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Kinetin regulates plant growth and biochemical changes during maturation and senescence of leaves, flowers, and pods of *Cajanus cajan* L.

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Abstract

Aspects of plant growth such as height, branch number, leaf number, leaf area, pod area, 100-seed mass, *etc*., were correlated with biochemical changes such as contents of chlorophyll (Chl), proteins, DNA, and RNA, and protease activity during development and senescent phases in leaves, flowers, and pods of *Cajanus cajan* L. cv. UPAS-120 after treatments with kinetin (Kn). A significant increase was noticed in branch number, leaf number, leaf area, and seed mass while other growth processes registered a small increase after Kn application. Effectiveness of 5 μM Kn was also noticed in minimizing the loss of Chls, proteins, and nucleic acids as well as reducing the protease activity during maturity and senescence. Chl *a/b* ratio maintained a high value up to 30-d followed by a decline in leaves while flowers registered much lower ratio at 20-d-age. Pods were unique in having relatively lower ratio of Chl *a*/*b* in comparison to leaves.

Additional key words: chlorophylls, DNA, growth parameters, protease activity, proteins, RNA.

Introduction

It was thought earlier that cytokinins (CKs) have a less marked or no effect on senescence of attached leaves (Muller and Leopold 1966). However, many cases are known of the protection of intact leaf senescence by cytokinins (Biswas and Choudhuri 1980, Nooden 1980, Purohit 1982). Besides minimizing the chlorophyll (Chl) loss (Beevers 1968, Zubkova *et al.* 1983, Kinoshita and Tsuji 1984, Chen and Kao 1986) CKs also reduce the degradation of RNA and soluble protein (Lamattina *et al.* 1987). Retardation of senescence in intact bean leaves by benzyladenine (BA) revealed the prevention in the loss of Chl, RNA, and proteins (Phillips *et al.* 1969, Venkatarayappa *et al.* 1984). Zeatin also increased the soluble protein content and decreased peroxidase activity (Gu *et al.* 1984). The content of individual CKs has been studied during bean cotyledon senescence (Wilhelmová *et al.* 2004) while seasonal changes of CKs have also been investigated in upper and lower canopy leaves of *Acer saccharum* (Held *et al.* 2005).

Kinetin (Kn) and other CKs promoted plant and leaf growth (Kuraishi and Okumura 1956, Richards and Wilkinson 1984, Nii and Kuroiwa 1986, Miller and Eldridge 1986, Goswami and Srivastava 1987), number of floral buds and pods (Clifford 1981, Crosby *et al.* 1981, Abu-Haider *et al.* 1985, Carlson *et al.* 1987, Dyer *et al.* 1987) and yield (Ray *et al.* 1983, Stevens and Westwood 1984). Multiple application of BA increased pod number as well as seed mass (Clifford 1981, Ray *et al.* 1983, Goswami and Srivastava 1987).

However, such studies are rare in which attempts have been made to correlate changes in various growth parameters with that of biochemical changes under control and various concentrations of Kn treatments on intact plants. Therefore, we measured alterations in plant height, number of branches and leaves, leaf area, stem diameter, 100-seed mass as well as changes in the amount of Chls, proteins, DNA, and RNA, and protease activity during growth and senescence stages of pigeonpea plants.

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Abbreviations: BA - benzyladenine; Chl - chlorophyll; CK - cytokinin; Kn - kinetin (Kn_0, Kn_1, Kn_2, and Kn_3 - kinetin 0, 5, 50 and 500 μM, respectively).

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Materials and methods

Seeds of *Cajanus cajan* L. cv. UPAS-120 were soaked in water, germinated, and sowing was carried out in 9 experimental beds (1 × 3 m). Six hundred uniform leaves and flower buds located in the same branch covering different plants were tagged when the plants were 15-week-old. Tagged leaves and flower buds were sprayed with Kn solution (5, 50 and 500 μM, 5 cm^3 per plant); control plants with 5 cm^3 of distilled water. Samples collected before the treatment was designated as 0-d. Further collections were made after 10, 20, 30, 40, 50, and 60 d in case of leaves. Flowers were analyzed on 0, 10 and 20 d while the analyses of pods were carried out on 30, 40, 50 and 60 d.

The amount of plant part which was used as a sample for biochemical analysis ranged between 100 - 500 mg. Chilled 80 % acetone (AR grade) and a pinch of calcium carbonate were used for the extraction of chlorophylls (Chl *a*, Chl *b*) and the absorbance was recorded at 645 and 663 nm using an *Electronic Corporation of India* (*ECI*, Hyderabad, India) spectrophotometer. The amount of Chl was estimated by the formula of Arnon (1949).

The amount of protein was estimated by the method of Lowry *et al.* (1951) using Folin and Ciocalteu's reagent. The procedure of protease extraction was a slight modification of that described by Yomo and Varner (1973) and Ihnen (1976). 100 mg of plant sample was homogenized in 100 mM phosphate buffer of pH 7.2 and the final volume of the extract was raised to 10 cm^3. It was centrifuged (Indian Equipment Corporation, Mumbai, India) at 24 000 *g* for 10 min at -4 °C. 1 % casein was prepared by dissolving it in minimal volume (12 cm^3) of 100 mM phosphate buffer of pH 7.6 (Mahadevan and Sridhar 1982). To 1 cm^3 of casein solution, 1 cm^3 of enzyme extract was added and the reaction mixture was incubated for 3 h at 37 °C. The pH of the reaction mixture was 7.5. In blank set, 2 cm^3 of cold 10 % trichloroacetic acid (TCA) was immediately added to stop the reaction. After incubation, 2 cm^3 of cold 10 % TCA was added to all the reaction sets and centrifuged them in IEC refrigerated centrifuge at 24 000 *g* for 10 min at -4 °C and discarded the residue. Out of the 4 cm^3 filtrate, 1 cm^3 was taken out and to this, 2 cm^3 of 0.5 M NaOH and one cm^3 of 1 M Folin Ciocalteu's reagent were added and allowed to stand for 30 min. The absorbance was recorded at 540 nm using an ECI spectrophotometer. Protease activity was expressed as tyrosine equivalent.

RNA and DNA were determined by a modified method of Ogur and Rosen (1950), the preliminary extraction removed the interfering compounds and defatting (adopted from Cherry 1962).

Results and discussion

The plant height reached the maximum value at 40-d where the order of the plant height was $Kn_2 > Kn_3 > Kn_1 > Kn_0$. The height decreased slightly at later stages due to drying effect of apical meristem (Table 1). The average number of branchs per plant was in order Kn_0, $Kn_3 > Kn_2 > Kn_1$ at 10-d. Branching number was significantly higher in Kn-treated plants, and a maximum was found at 40-d, thereafter a decreasing trend could be noticed in both control and treated plants.

Leaf number and leaf area also exhibited a steady increment up to 40-d stage. However, with the onset of senescence and abscission, as revealed from the data of 50-d stage, a sharp decline was noticed in leaf number. The effectiveness of Kn was evident at all stages having significantly higher values than control; the highest leaf number was recorded with Kn_0 whereas maximum leaf area was found with Kn_2. The increment in stem diameter followed a pattern similar to that of leaf area.

Kn treatments increased the pod length and pod area in comparison to control and the value was significantly higher at 40-d stage. The size of pods registered an increment upto 50-d thereafter a slight decline was noticed. Seed mass values also increased in Kn-treated plants, the maximum rise was noticed at Kn_2 (Table 1).

Several reports in the literature agree with our results on the increase in plant height (Letham 1969, Richards and Wilkinson 1984), branching (Mulgrew and Williams 1985, Tanimoto and Harada 1989), leaf area (Abdullah *et al.* 1986, Goswami and Srivastava 1987), pod length and pod area (Powell and Howell 1985), and seed mass (Carlson *et al.* 1987) after Kn application. The possible mechanisms of growth enhancement by Kn are *a*) cell division promotion, *b*) suppression of senescence promoting enzymes, and *c*) minimizing contents of abscisic acid, ethylene or other growth retardants. We found a stimulation of cell division and suppression of proteolysis as the contents of DNA and RNA increased and a decline in protease activity was noticed after Kn treatment (Table 3).

Contents of Chl *a* and Chl *b* showed a steady increase from 0 to 30-d of plant growth followed by a gradual decline during 40 to 50-d and a sharp fall in leaves at 60-d (Table 2). Kn was effective in enhancing the Chl contents, the most effective was Kn_2. The Chl *a*/*b* ratio maintained a high value upto 30-d, decreased slightly between 40 to 50-d and declined substantially at 60-d in leaf samples.

The amount of Chls was much higher in leaves than in flowers and pods. Kn was slightly effective in retaining Chls in flowers while it was least effective in pods. Flowers at 20-d exerted a sharp fall in Chl *a*/*b* ratio. In general, Chl *a*/*b* ratio in pods was lower than that of leaves.

Table 1. Growth and yield parameters at different stages of growth, development, and senescence as affected by different kinetin (Kn) concentrations (0, 5, 50 and 500 μM). Means ± SE, *n* = 3, * - values significantly different from control at 5 % level.

Time [d]	Kn	Plant height [cm]	Branch number [plant^{-1}]	Leaf number [plant^{-1}]	Leaf area [cm^2]	Stem diameter [cm]	Pod length [cm]	Pod area [cm^2]	100 seed mass [mg]
0	Kn_0	264.2±2.0	38.6±1.6	408.1±2.8	12.1±0.6	1.38±0.02	-	-	-
10	Kn_0	267.2±1.4	41.2±1.0	421.2±3.0	18.0±0.4	1.40±0.01	-	-	-
	Kn_1	266.8±1.9	46.4±1.2*	448.0±2.1*	20.6±1.0*	1.48±0.04*	-	-	-
	Kn_2	268.4±2.1	45.2±1.0*	439.9±2.6*	22.4±1.1*	1.60±0.02*	-	-	-
	Kn_3	266.2±1.2	46.2±1.1*	434.7±3.0*	21.2±0.9*	1.59±0.01*	-	-	-
20	Kn_0	269.9±2.6	43.8±1.4	428.9±3.1	21.0±0.5	1.57±0.04	-	-	-
	Kn_1	269.0±2.8	49.2±1.0*	462.1±2.0*	24.6±0.7*	1.60±0.01	-	-	-
	Kn_2	270.8±1.9	46.2±1.7	454.0±2.5*	25.2±1.0*	1.78±0.01*	-	-	-
	Kn_3	269.2±1.8	46.7±1.1	454.2±2.8*	24.8±1.0*	1.68±0.02*			
30	Kn_0	271.2±3.2	44.5±1.0	436.0±2.9	21.3±0.6	1.58±0.02	2.76±0.10	1.98±0.21	236± 1
	Kn_1	271.0±2.8	51.8±1.1*	473.2±2.1*	26.3±0.6*	1.70±0.01*	2.98±0.18	2.08±0.10	248± 3*
	Kn_2	273.1±2.1	50.0±1.6*	462.1±2.6*	26.9±1.0*	1.82±0.02*	3.08±0.13	2.00±0.21	259± 7*
	Kn_3	272.5±1.9	50.9±1.2*	466.7±2.8*	26.4±1.0*	1.71±0.04*	2.84±0.14	1.96±0.12	. 241± 1*
40	Kn_0	271.9±2.4	46.2±1.0	441.3±2.7	21.9±0.8	1.60±0.04	4.02±0.12	4.08±0.12	912± 3
	Kn_1	272.1±2.6	53.1±1.2*	482.0±1.8*	26.8±0.7*	1.71±0.01*	4.98±0.14*	4.82±0.24*	982± 3*
	Kn_2	273.8±2.0	51.9±1.7*	473.1±2.5*	27.0±1.1*	1.83±0.02*	5.32±0.13*	4.60±0.10*	998± 1*
	Kn_3	273.4±2.0	52.2±1.1*	476.2±2.3*	26.8±0.8*	1.74±0.02*	4.80±0.10*	4.36±0.12*	954± 5*
50	Kn_0	271.2±2.1	46.0±1.0	424.2±2.9	20.8±0.6	1.58±0.01	4.24±0.10	4.52±0.20	4658±11
	Kn_1	272.0±2.3	51.8±1.0*	476.6±1.7*	24.9±0.7*	1.71±0.01*	4.98±0.16*	4.98±0.12	4708±12*
	Kn_2	272.6±2.0	49.3±1.5	464.1±2.0*	25.2±0.9*	1.80±0.01*	5.36±0.18*	4.92±0.30	5750±23*
	Kn_3	272.0±1.7	50.1±1.0*	468.3±1.9*	24.6±0.8*	1.72±0.03*	4.81±0.12*	4.79±0.12	4716±11*
60	Kn_0	270.0±2.0	44.2±1.0	402.1±2.8	20.4±0.4	1.54±0.01	4.18±0.10	4.30±0.12	4702±20
	Kn_1	268.1±1.9	48.6±0.9*	448.6±1.8*	24.2±0.7*	1.68±0.01*	4.82±0.14*	4.58±0.20	5784±21
	Kn_2	270.3±2.0	47.4±1.2	442.0±2.0*	24.8±0.8*	1.74±0.02*	5.24±0.12*	4.62±0.36	5824±18*
	Kn_3	268.8±2.0	49.2±1.0*	449.0±1.9*	24.3±0.6*	1.70±0.04*	4.64±0.10*	4.70±0.60	4792±12*

The protein content reached a maximum value at 30-d where the control leaves had about 47 % increment and Kn treatments resulted in further significant rise. At 40-d stage, with the onset of senescence, the amount declined irrespective of Kn treatments, however, Kn significantly slowed down the protein breakdown. In flowers, the amount of protein was considerably higher than in leaves and pods exhibited maximum values (Table 3). With the progress of pod development, a steady increase in protein was observed which was further increased by Kn applications.

Protease activity increased in both Kn-treated and untreated leaves with the development and initiation of senescence. Upto 30 d, Kn_3 was most effective whereas during leaf senescence Kn_2 had the greatest effect in bringing down the enzymatic activity. Protease activity of flowers and pods was higher than in leaves and Kn applications also decreased the activity appreciably.

Generally, Kn applications control the Chl breakdown in leaves (Richmond and Lang 1957, Fletcher 1969, Thimann 1985). Venkatarayappa *et al.* (1984) reported that Kn could increase Chl content in leaves of *Phaseolus vulgaris*. We found an effectiveness of Kn in minimizing Chl loss not only in leaves but also in flowers and pods at various stages including senescence. The treatment effectively brought down the protease activity, a senescence promoting enzyme. Thimann (1980) pointed out earlier how Kn was effective in suppressing senescence enzymes.

The amount of RNA increased from 0 to 30-d of leaf growth followed by a marked decrease at 40-d. Further advancement in senescence showed a gradual decline in RNA. Kn applications, however, showed significantly higher values of RNA in leaves as compared with control. Effectiveness of Kn was also seen in flowers and pods (Table 3). The amount of DNA was considerably lower than RNA in all the plant parts. Like RNA, DNA content was also much higher after Kn treatment. In most of the cases, Kn_2 was most effective in maintaining the content of nucleic acids.

The increment in overall plant growth by Kn application can be correlated with the significant increase in DNA, RNA, and protein contents during maturity and senescent phase. Leshem *et al.* (1986) found that RNA content first increased and then decreased by less than 50 % during flower senescence and Kn could check this breakdown.

Table 2. Chlorophyll (Chl) [g kg^{-1}(FM)] contents in the leaves, flowers (0 - 20 d), and pods (30 - 50 d) during their growth, development, and senescence as affected by different kinetin concentrations (0, 5, 50 and 500 μM). Means ± SE, n = 3, * - values significantly different from control at 5 % level.

Time [d]	Kn	Leaves Chl *a*	Chl *b*	Chl (*a*+*b*)	Chl *a*/*b*	Flowers/pods Chl *a*	Chl *b*	Chl (*a*+*b*)	Chl *a*/*b*
0	Kn_0	1.89±0.06	0.51±0.03	2.40±0.09	3.72	0.60±0.03	0.18±0.01	0.78±0.04	3.34
10	Kn_0	2.59±0.03	0.56±0.02	3.15±0.05	4.59	0.21±0.01	0.07±0.00	0.28±0.02	3.16
	Kn_1	2.83±0.01*	0.53±0.01	3.36±0.02*	5.34	0.28±0.01*	0.08±0.00*	0.37±0.01*	3.38
	Kn_2	2.97±0.02*	0.58±0.01	3.55±0.03*	5.12	0.28±0.01	0.08±0.00*	0.38±0.01*	3.38
	Kn_3	2.65±0.035	0.56±0.01	3.21±0.04	4.70	0.24±0.01	0.07±0.00*	0.31±0.01	3.33
20	Kn_0	2.63±0.01	0.60±0.04	3.23±0.05	4.37	0.08±0.00	0.05±0.00	0.13±0.00	1.75
	Kn_1	3.00±0.02*	0.63±0.02	3.63±0.04*	4.75	0.09±0.00	0.06±0.00*	0.15±0.00*	1.53
	Kn_2	3.25±0.01*	0.64±0.01	3.89±0.02*	5.07	0.08±0.00	0.05±0.00	0.13±0.00	1.72
	Kn_3	2.97±0.02*	0.61±0.02	3.58±0.04*	4.88	0.10±0.00*	0.07±0.00*	0.17±0.00*	1.36
30	Kn_0	2.90±0.02	0.70±0.01	3.60±0.03	4.16	0.71±0.01	0.24±0.00	0.94±0.01	3.12
	Kn_1	3.20±0.02*	0.68±0.02	3.88±0.04*	4.71	0.67±0.00*	0.24±0.00*	0.91±0.01*	2.76
	Kn_2	3.31±0.03*	0.71±0.01	4.02±0.04*	4.65	0.71±0.00	0.22±0.00*	0.94±0.00	3.18
	Kn_3	3.12±0.01*	0.67±0.01	3.79±0.02*	4.64	0.75±0.00*	0.26±0.00*	1.02±0.00*	2.86
40	Kn_0	2.31±0.03	0.71±0.02	3.02±0.05	3.27	1.00±0.01	0.38±0.01	1.38±0.02	2.60
	Kn_1	2.64±0.02*	0.74±0.01	3.38±0.03*	3.56	0.99±0.01	0.37±0.01	1.36±0.02	2.67
	Kn_2	3.01±0.01*	0.78±0.01*	3.79±0.02*	3.84	1.03±0.01	0.41±0.01	1.44±0.02	2.50
	Kn_3	2.50±0.05*	0.72±0.01	3.22±0.06	3.45	1.01±0.01	0.40±0.01	1.41±0.02	2.51
50	Kn_0	1.66±0.02	0.58±0.01	2.24±0.03	3.14	0.39±0.01	0.22±0.01	0.62±0.02	1.75
	Kn_1	1.84±0.02*	0.58±0.01	2.42±0.03*	3.16	0.36±0.00*	0.22±0.01	0.59±0.01	1.63
	Kn_2	1.96±0.01*	0.64±0.01*	2.60±0.02*	3.05	0.39±0.01	0.24±0.00	0.63±0.01	1.63
	Kn_3	1.67±0.01	0.59±0.01	2.29±0.02	2.88	0.40±0.00	0.24±0.00	0.64±0.01	1.68
60	Kn_0	0.49±0.01	0.30±0.01	0.79±0.02	1.63	0.08±0.00	0.09±0.00	0.18±0.00	0.91
	Kn_1	0.60±0.01*	0.30±0.01	0.90±0.02*	1.99	0.10±0.00*	0.08±0.00	0.18±0.00	1.17
	Kn_2	0.64±0.01*	0.32±0.01	0.96±0.02*	2.02	0.08±0.00	0.07±0.00*	0.16±0.00*	1.17
	Kn_3	0.06±0.01	0.30±0.01	0.80±0.02	1.68	0.09±0.00*	0.08±0.00	0.18±0.01	1.12

Table 3. Protein content [g kg^{-1}(DM)], protease activity [nmol(tyrosine) kg^{-1}(FM) s^{-1}], and contents of RNA and DNA [mg kg^{-1}(DM)] in the leaves, flowers (0 - 20 d), and pods (30 - 50 d) during their growth, development, and senescence as affected by different kinetin concentrations (0, 5, 50 and 500 μM). Means ± SE, n = 3, * - values significantly different from control at 5 % level.

Time [d]	Kn	Protein leaves	flowers/pods	Protease activity leaves	flowers/pods	RNA leaves	flowers/pods	DNA leaves	flowers/pods
0	Kn_0	49.2±0.01	5.84±0.01	589±27	722±30	0.302±0.003	0.140±0.001	0.072±0.003	0.036±0.003
10	Kn_0	58.4±0.01	7.08±0.01	792±30	1219±29	0.398±0.001	0.184±0.003	0.092±0.001	0.054±0.001
	Kn_1	59.2±0.01*	6.98±0.01*	723±30	1240±22	0.434±0.003*	0.184±0.003	0.108±0.003*	0.058±0.003
	Kn_2	61.0±0.01*	7.32±0.01*	671±31*	1121±27	0.456±0.001*	0.198±0.001*	0.120±0.001*	0.066±0.005
	Kn_3	63.2±0.12*	7.28±0.01*	639±27*	1183±28	0.456±0.003*	0.198±0.001*	0.124±0.001*	0.072±0.001*
20	Kn_0	69.8±0.01	8.92±0.01	948±27	1402±31	0.432±0.005	0.080±0.001	0.112±0.005	0.072±0.003
	Kn_1	69.8±0.01	9.28±0.01	916±30	1314±28	0.436±0.003	0.108±0.005*	0.118±0.003	0.090±0.001*
	Kn_2	72.4±0.01*	9.56±0.01*	836±27*	1295±22*	0.498±0.005*	0.154±0.003*	0.136±0.001*	0.108±0.005*
	Kn_3	74.6±0.01*	9.56±0.01*	803±28*	1305±27	0.508±0.001*	0.142±0.008*	0.136±0.003*	0.098±0.003*
30	Kn_0	72.4±0.01	8.42±0.01	1067±30	1027±30	0.501±0.001*	0.208±0.001	0.134±0.003*	0.059±0.003
	Kn_1	75.2±0.02*	8.60±0.01*	1067±27	1027±22	0.539±0.005*	0.264±0.005*	0.149±0.003*	0.076±0.001*
	Kn_2	79.8±0.03*	8.98±0.01*	970±22	917±27	0.560±0.003*	0.272±0.001*	0.168±0.001*	0.098±0.001*
	Kn_3	79.8±0.01*	8.60±0.01*	919±28*	949±18	0.572±0.003*	0.268±0.003*	0.174±0.003*	0.082±0.001*
40	Kn_0	61.2±0.01	10.08±0.01	1194±27	1056±30	0.398±0.008	0.332±0.005	0.168±0.001	0.076±0.001
	Kn_1	65.2±0.01*	10.92±0.02*	1112±28	1072±18	0.448±0.005*	0.354±0.003*	0.168±0.005	0.092±0.001*
	Kn_2	68.4±0.01*	11.20±0.01*	1129±22	949±27	0.472±0.008*	0.382±0.008*	0.192±0.001*	0.108±0.001*
	Kn_3	68.9±0.02*	12.08±0.01*	1144±18	891±27*	0.472±0.001*	0.402±0.005*	0.188±0.003*	0.118±0.003*

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continued

50	Kn_0	42.8±0.01	13.52±0.02	1452±31	1138±28	0.312±0.001	0.364±0.003	0.192±0.003	0.092±0.003
	Kn_1	47.2±0.01*	13.84±0.02*	1406±22	1081±27	0.360±0.001*	0.364±0.005	0.202±0.003	0.098±003
	Kn_2	50.1±0.01*	15.29±0.01*	1340±27	946±18*	0.384±0.005*	0.428±0.001*	0.238±0.001*	0.124±0.005*
	Kn_3	48.8±0.01*	13.92±0.02*	1389±18	1027±28	0.368±0.003*	0.384±0.008	0.216±0.003*	0.106±0.001*
60	Kn_0	30.8±0.01	16.98±0.01	1531±27	1245±27	0.252±0.005	0.302±0.001	0.184±0.001	0.080±0.005
	Kn_1	32.4±0.01*	18.96±0.02*	1472±28	1169±31	0.278±0.001*	0.312±0.005	0.192±0.001*	0.092±0.001
	Kn_2	37.2±0.00*	16.94±0.01	1396±18*	1137±18	0.301±0.003*	0.360±0.003*	0.208±0.003*	0.108±0.003*
	Kn_3	36.0±0.01*	18.60±0.01	1461±22	1166±27*	0.284±0.001*	0.332±0.001*	0.196±0.001	0.102±0.003*

References

Abdullah, A.A., Grace, R., Yeoman, M.M.: Rapid micropropagation of Calabrian pine from primary and secondary buds on shoot explants. - Can. J. Forest Res. **16**: 637-641, 1986.

Abou-Haider, S.S., Miginiac, E., Sachs, R.M.: C^{14} assimilate partitioning in photoperiodically induced seedling of *Pharbitis nil*. The effect of benzyladenine. - Physiol. Plant. **64**: 265-270, 1985.

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

Beevers, L.: Growth regulator control of senescence in leaf discs of nasturtium (*Tropaeolum majus*). - In: Wightman, F., Scatterfield, G. (ed.): Biochemistry and Physiology of Plant Growth Substances. Pp. 1417-1437. Runge Press, Ottawa 1968.

Biswas, A.K., Choudhuri, M.A.: Mechanism of monocarpic senescence in rice. - Plant Physiol. **46**: 806-811, 1980.

Carlson, D.R., Dyer, D.J., Cotterman, C.D., Durley, R.C.: The physiological basis for cytokinin induced increases in pod set in 1x93-100 soybeans. - Plant Physiol. **84**: 233-239, 1987.

Chen, C.T., Kao, C.H.: Localized effect of 1,3-diaminopropane and benzyladenine on chlorophyll loss in soybean primary leaves. - Bot. Bull. Acad. sin. **27**: 97-100, 1986.

Cherry, J.H.: Nucleic acid determination in storage tissues of higher plants. - Plant Physiol. **37**: 670-678, 1962.

Clifford, P.E.: Control of reproductive sink yield in mung beans. - Z. Pflanzenphysiol. **102**: 173-181, 1981.

Crosby, K.E., Aung, L.H., Buss, G.R.: Influence of 6-benzylaminopurine on fruit-set and seed development in two soybean, *Glycine max* (L.) Merr. genotypes. - Plant Physiol. **68**: 985-988, 1981.

Dyer, D.J., Carlson, D.R., Cotterman, C.D., Sikorski, J.A., Ditson, S.L.: Soybean pod set enhancement with synthetic cytokinin analogs. - Plant Physiol. **84**: 240-243, 1987.

Fletcher, R.A.: Retardation of leaf senescence by benzyladenine in intact bean plants. - Planta **89**: 1-8, 1969.

Goswami, B.K., Srivastava, G.C.: Modification of leaf senescence and seed set in sunflower (*Helianthus annuus)* by benzyladenine and urea. - Indian J. Plant Physiol. **30**: 337-343, 1987.

Gu, Z.M., Shen, Z.Y., Zhang, Z.L., Yan, J.Q.: The effect of cytokinin on the growth of water stressed wheat coleoptiles. - Acta phytophysiol. sin. **10**: 353-361, 1984.

Held, M.A., Quesnelle, P.E., Emery, R.J.N.: Seasonal changes of cytokinins in upper and lower leaves of a sugar maple crown. - Biol. Plant. **49**: 455-458, 2005.

Ihnen, J.L.: Lung Proteases and Protease Inhibitors. - M.S. Thesis, University of Iowa, Iowa City 1976.

Kinoshita, I., Tsuji, H.: Benzyladenine induced increase in DNA content per chloroplast in intact bean leaves. - Plant Physiol. **76**: 575-578, 1984.

Kuraishi, S., Okumura, F.: The effect of kinetin on leaf growth. - Bot. Mag. (Tokyo) **69**: 300-306, 1956.

Lamattina, L., Viviana, A., Conde, R.D., Lezica, R.P.: Quantification of kinetin effect on protein synthesis and degradation in senescing wheat leaves. - Plant Physiol. **83**: 497-499, 1987.

Leshem, Y.Y., Halevy, A., Frenkel, C.: Processes and Control of Plant Senescence. - Elsevier Scientific Publ., New York 1986.

Letham, D.S: Cytokinins and their relations to other phytohormones. - BioSciences **19**: 309-316, 1969.

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

Mahadevan, A., Sridhar, R.: Methods in Physiological Plant Pathology. 2nd Ed. - Sivakami Publications, Madras 1982.

Miller, S.S., Eldridge, B.J.: Use of 6-benzylaminopurine and prolamin for improved canopy development in selected apple cultivars. - Sci. Hort. **28**: 355-368, 1986.

Mulgrew, S.M., Williams, D.J.: Effect of benzyladenine on the promotion of bud development and branching of *Picea pungens*. - Hort. Sci. **20**: 380-381, 1985.

Muller, K., Leopold, A.C.: The mechanism of kinetin induced transport in corn leaves. - Planta **68**: 186-205, 1966.

Nii, N., Kuroiwa, T.: Morphological and anatomical development of peach shoot and leaves as influenced by 6-benzyladenine purine. - Acta Hort. **179**: 267-268, 1986.

Nooden, L.D.: Senescence in the whole plant. - In: Thimann, K.V. (ed.): Senescence in Plants. Pp. 219-258. CRC Press, Boca Raton 1980.

Ogur, M., Rosen, G.: The nucleic acids of plant tissues. I. The extraction and estimation of deoxypentose nucleic acid and pentose nucleic acid. - Arch. Biochem. **25**: 262-264, 1950.

Phillips, D.R., Horton, R.F., Fletcher, R.A.: Ribonuclease and chlorophyllase activities in senescing leaves. - Physiol. Plant. **22**: 1050-1054, 1969.

Powell, A.A., Howell, J.C.: Peach growers. Increase size with girdling. - Amer. Fruit Grower **105**: 12-14, 1985.

Purohit, S.S.: Prevention by kinetin of ethylene induced chlorophyllase activity in senescing detached leaves of *Helianthus annuus*. - Biochem. Physiol. Pflanz. **177**: 625-627, 1982.

Ray, S., Mondal, W.A., Choudhuri, M.A.: Regulation of leaf senescence, grain filling and yield of rice by kinetin and abscisic acid. - Physiol. Plant. **59**: 343-346, 1983.

Richards, D., Wilkinson, R.I.: Effect of manual pinching, potting-on and cytokinins on branching and flowering of *Comellina, Rhododendron* and *Rosa* .- Scientia Hort. **23**: 75-83, 1984.

Richmond, A.E., Lang, A.: Effect of kinetin on protein content and survival of detached *Xanthium* leaves. - Science **125**: 650-651, 1957.

Stevens, G.A., Jr., Westwood, M.N.: Fruit set and cytokinin like activity in the xylem sap of sweet cherry (*Prunus avium*) as affected by rootstock. - Physiol. Plant. **61**: 464-468, 1984.

Tanimoto, S., Harada, H.: Involvement of calcium in adventitious bud initiation in Torenia stem segments. - Plant Cell Physiol. **27**: 1-10, 1986.

Thimann, K.V.: The senescence of leaves. - In: Thimann, K.V. (ed.): Senescence in Plants. Pp. 85-115. CRC Press, Boca Raton 1980.

Thimann, K.V.: The interaction of hormonal and environmental factors in leaf senescence. - Biol. Plant. **44**: 120-124, 1985.

Venkatarayappa, T., Fletcher, R.A., Thompson, J.E.: Retardation and reversal of senescence in bean leaves by benzyladenine and decapitation. - Plant Cell Physiol. **25**: 407-418, 1984.

Wilhelmová, N., Procházková, D., Macháčková, I., Vágner, M., Srbová, M., Wilhelm, J.: The role of cytokinins and ethylene in bean cotyledon senescence. The effect of free radicals. - Biol. Plant. **48**: 523 - 529, 2004.

Yomo, H., Varner, J.E.: Control of the formation of protease in attached and detached cotyledons of *Phaseolus vulgaris* L. - Plant Physiol. **52**: 671-673, 1973.

Zubkova, N.F., Bukashikino, Z.V., Sharenkova, K.A., Kulaeva, O.N.: [Defoliating activity of substances of cytokinin type.] - Doklady bot. Sci. **271/273**: 106-109, 1983. [In Russ.]

Montagnini, F. (ed.): **Environmental Services of Agroforestry Systems.** - Food Products Press. An Imprint of the Haworth Press, New York - London - Victoria 2005. Pp. 126. USD 18.39. ISBN 978-1-56022-131-9. Co-published simultaneously as Journal of Sustainable Forestry 21, No. 1, 2005.

The reviewed book consists of 6 contributions, Preface and Index. The individual chapters have been written by 16 authors. In the 1st chapter, R. Lal reviews soil carbon sequestration in forest ecosystems. M.C. Amézquita, M. Ibrahim, T. Llanderal, P. Buurman and E. Amézquita deal with carbon sequestration in Latin American Tropics. The editor with D. Cusack, B. Petit and M. Kanninen describe environmental services of both native tree plantation and agroforestry systems in Latin America. J.B. Aune, A.T. Alemu and K.P. Gautam analyse the usefulness of carbon sequestration in rural communities. S. Davidson offers a survey on shade-coffee agro-ecosystems in Mexico. The last chapter written by A.R. Brenes is devoted to agroforestry systems in Costa Rica.

The title of the book promises a monograph on agroforestry and its environmental services. No doubt, agroforestry represents a useful technological system combining an effective crop production with an improved soil management. It also offers possibilities to better manage the whole landscape. Similarly, an analysis of environmental services is highly desired because of the lack of appreciation of both natural systems and crop canopies for human welfare and sustainability. However, this book practically presents only papers dealing with specialized problems of agroforestry in some locations. There was no attempt to include more general information on the topic represented by the volume title and to adequately cover the most important problems of ecosystem services in agroforestry.

Of course, individual experimental studies are needed in order to subsequently provide general conclusions. However, the editor did not sum up a representative amount of either experimental results or general evaluation of agroforestry ecosystem services. Similarly, the reader will not find comprehensive information on either environmental services or theory and practice of agroforestry.

In no way do I want to say that the individual chapters are of no value. However, I do not understand why their publication was not limited to the Journal of Sustainable Forestry. In other words: A monograph on agroforestry ecosystem services is highly desirable to be published. The reviewed book treats some of the title's topic only. Neither students nor researchers could gain their first understanding or concise knowledge by reading this volume. Nevertheless, if to some readers the Journal of Sustainable Forestry is not available, this book presents some of its papers.

L. NÁTR (*Praha*)