Effect of Plant Hormones on the Invertase Activity in the Senescing Leaves of *Phaseolus radiatus*

Dong Hee Lee, Chin Bum Lee* and Young Sang Kim

Department of Biology, Pusan National University, Pusan 609-735, Korea
Department of Biology, Dong-Eui University, Pusan 614-714, Korea

(Manuscript received on 8 April, 1998)

Effect of plant hormones on the leaf senescence of mung bean (*Phaseolus radiatus*) was investigated by measuring the changes of reducing sugar contents and invertase isozyme activities in detached leaves treated with NAA, GA_3 or BA. During dark-induced senescence, reducing sugar contents in the detached leaves increased temporarily at 4 d, thereafter decreased rapidly and reached minimum values within 7-14 d. The pattern of soluble acid invertase activity in the senescing leaves kept in the dark was similar to that of reducing sugar accumulation, whereas the activities of alkaline and extracellular invertases were not significantly changed during leaf senescence. Therefore, these results suggest that soluble acid invertase, but not alkaline and extracellular invertases, induces the accumulation of reducing sugar during leaf senescence of mung bean plants. Exogenous NAA application had little or no effect in the increase of soluble acid invertase activity during dark-induced senescence compared to the control. However, exogenous applications of GA_3 and BA led to the increase of soluble acid invertase activity in the senescing leaves. Particularly, BA application was very effective in enhancing the activity of soluble acid invertase as well as in delaying chlorophyll breakdown during dark-induced senescence. These results suggest, therefore, that BA regulates the activity of soluble acid invertase, which leads to the accumulation of reducing sugar, and the stability of photosynthetic apparatus to delay leaf senescence.

Key words: senescence, invertase, *Phaseolus radiatus*, NAA, GA_3, BA

1. Introduction

Senescence is a phase of leaf development marked by declining cellular components and biological activities after it is fully developed, and which subsequently causes cellular breakdown and death (Brady, 1988; Nooden, 1988). Although leaf senescence occurs in an age-dependent manner (Hensel, 1993; Jiang, 1993), initiation of leaf senescence can be caused not only by external factors such as drought, mineral deficiency, temperature, and pathogen infection; but also by internal factors such as plant growth regulators, reproduction, and shading (Thomas, 1980; Lohman,

1994; Smart, 1994). One of the most dramatic features of senescence is leaf yellowing, which is due to the preferential degradation of chlorophylls in comparison with carotenoids. Leaf senescence is accompanied by the disassembly of chloroplast. Prior to senescence, the leaves serve as source organs of photosynthetic assimilates. As senescence progresses, leaves function as sink organs of the assimilates by disassembly of photosynthetic apparatus (Blank, 1991).

Of particular interest is the fact that leaf senescence profoundly affects not only structural changes of photosynthetic apparatus but also metabolic changes of carbohydrates such as

glucose, sucrose and starch. Sucrose levels have not shown a significant decrease during natural senescence, and contents of glucose have slightly increased in the early state of natural senescence (Chung, 1997). In contrast with natural senescence, contents of sucrose and reducing sugar rapidly decrease in transgenic tobacco leaves during dark-induced senescence, and reduction of sugar levels induces expression of *sen1*, a senescence-associated gene (Oh, 1996; Chung, 1997). Exogenous application of carbohydrates such as sucrose, glucose, and fructose is proposed to induce suppression of *din1*, a senescence-associated gene, in the cotyledons of radish (Azumi, 1991). Thus carbohydrates levels, particularly sucrose and glucose which regulate senescence-associated genes may be closely correlated with senescence progress, while invertase isozyme hydrolyzes sucrose into glucose and fructose may play an important role in the leaf senescence. However, changes of invertase activity during leaf senescence are not fully understood, though there are some studies on the metabolism of carbohydrates in senescing leaves (Gogorcena, 1997).

Factors affecting the initiation of senescence include plant hormones, light, nutrients, environmental stress, such as extremes of temperature and water, and invasion of pathogens (Thomas, 1980; Robert, 1988). Particularly, application of plant hormones may influence leaf senescence by affecting the transport of available nutrients and photosynthetic assimilates, or alternatively may exhibit more direct effect on chlorophyll turnover (Patrick, 1989; Jordi, 1993). Jordi et al. (1993) have demonstrated that significant differences in carbohydrate level were not shown in the leaves of GA_3-treated cut flowers compared with those of control flowers until the middle state of senescence. Additionally, leaves of GA_3-treated cut flowers exhibit an increase of reducing sugar level compared to those of control flowers during

prolonged senescence progress. There are two types of evidence for the role of cytokinin in the control of senescence. First, external application of cytokinin causes dramatic senescence retardation in detached leaves (Stoddart, 1982; Singh, 1992). Secondly, decrease of endogenous cytokinin levels may induce foliar senescence (Colbert, 1981; Van Staden, 1988). However, very little information has been reported on invertase activity affected by plant hormones, such as auxin, gibberellin, and cytokinin during leaf senescence. On the other hand, there are many studies of leaf senescence using detached leaves *in vitro* because of the relative ease of manipulating the experimental protocol and the certainty of the exact time of onset of senescence compared to attached leaves exhibited complexity and uncertain onset in senescence (Thomas, 1990; Thomas, 1992). Therefore, experimental systems using detached leaves offer an excellent opportunity in which to investigate not only changes of invertase activity but also the effect of plant hormones affecting invertase activity during leaf senescence.

The present study investigated to identify the changes of chlorophyll and reducing sugar contents, and invertase isozyme activities in detached leaves of *Phaseolus radiatus* during senescence. The effect of plant hormones on changes of invertase activity during leaf senescence was also investigated.

2. Materials and Methods

2.1. Plant material

Mung bean (*Phaseolus radiatus* L.) seeds were soaked in running tap water for 6 h, planted on a pot, and grown in a growth chamber at 25℃/18℃ (light/dark) under an 18 h of light /6 h of dark regime with 70% humidity for 25 days.

Additionally the leaves of 25 day-old plants were excised by razor blades and the detached leaves incubated hydroponically for 14 days in 3 mM MES (2-(N-morpholine) ethanesulfonic acid) buffer (pH 5.8) with or without plant hormones under dark condition or white light condition. The concentrations of NAA (napthaleneacetic acid), GA$_3$ (gibberellic acid) and BA (benzyladenine) were ascertained to be 5×10^{-3}, 5×10^{-2} and 5μ M, respectively, which were chosen for optimal concentration as previously described (Lee et al, 1995).

2.2. Crude invertase extraction

Extraction of crude soluble invertases containing soluble acid and alkaline forms was performed by the modified Chen and Black method (1992). Detached leaves rinsed three times with distilled water were homogenized with a Waring blender in an initial plant extraction buffer consisting of 50 mM sodium phosphate, pH 7.0, 1 mM Mg-acetate, 1 mM Na-EDTA (ethylenediaminetetraacetic acid), 1 mM DTT (dithiothreitol) and 1mM PMSF (phenylmethylsulfonylfluoride) with a ratio of 1 g of fresh leaves: 1 ml of the buffer. The homogenate was filtered through four layers of cheesecloth, and then centrifuged at 15,000 x g for 15 min. The supernatant was used as the crude soluble invertase extract. Crude extract of extracellular invertase was prepared by the modified Fahrendorf and Beck method (1990). The final pellet, which was used for the preparation of extracellular invertase, was extracted a second time with the same buffer and subsequently rinsed with distilled water until the effluent was free of soluble protein extracellular invertase was extracted. From the suspension, by 12~18 h incubation in 25 mM Tris-HCl buffer (pH 8.0) which contained a high concentration (1.5 M) of NaCl, in addition to 1 mM Mg-acetate, 1 mM

Na-EDTA, 1 mM DTT and 1 mM PMSF. The suspension was centrifuged at 18,000 x g for 20 min, and the supernatant was designated as the crude extract of extracellular invertase. All purification steps were carried out at 4℃.

2.3. Measurement of chlorophyll and reducing sugar content

After thorough extraction of chlorophyll in 80% (v/v) acetone, the amount of chlorophyll was determined with a spectrophotometer according to the method of Lichtenthaler (1987). The amount of reducing sugar was determined according to the modified Somogyi-Nelson method (1944).

2.4. Assays for invertase activities

Invertase activity was determined by measuring glucose content formed by sucrolysis. For the assay of alkaline invertase, a 1.6 ml aliquot of reaction mixture contained 50 mM HEPES (N-2-hydroxyethylpiperazine-N′-ethanesulfonic acid), 1 mM Mg-acetate, 100 mM sucrose, and a suitable amount of enzyme solution at a pH 7.0. Whereas in the case of soluble acid and extracellular invertases, a 1.6 ml aliquot of reaction mixture, consisted of 50 mM phosphate-citrate, 1 mM Mg-acetate, 100 mM sucrose, and a suitable amount of enzyme solution at a pH 5.0. The reaction mixture containing enzyme was incubated at 25℃ for 1 h, and then boiled for 3-5 min to end the reaction. The amount of glucose formed was measured by the modified glucose oxidase-peroxidase method (Bergmeyer and Bernt, 1974). A 0.8 ml aliquot of the glucose oxidase-peroxidase mixture (0.8 unit of each enzyme) containing 400 μg of o-dianisidine dihydrochloride was added to reaction mixture and incubated for at least 30 min. After the addition of a 0.8 ml aliquot of 5 N HCl, the amount of glucose was

determined at 540 nm with a spectrophotometer (Shimadzu, UV 240, Japan). A unit (U) was defined as the formation of 1 μmol of glucose from sucrose per min per ml of enzyme solution at 25℃ at pH 7.0 and 5.0 for alkaline invertase, and soluble acid and extracellular invertases, respectively.

3. Results

3.1. Changes in the contents of chlorophyll during leaf senescence

Detached leaves undergoing senescence in mung bean plants, harvested daily for 14 d, were used to determine the changes of chlorophyll contents at various times under different experimental conditions. Although leaf yellowing during natural senescnece start at the tip of leaves (Lohman, 1994), in this experiment leaf yellowing started in all areas of the detached leaves during dark-induced senescence. Changes in the contents of chlorophyll from detached leaves which were kept in the dark or light condition during leaf senescence are shown in Fig. 1. The control was defined as the mung bean leaves incubated hydroponically in 3 mM MES solution without plant hormones under darkness or light. Detached leaves that were incubated under dark conditions showed a gradual decrease of chlorophyll along with incubation time; thus, the decrease in the chlorophyll content of control under dark conditions was about 74% of the initial value. Light treatment strongly delayed chlorophyll breakdown during leaf senescence compared to darkness, showing a decreased level of chlorophyll of about 46%. To investigate if plant hormones induce changes in the chlorophyll status of the senescing leaves, we determined chlorophyll contents of detached leaves treated with plant

hormones for 14 d. The changes of chlorophyll content in the NAA- and GA3-treated leaves during senescence were similar to those in the control. Exogenous BA application, however, was more effective in retardation of chlorophyll loss of leaves upon prolonged period of senescence. Specifically, BA application collaborated with light to delay chlorophyll breakdown of senescing leaves. These results show that BA, but not NAA and GA3, causes retardation of chlorophyll breakdown in the senescing mung bean leaves, and that interaction between BA and light induces a synergistic effect on the suppression of chlorophyll breakdown.
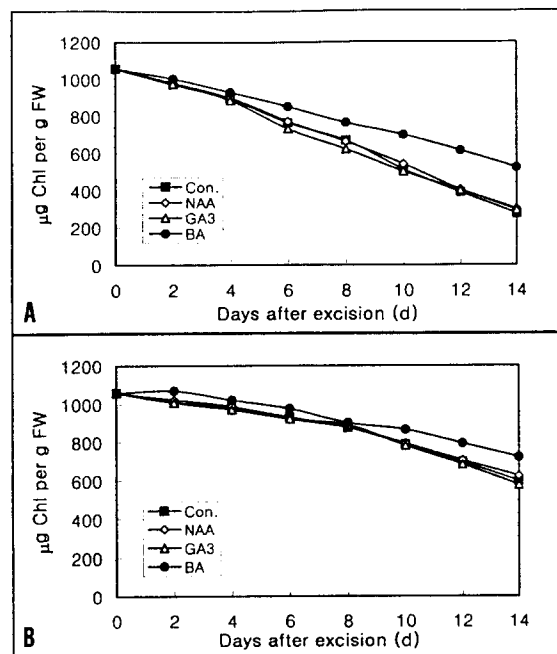
As the detached leaves of 25 day-old mung

Fig. 1. Changes in the contents of chlorophyll from detached leaves of *Phaseolus radiatus* treated with plant hormones under dark (A) or light condition (B) for 14 d. The values are the average of three independent experiments. -■-, control; -◇-, NAA; -△-, GA3; -●-, BA.

bean plants, the Chl a/b ratio ranged from 2.45 to 3.24 (Fig. 2). The Chl a/b ratio of the control leaves kept in the dark did not significantly change during 6 d of dark-induced senescence, and then the leaves followed by a gradual increase in the ratio of Chl a/b as senescence continued. Whereas both light and BA treatment caused the stability of Chl a/b ratio during leaf senescence, light treatment was more effective in this respect upon prolonged senescence than was BA, suggesting that light may be an important factor in delaying the breakdown of light harvesting chlorophyll complexes containing Chl a and b compared to the action of plant hormones, particularly BA.
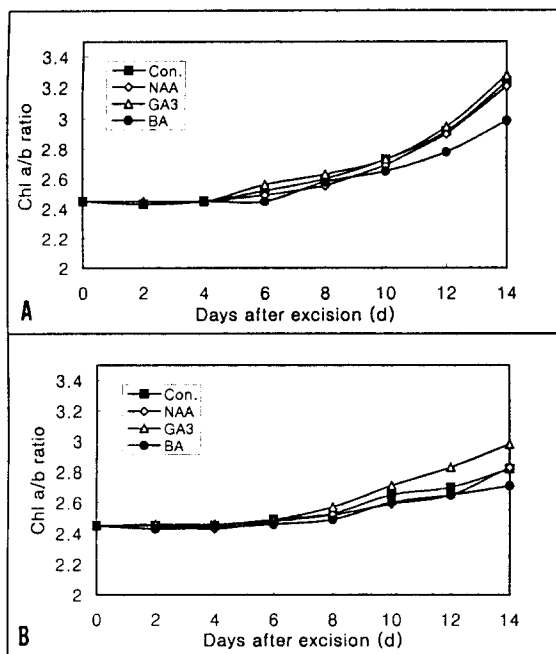
3.2. Changes in the contents of reducing sugar during leaf senescence

As senescence-related chlorophyll loss may be associated with the changes of reducing sugars, reducing sugars were measured in the senescing mung bean leaves. In detached leaves kept in the dark, the contents of reducing sugars decreased rapidly after 4 d and reached minimum values within 7-14 d (Fig. 3A). Of particular interest was the result that reducing sugar content in the leaves kept in the dark increased temporarily at 4 d, suggesting that the increase may be accompanied by the conversion of cellular starch and sucrose into reducing sugars during leaf senescence. The
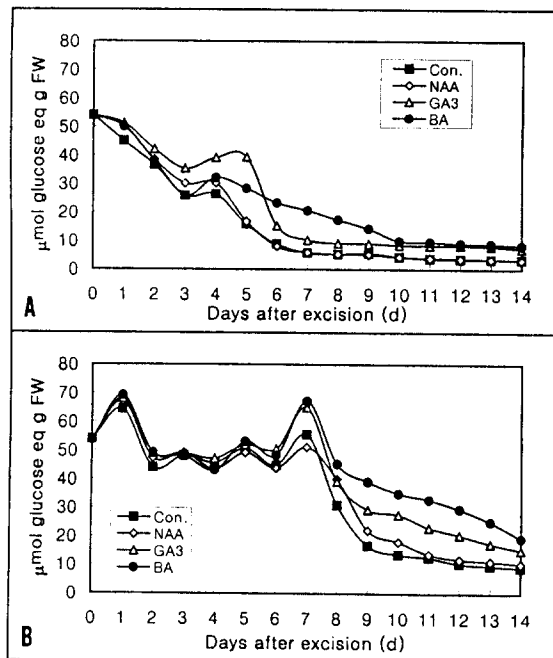

Fig. 2. Changes in the ratios of Chl a/b from detached leaves of *Phaseolus radiatus* treated with plant hormones under dark (A) or light condition (B) for 14 d. The values are the average of three independent experiments. -■-, control; -◇-, NAA; -△-, GA₃; -●-, BA.

Fig. 3. Changes in the contents of reducing sugar from detached leaves of *Phaseolus radiatus* treated with plant hormones under dark (A) or light condition (B) for 14 d. The values are the average of three independent experiments. -■-, control; -◇-, NAA; -△-, GA₃; -●-, BA.

changes of reducing sugar in the NAA-treated leaves during dark-induced senescence was similar to those in the controls kept in the dark. GA₃ and BA applications in the detached leaves were more effective in the accumulation of reducing sugars during dark-induced senescence than in the controls. In particular, exogenous BA application, induced a promotive effect in the accumulation of reducing sugar at the late stage of dark-induced senescence. On the other hand, the reducing sugar levels in the illuminated leaves were slightly different from the results of dark-induced senescence (Fig. 3B). In the light treatment, reducing sugar levels temporarily increased at 1 d, and did not drop significantly until 7 d, then reached minimum levels within 10-14 d. These results suggest that light induces a promotive accumulation of reducing sugars in detached mung bean leaves until the late stage of leaf senescence. Effects of plant hormones in the illuminated leaves were similar to those in the leaves kept in darkness with regards the accumulation of reducing sugar during leaf senescence.

3.3. Changes in the invertase activity during leaf senescence

The senescence-associated changes of reducing sugar contents can be considered to be a physiological event by which senescing leaves acquire an alternative carbon source and energy to sustain metabolic integrity. Our purpose was to see whether invertases, which play an important role in the hydrolysis of sucrose into glucose and fructose, regulated changes of reducing sugar levels in senescing leaves. Therefore, the activation pattern was monitored using detached leaves harvested daily during dark-induced senescence (Fig. 4A). Soluble acid invertase was activated at 4 d, and peaked at 5 d. Additionally, the activity of soluble acid invertase dramatically decreased

after 5 d, reaching its basal level within 10-14 d. These results agree with the data obtained by experiments on changes of reducing sugar in senescing leaves kept in the dark condition. The activity of alkaline invertase in the detached leaves, however, did not show significant changes during dark-induced senescence. The overall levels of extracellular invertase activity were very low during leaf senescence when compared to those of soluble acid and alkaline invertase activities. The activity of extracellular invertase slightly increased until 7 d, followed by a gradual decrease in enzyme activity. Changes in invertase activities may be attributed to the proposal that soluble acid invertase, but not alkaline and extracellular
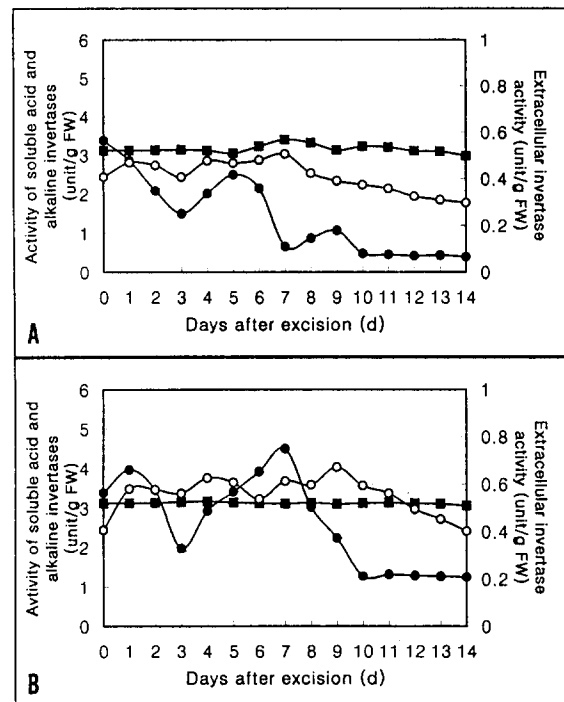

Fig. 4. Changes in the activities of invertase isozymes from detached leaves of *Phaseolus radiatus* kept in the dark (A) or light condition (B) for 14 d. The values are the average of three independent experiments. -●-, soluble acid invertase; -■-, alkaline invertase; -○-, extracellular invertase.

invertases, induces on accumulation of reducing sugars during leaf senescence. Effects of light on the changes of invertase activity during leaf senescence are shown in Fig. 4B. The activity of soluble acid invertase temporarily increased at 1 d, and peaked at 7 d of the illuminated leaf senescence. Illumination was more effective in enhancing enzyme activity during leaf senescence than was the dark condition. However, illumination had little affect on the increase of alkaline and extracellular invertases activities when compared to the dark condition. These results suggest that light may participate in the activity of soluble acid invertase during leaf senescence.

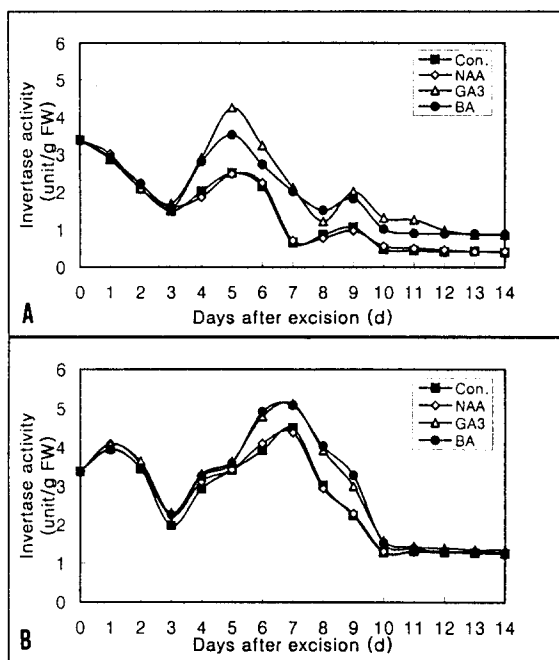To elucidate the effect of plant hormones on

Fig. 5. Changes in the activities of soluble acid invertase from detached leaves of *Phaseolus radiatus* treated with plant hormones under dark (A) or light condition (B) for 14 d. The values are the average of three independent experiments. -■-, control; -◇-, NAA; -△-, GA₃; -●-, BA.

the activities of invertase isozymes during leaf senescence, changes of invertase activity in the detached leaves treated with plant hormones for 14 d were investigated. Leaves treated with NAA under dark conditions were little changed in the activity of soluble acid invertase during leaf senescence compared to the controls which were kept in the dark. Leaves treated with GA₃ and BA exhibited a higher level in the activity of soluble acid invertase within 4-6 d of dark-induced senescence compared to controls; GA₃ was more effective than BA in this regard (Fig. 5A). Under illumination, however, BA application was as effective as GA₃ application on the activity of soluble acid invertase activity during leaf senescence (Fig. 5B). On the other hand, alkaline and extracellular invertases were not affected by the applications of plant hormones or light illumination (Tables 1 and 2). In summary, these results suggest that GA₃ and BA affect soluble acid invertase which participates actively in the accumulation of reducing sugar during leaf senescence, whereas GA₃ and BA have little affect on the activity of alkaline and extracellular invertases.

4. Discussion

One of the most conspicuous syndromes during dark-induced senescence is a gradual chlorophyll loss in detached leaves. Exposure to darkness is one of the most potent stimuli to accelerate leaf senescence and has long been used for the experimental induction of leaf senescence. The possibility that plant hormones can retard chlorophyll breakdown during leaf senescence was investigated by studying changes of chlorophyll content in the detached leaves of mung bean plants treated with plant hormones for 14 d. Exogenous application of BA significantly inhibited chlorophyll breakdown during dark-induced senescence when

Table 1. Changes in the activities of alkaline invertase from detached leaves of *Phaseolus radiatus* treated with plant hormones under dark or light condition for 14 d.

Unit/g fresh weight

Days after excision (d)		control	NAA	GA$_3$	BA
Dark	0	3.12[a]	3.12	3.12	3.12
	1	3.12	3.10	3.13	3.12
	2	3.13	3.15	3.14	3.12
	3	3.14	3.17	3.12	3.15
	4	3.12	3.14	3.10	3.19
	5	3.05	3.11	3.20	3.14
	6	3.23	3.10	3.23	3.11
	7	3.40	3.12	3.17	3.20
	8	3.32	3.12	3.13	3.11
	9	3.13	3.14	3.11	3.10
	10	3.23	3.17	3.11	3.09
	11	3.21	3.20	3.13	3.12
	12	3.11	3.14	3.12	3.11
	13	3.10	3.11	3.05	3.02
	14	2.98	3.00	2.82	2.99
Light	0	3.12	3.12	3.12	3.12
	1	3.12	3.13	3.13	3.12
	2	3.13	3.13	3.13	3.12
	3	3.15	3.20	3.12	3.15
	4	3.17	3.14	3.08	3.23
	5	3.14	3.11	3.06	3.25
	6	3.12	3.05	3.09	3.17
	7	3.11	3.11	3.13	3.12
	8	3.13	3.12	3.14	3.09
	9	3.10	3.15	3.12	3.08
	10	3.12	3.13	3.11	3.14
	11	3.13	3.11	3.11	3.12
	12	3.11	3.10	3.05	3.11
	13	3.10	3.05	3.02	3.01
	14	3.05	3.03	3.01	3.00

[a]The values are the average of three independent experiments.

Table 2. Changes in the activities of extracellular invertase from detached leaves of *Phaseolus radiatus* treated with plant hormones under dark or light condition for 14 d.

Unit/g fresh weight

Days after excision (d)		control	NAA	GA$_3$	BA
Dark	0	0.407[a]	0.407	0.407	0.407
	1	0.468	0.442	0.400	0.417
	2	0.456	0.457	0.416	0.420
	3	0.407	0.411	0.416	0.410
	4	0.476	0.477	0.443	0.446
	5	0.466	0.569	0.502	0.457
	6	0.479	0.468	0.472	0.472
	7	0.505	0.497	0.495	0.512
	8	0.423	0.423	0.474	0.448
	9	0.391	0.388	0.528	0.427
	10	0.374	0.369	0.443	0.423
	11	0.358	0.345	0.454	0.397
	12	0.325	0.323	0.420	0.376
	13	0.310	0.309	0.414	0.370
	14	0.297	0.287	0.392	0.364
Light	0	0.407	0.407	0.407	0.407
	1	0.580	0.528	0.518	0.623
	2	0.577	0.590	0.700	0.656
	3	0.561	0.598	0.659	0.590
	4	0.626	0.636	0.679	0.669
	5	0.607	0.602	0.684	0.683
	6	0.538	0.512	0.643	0.528
	7	0.613	0.608	0.603	0.558
	8	0.597	0.581	0.705	0.590
	9	0.672	0.699	0.888	0.734
	10	0.594	0.583	0.531	0.646
	11	0.561	0.551	0.653	0.640
	12	0.495	0.463	0.572	0.528
	13	0.453	0.442	0.463	0.466
	14	0.402	0.399	0.402	0.432

[a]The values are the average of three independent experiments.

compared to the controls (Fig. 1A). Similarly, GA$_3$ have been shown to little or no effect on the senescence rate of chlorophyll loss in wheat seedlings (Wittenbach, 1977). Horton and Brourguin (1992) demonstrated, however, that gibberellic acid inhibited chlorophyll breakdown of juvenile ivy; other reports also showed the same effect of GA$_3$ on the inhibition of chlorophyll loss (Hicklenton, 1991; Van Doorn, 1992). The contrary effect of GA$_3$ on the breakdown of chlorophyll may be attributed to regulatory degrees of chlorophyll turnover or different degrees of delaying export of nutrients from the leaves to other sink organs during senescence. Cytokinin prevented the

senescence-related decline in the amount of chlorophyll in the present study. These results are consistent with the observations from many plant species (Singh, 1992; Van Doorn, 1992; Wingler, 1998). The facts may be attributed to the positive effect of cytokinin in retarding the loss of ALA (aminolevulinic acid) dehydratase and PBG (porphobilinogen) deaminase, both of which are involved in chlorophyll biosynthesis (Hukmani, 1994). Light was also a major factor in retarding chlorophyll loss during leaf senescence, and had a stronger effect than cytokinin (Fig. 1B). Particularly, interaction between light and cytokinin induced a synergistic effect on the retardation of chlorophyll breakdown. The effects of light delaying chlorophyll loss during leaf senescence in mung beans appears to be similar to those in rice and *Lolium tumulentum* (Okada, 1992; Mae, 1993).

The Chl a/b ratio of the controls which were kept in the dark did not induce significant changes during 6 d of dark-induced senescence, and the leaves followed by a gradual increase in the ratio of Chl a/b as senescence continued (Fig. 2). The gradual increase of Chl a/b at the late stage of dark-induced senescence significantly was inhibited by exogeneous BA application. The increase of Chl a/b ratio may be induced by degradation of light harvesting complexes containing Chl a and b; thus, it gives rise to dismantling of the photosynthetic apparatus (Lee, 1998). Therefore, these results imply that BA delays chlorophyll loss and degradation of light harvesting complexes to offer stability of photosynthetic apparatus during leaf senescence.

The possibility that the dismantling of the photosynthetic apparatus induced by chlorophyll breakdown and an increase of Chl a/b ratio may be associated with reducing sugar levels, was further investigated by studying changes of reducing sugars during dark-induced senescence. Glucose is known to be involved in the control of a number of metabolic processes in such a way as to maintain a constant level of intracellular carbons for both catabolic and anabolic purposes (Jang, 1997). It is therefore reasonable to believe that levels of reducing sugar somehow regulate the senescence process. The reducing sugar contents in the detached leaves kept in the dark increased temporarily at 4 d, thereafter decreasing rapidly and reaching minimum values within 7-14 d (Fig. 3A). The temporary increase in the contents of reducing sugar may be induced by conversion of cellular starch and sucrose during leaf senescence. Wingler et al. (1998) have also suggested that accumulation of glucose and fructose in old leaves is accompanied by lower starch and sucrose contents. Accumulation of reducing sugar during senescence is known to regulate the transcription of photosynthetic genes, probably acting via hexokinase as a sugar sensor (Sheen, 1990; Jang, 1994; Jang, 1997). It is thus reasonable to speculate that the accumulation of reducing sugars by the breakdown of cellular starch and sucrose during leaf senescence regulates senescence-related changes in the photosynthetic apparatus.

It is likely that accumulation of reducing sugars during leaf senescence may be regulated by enzymes participating in the metabolism of carbohydrates, such as sucrose and starch. Among the enzymes involved in carbohydrate metabolism, invertase was chosen for the present study, since it has been known to play an important role in hydrolysis of sucrose into its constituent monosaccharides, glucose and fructose. Invertase is present in multiple forms in mung bean plants (Lee, 1995a, 1995b; Lee, 1998). Two major forms were distinguished on the basis of their optimal pH, acid (pH 4.0-5.0) and alkaline (pH 7.0-8.0) forms (Lee, 1995a, 1995b). The former was subdivided into soluble (intracellular) and extracellular forms according to its subcellular

localization (Lee, 1998). The pattern of soluble acid invertase activation in the senescing leaves kept in the dark was similar to that of reducing sugar accumulation, whereas the activity of alkaline invertase did not show significant changes during leaf senescence (Figs. 3A and 4A). The activity of extracellular invertase slightly increased until 7 d, followed by a gradual decrease of enzyme activity. Of particular interest was the result that the overall levels of extracellular invertase activity were very low during leaf senescence compared to those of soluble acid and alkaline invertase activities. These results are consistent with the notion that the extracellular invertase exhibited lower levels in enzyme activity, whereas soluble invertases exhibited higher levels in the enzyme activity at the same tissues (Lee, 1998). Therefore, these results suggest that soluble acid invertase, but not alkaline and extracellular invertases, induces the accumulation of reducing sugars during the middle stage of leaf senescence in mung bean plants. However, it is not clear whether accumulation of reducing sugars during the middle stage of leaf senescence is induced by soluble acid invertase, or by other enzymes participating in carbohydrate metabolism. Therefore, further studies are required to elucidate the accurate mechanism of carbohydrate metabolism during leaf senescence.

Plant hormones, such as auxin, gibberellin and cytokinin, are known to regulate chlorophyll loss of senescing leaves in many plant species (Thiamann, 1980; Dai, 1991; Hicklenton, 1991). However, there is little informations on the effect of plant hormones in the changes of reducing sugar content and invertase activity during leaf senescence. In the present study, we have studied the affect of plant hormones on the changes of reducing sugar accumulation and invertase activity in senescing leaves. Exogenous GA_3 and BA applications were more effective in enhancing the activity of soluble acid invertase which led to the accumulation of reducing sugar during leaf senescence when compared to the control kept in the dark (Figs. 3A and 5A). However, leaves treated with NAA under dark condition exhibited little change in the activity of soluble acid invertase. Both alkaline and extracellular invertase had little effect on the accumulation of reducing sugar during leaf senescence and were not affected by treatment with plant hormones or light (Tables 1 and 2). Therefore, these results suggest that GA_3 and BA initiate activation of soluble acid invertase and regulate levels of reducing sugar during leaf senescence. Jordi et al. (1993) reported similar results on the effect of GA_3 with *Alstroemeria* cut flowers, and Masuda et al. (1988) also suggested a similar possibility on the effect of BA. On the other hand, GA_3 showed little effect on the retardation of chlorophyll breakdown, whereas it was effective in the activation of soluble acid invertase during leaf senescence. It is likely that GA_3 does not exert its photosynthetic apparatus- preserving effect via regulation of reducing sugars in the senescing leaves. Light was a major factor not only in the activation of soluble acid invertase leading to the regulation of reducing sugars but also in the stability of the photosynthetic apparatus: Illumination had a stronger effect than BA during leaf senescence (Figs. 3B, 4B and 5B). These findings imply that light strongly delays leaf senescence involved in the changes of carbohydrate metabolism and the photosynthetic apparatus. Particularly, interaction between light and BA induced a synergistic effect on the activation of soluble acid invertase during leaf senescence. This is an agreement with prior results obtained from radish cotyledons (Huff, 1975). It is concluded that BA among plant hormones regulates the activation of soluble acid invertase and the stability of the photosynthetic

apparatus to delay leaf senescence, although these facts are insufficient to determine the accurate mechanism of plant hormones on carbohydrate metabolism and the photosynthetic apparatus.

References

Azumi, Y. and A. Watanabe, 1991, *Plant Physiol.*, 95, 577-583.

Bergmeyer, H. U. and E. Bernt, 1974, D-Glucose, *In* Methods of Enzymatic Analysis, H. U. Bergmeyer ed., Academic Press, New York, Vol. 2, pp 1205-1212.

Blank, K. and T. A. Mckeon, 1991, *Plant Physiol.*, 97, 14090-1413.

Brady, C. J., 1988, Nucleic acid and protein synthesis, *In* Senescence and Aging in Plants, Nooden, L. D. and A. C. Leopold eds., Academic Press, San Diego, pp 147-179.

Chen, J. Q. and C. C. Black, 1992, *Arch. Biochem. Biophys.,* 296, 1-9.

Chung, B. C., S. Y. Lee, S. A. Oh, T. H. Rhew, H. G. Nam and C. H. Lee, 1997, *J. Plant Physiol.*, 151, 339-345.

Colbert, K. A. and J. E. Beever, 1981, *J. Exp. Bot.*, 32, 121-127.

Dai, J. W. and R. E. Paui, 1991, *Hortscience*, 26, 314.

Gogorcena, Y., A. J. Gordon, P. R. Escuredo, F. R. Minchin, J. F. Witty, J. F. Moran and M. Becana, 1997, *Plant Physiol.*, 113, 1193-1201.

Hensel, L. L., V. Grbic, D. A. Baumgarten and A. B. Bleecter, 1993, *Plant Cell*, 5, 553-564.

Hicklenton, P. R., 1991, *Hortscience*, 26, 1198-1199.

Horton, R. F. and N. Bourguoin, 1992, *Plant Physiol. Biochem.*, 30, 119-122.

Huff, A. and C. Ross, 1975, *Plant Physiol.*, 56, 429-433.

Hukmani, P. and B. C. Tripathy, 1994, *Plant Physiol.*, 105, 1295-1300.

Jang, J. C. and J. Sheen, 1997, *Trends Plant Sci.*, 2, 208-214.

Jang, J. C., P. Leon, L. Zhou and J. Sheen, 1997, *Plant Cell*, 9, 5-19.

Jiang, C. Z., S. R. Rodermel and R. M. Shibles, 1993, *Plant Physiol.*, 101, 105-112.

Jordi, W., H. M. Dekhuijzen, G. M. Stoopen, and J. H. M. Overbeek, 1993, *Physiol. Plant.*, 87, 426-432.

Lee, D. H. and Y. S. Kim, 1995a, *J. Plant Biol.*, 38, 251-258.

Lee, D. H. and Y. S. Kim, 1995b, *J. Plant Biol.*, 38, 349-357.

Lee, D. H., J. H. Hong and Y. S. Kim, 1995, *J. Korean Environ. Sci. Soc.*, 4, 323-333.

Lee, D. H., Y. H. Moon and Y. S. Kim, 1998, *J. Plant Biol.*, 41, 16-23.

Lichententhaler, H. K., 1987, Chlorophyll and carotenoids: Pigments of photosynthetic biomembrane, *In* Methods in Enzymology, Lester Packer and Roland Doncd eds., Academic Press, New York, Vol. 148, pp 350-367.

Lohman, K. N., S. Gan, M. C. John and R. M. Amasino, 1994, *Physiol. Plant.*, 92, 322-328.

Mae, T., T. Howard, G. P. Alan, M. Amane and H. Jun, 1993, *Plant Cell Physiol.*, 34(3), 391-399.

Masuda, H, T. Takahashi and S. Sugawara, 1988, *Plant Physiol.*, 86, 312-317.

Nelson, M. F., 1944, *J. Biol. Chem.*, 135, 375-380.

Nooden, L. D., 1988, Whole plant senescence, *In* Senescence and Aging in Plants, Nooden, L. D. and A. C. Leopold eds., Academic

Press, San Diego, pp 391-439.

Oh, S. A., S. Y. Lee, I. K. Chung, C. H. Lee, A. Watanabe and H. G. Nam, 1996, *Plant Mol. Biol.*, 30, 739-754.

Okada, K., Y. Inoue, K. Satoh and S. Satoh, 1992, *Plant Cell Physiol.*, 33, 1183-1191.

Patrick, J. W. and D. R. Mulligan, 1989, *Ann. Bot.*, 63, 581-587.

Roberts, J. A., and R. Hooley, 1988, Leaf, flower and fruit development, *In* Plant Growth Regulators, Blackie, London, pp 133-143.

Sheen, J., 1990, *Plant Cell*, 2, 1027-1038.

Singh S., L. M. S. Palni, and D. S. Letham, 1992, *J. Plant Physiol.*, 139, 279-283.

Smart, C. M., S. R. Scofield, M. W. Bevan and T. A. Dyer, 1991, *Plant Cell*, 3, 647-656.

Stoddart, J. L. and H. Thomas, 1982, Leaf senescence, *In* Encyclopedia of Plant Physiology, Boulter, D. and M. G. Pitman eds., Springer-Verlag, Berlin, Vol. 14, pp 592-636.

Thiamann, K. V., 1980, The senescence of leaves, *In* Senescence in Plants, Thiamann, K. V. eds., CRC Press, Boca Raton, Florida, pp 5803-5805.

Thomas, H. and J. L. Stoddart, 1980, *Rev. Plant physiol.*, 31, 83-111.

Thomas, H., 1990, *J. Plant physiol.*, 136, 45-50.

Thomas, H., H. J. Ougham, T. G. E. Davies, 1992, *J. Plant physiol.*, 139, 403-412.

Van Doorn, W. G., J. Hibma and J, de Wit, 1992, *Plant Growth Regul.*, 11, 59-62.

Van Staden, J., E. L. Cook and L. D. Nooden, 1988, Cytokinins and senescence, *In* Senescence and Aging in Plants, Nooden, L. D. and A. C. Leopold, eds., Academic Press, San Diego, pp 281-328.

Wingler, A., A. von Schaewen, R. C. Leegood, P. J. Lea and W. P. Quick, 1998, *Plant Physiol.*, 116, 329-335.

Wittenbach, V. A., 1977, *Plant Physiol.*, 59, 1039-1042.