ± 32.67	218.71 ± 2.50	562.56 ± 23.76
	Free Zeatin	9.68 ± 1.67	7.52 ± 1.36	42.83 ± 2.01	8.65 ± 0.09	11.33 ± 1.17	4.24 ± 0.09	61.15 ± 8.87	42.00 ± 1.06	28.18 ± 2.60
ZEATİN	Bound Zeatin	4.41 ± 0.91	8.28 ± 0.73	50.95 ± 5.95	5.83 ± 1.88	2.49 ± 0.21	9.42 ± 0.29	12.58 ± 2.72	5.41 ± 0.23	20.48 ± 2.03
	Total Zeatin	14.09 ± 2.35	15.80 ± 1.61	93.78 ± 7.53	14.48 ± 1.93	13.82 ± 1.38	13.66 ± 0.34	73.73 ± 8.86	47.41 ± 0.94	48.66 ± 1.86

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