韓國環境科學會誌 第3卷(第2號), 141~155, 1994
J. of the Korean Environmental Sciences Society

The Effect of Growth Regulators and Light Quality on the Changes in Protein Pattern of Callus from Intergeneric Protoplast Fusion between *Nicotiana tabacum* and *Solanum nigrum*

Young-Sang Kim and Dong-Hee Lee

Department of Biology, College of Natural Sciences, Pusan National University
(Manuscript received 6 May 1994)

Abstract

The effect of growth regulators (NAA, BA and GA_3) and light (blue, red and far-red) on the changes in total protein and thylakoid membrane protein pattern of callus from intergeneric protoplast fusion between *Nicotiana tabacum* and *Solanum nigrum* were investigated.

When the callus were irradiated with different wavelengths of light, blue and red light accelerated the synthesis of total proteins and thylakoid membrane proteins. Particularly, red light led to an increase in the protein synthesis compared to blue light. When the callus were subjected to various combinations of growth regulators, NAA+GA_3 and NAA+BA treatments induced remarkable increase of total proteins and thylakoid membrane proteins accumulation, particulary in the combination of NAA+GA_3. NAA+GA_3 treatment with irradiation of red light showed highest value in the accumulation of total proteins and thylakoid membrane proteins. We conclude that simultaneous application of red light and NAA+GA_3 treatment may induce synergistic effect in the synthesis of total proteins and thylakoid membrane proteins.

Key words : *Nicotiana tabacum*, *Solanum nigrum*, protoplast fusion, protein, thylakoid membrane protein, growth regulators, light quality

1. INTRODUCTION

Plant protoplasts have been used extensively as a key tool in somatic hybridization and genetic manipulation (Bonnema and O'Connell, 1992; Derks *et al.*, 1992; Ratushnyak *et al.*, 1993; Wolters *et al.*, 1993). A better understanding of biochemical events occurring in protoplast cultures would probably help in studing resultant protoplast culture systems. Then the preparation of viable protoplasts necessitates the use of physiologically proper medium containing various components. Of major interest is the fate of growth regulators in growth medium.

The factors of growth regulators influencing organogenesis in plant cell and tissue culture have been investigated by many researchers. Auxin-cytokinin-mediated mechanism of organogenesis has been the subject of extensive studies and discussed about many works employing different tissues and organs of various plant species (Frisch and Camper, 1987; Kim and An, 1990). Some effects of auxin, cytokinin and gibberellin on *in vitro*

The present investigation was supported by the genetic engineering (GE) program (#Ministry of Education of ROK), 1988-1989.

organogenesis of *Rudbeckia* was observed in various organs and over different photoperiods (Tanimoto and Harada, 1982). Many pathways of morphogenesis and regenerational capacity of the callus via organogenesis or somatic embryogenesis, with respect to its orgin and the relative role of growth regulators in inducing morphogenesis, have been observed in the callus derived from various explants of sugarbeet (Sauders and Daub, 1984; Van-Geyt and Jacobs, 1985; Tetu *et al.*, 1987).

Factors such as duration and intensity of light during growth of source plants critically affect the viability and growth of protoplasts. The photomorphogenetic action of light is restricted to only the blue and red waveband of the spectrum (Morh and Shrosphire, 1983). Phytochrome is considered as the undisputed photoreceptor for preceiving red light, and it has been hypothesized that phytochrome also participates in some blue light triggered photoresponses (Gaba and Black, 1987). Auxins have long been thought to mediate the red light-induced changes in etiolated seedlings (Vanderhoef and Briggs, 1978; Shinkle and Briggs, 1984), and cytokinin was known to substitute red light requirement in plant development (Bevan and Northcote, 1981; Qamaruddin and Tillberg, 1989).

It was reported that red light induced the swelling of etiolated protoplast, and that was manipulated by the phytochrome that induce the gibberellin uptake (Blakeley *et al.*, 1983; Keppler and Mertz, 1986; Kim *et al.*, 1986). Chung *et al.* (1988) reported that the combination of red light and GA_3 showed a synergistic effect on the enlargement of protoplast size.

During the development of higher plants in the presence of light, a rapid formation of the thylakoid membranes occurs pararell to the chlorophyll formation, leading to the assembly of a photosynthetically component chloroplast (Mayfield and Huff, 1986). The major chloroplast components involved in the two light reactions of green plant photosynthesis are embedded in the chloroplast thylakoid membrane. Most of chlorophyll molecules in thylakoids are bound to proteins in the form of chlorophyll-proteins which play an important role in the light harvesting and photochemistry of photosynthesis (Leong and Anderson, 1983; Cho and Thompson, 1989). Two major chlorophyll-protein complexes are associated with the chloroplast internal membranes of higher plants (Thornber and Highkin, 1974). These two complexes, commonly referred to as CPI and CPII differ in amino acid composition and molecular weight, as well as in their postulated roles in the photosynthetic process (Murphy, 1986).

Chlorophyll accumulation is regulated by many factors such as light, temperature, growth regulators and age of tissues (Dalling and Nettleton, 1986; Mathis and Burkey, 1987; Ovaska *et al.*, 1990). But interactions between growth regulators and light on the growth and chloroplast development of callus obtained from protoplast fusion were not known.

The present study was undertaken to investigate the effect of growth regulators and light on the growth and protein pattern of callus derived from the intergeneric protoplast fusion of mesophyll cells between *Nicotiana tabacum* and *Solanum nigrum*.

2. MATERIALS AND METHODS

2.1. Plant materials

Nicotiana tabacum L., cv. and *Solanum nigrum* L., cv. were grown in compost-soil mixture in a growth chamber at 25±1℃ under 70% relative humidity and 16 h / 8 h light/dark cycle. Plants were illuminated with white fluorescent light at a intensity of ca. 7,000 lux.

2.2. Preparation of protoplasts

Leaves were harvested from young plants and rinsed briefly in tap water and sterilization

and pretreatment for protoplast isolation were followed by Bhatt and Fassuliotis (1981) in 0.6 M mannitol + cell and protoplast washing (CPW) inorganic salts (Power and Chapman, 1985). The leaves were transferred to 100 m*l* Erlenmeyer flask containing 20 m*l* of a filter-sterilized enzyme solution consisted of 1.5% Cellulase Onozuka R-10 (Yacult honsha Co., Japan), 0.5% Macerozyme R-10 (Yacult honsha Co., Japan), 0.01 M 2[N-morpholino]ethanesulfonic acid (MES), 0.2% bovine serum albumin (BSA) and 0.6 M mannitol containing CPW inorganic salts, pH 5.8 for *Nicotiana tabacum,* and 1.5% Cellulase, 0.3% Macerozyme, 0.01 M MES, 0.2% BSA and 0.6 M mannitol containing CPW inorganic salts, pH 5.8 for *Solanum nigrum,* and incubated for 90-150 min at 34℃ on a reciprocal shaker of 30 strokes per minute. The procedure of elimination of enzyme solution and the collection of fresh protoplast were also followed by the modified Bhatt and Fassuliotis (1981).

Finally, the protoplasts washed with 0.6 M mannitol + CPW inorganic salts were suspended in Kao and Michayluk (KM) culture medium (Kao and Michayluk, 1975).

2.3. Protoplast fusion and culture

Equal numbers of the purified protoplasts ($1x10^5$ cells/m*l*) from *Nicotiana tabacum* and *Solanum nigrum* were suspended in test tubes. One or two drops (0.8-1.0 m*l*) of protoplast suspension were placed in petri dish (50×12 mm) for 10 min. 40% polyethylene glycol (PEG) (MW 1540) solution containing 0.25 M glucose, 15 mM $CaCl_2 \cdot 2H_2O$ and 1 mM KH_2PO_4, pH 5.8, was gently mixed around the suspension. After 5 min, the suspension was eluted with same volume of eluting solution containing 50 mM glycine, 100 mM $CaCl_2 \cdot 2H_2O$, and 0.5 M glucose, pH 10.5, replaced by PEG solution and the eluting procedures were repeated in twice. Fused protoplast coagulation was stabilized in 0.6 M KM culture medium with rinse of the eluting solution three times and cultured in density of 1×10^5 cells/m*l* under dark condition at 25±1℃. The culture medium contained combinations of hormones: 1.0 mg/*l* naphthaleneacetic acid (NAA), 0.5 mg/*l* benzyladenine (BA) and 0.2 mg/*l* 2,4-dichlorophenoxyacetic acid (2,4-D). After 7 days from culture, the culture medium was replaced by 0.5 M KM medium with 0.1 M mannitol under dim light (100 lux). After 14 days from culture, the culture medium was replaced by modified Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 0.5 mg/*l* BA and 1.0 mg/*l* NAA at a week interval under 300-400 lux. Calli of 2-3 mm in diameter were transferred to modified MS medium containing 0.5 mg/*l* BA, 1.0 mg/*l* NAA, 30 g/*l* sucrose and 2 g/*l* gerlite for regeneration. The cultures were then incubated under fluorescent light (2,000 lux) of 16 h / 8 h light/dark cycle.

2.4. Growth regulators and light treatments

A preliminary study was performed to determine the effect of growth regulators on the growth of callus. The optimal concentrations for callus growth were 0.5 mg/*l* BA and 1.0 mg/*l* NAA, respectively. The optimal concenteration of GA_3 was too selected 1.0 mg/*l* (30 μM) concentration for these experiments. Callus cultured for a month in basal MS medium mixtured these three growth regulators.

Light source was a microscope illuminator (Kyowa optical Co., Ltd., Japan) attacted with band-pass filters (Corion, . S-10-660-S, S-40-450-S) for red (660 nm) and blue light (450 nm), and Schott glass filter (Corion, RG-6-S) for far-red light (730 nm).

Each culture was reimposed upon three different wavelenghts of light for 15 min at 2 h intervals in 16 h photoperiod under fluorescent light of 2,000 lux irradiation.

2.5. Chloroplast isolation and protein analysis

Callus (8-10 g) treated with growth regulators

and light for 7 days was homogenized in a chilled mortar and pestle with isolation medium containing 0.33 M glucose, 50 mM Na_2HPO_4, 50 mM KH_2PO_4, 5 mM $MgCl_2$, 0.1% NaCl, 0.2% sodium isoascorbate and 0.1% BSA adjusted pH 6.5 with HCl. The homogenate was filtered through eight layers of cheesecloth and centrifuged at 100 x g for 5 min. The supernatant was centrifuged at 4,000 x g for 15 min to obtain intact chloroplast. All procedures of chloroplast isolation and subsequent preparation of materials used for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis were performed at 4℃.

Callus were ground in a chilled mortar and pestle with grinding buffer (2% SDS, 10% β

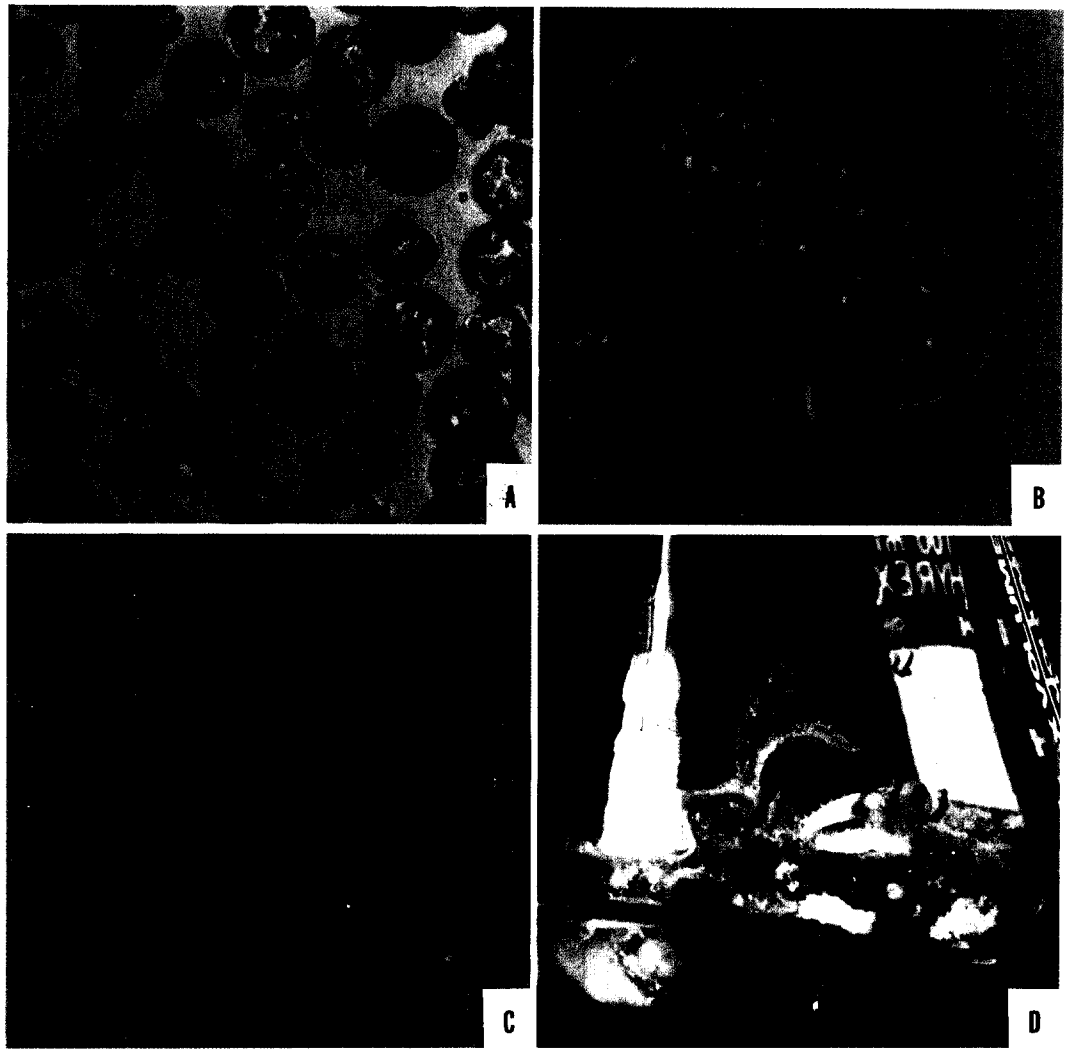

Fig. 1. Callus formation and regeneration from protoplasts of *Nicotiana tabacum* and *Solanum nigrum*. A, Freshly isolated protoplasts from *Nicotiana tabacum* and *Solanum nigrum*; B, Single fused protoplast in 40% PEG (MW 1540) solution; C, Aggregation of protoplasts after PEG treatment; D, Explant by callus regeneration in modified MS medium.

-mercaptoethanol, 30 mM Tris-HCl, 0.1 M NaCl and 0.1 M ethylenediaminetetraacetic acid (EDTA), pH 7.8). For protein collection the homogenate was centrifuged at 8,000 x g for 1 h at 4℃ and supernatant was mixed with an equal volume of 2 times diluted stock solution of stacking gel buffer containing 20% sucrose. The samples were heated over boiling water for 90 sec and used for electrophoresis. Polyacrylamide gel electrophoresis in the presence of SDS was performed as described by Laemmli (1970).

For the preparation of thylakoid membrane proteins the intact chloroplasts (washed membranes) were dissolved 0.0625 M Tris-HCl (pH 6.8), 2% SDS and 10% sucrose and centrifuged at 10,000 x g for 90 sec. The samples were loaded onto the well after adding 5% β-mercaptoethanol and 0.002% bromophenol blue. Thylakoid membrane proteins were analyzed by modified Laemmli (1970).

3. RESULTS

3.1. Ascertainment of fusion

The protoplasts isolated from two different plant sources were fused with 40% PEG (MW 1540) solution and cultured till callus was initiated (Fig. 1). In order to ascertain the fusion state the protein extracts from callus were electrophorized. Fig. 2 shows one-dimensional gel and the difference of protein patterns between the fused callus and the nonfused callus. There were significant differences in the protein pattern of callus compared to two parental species in the bands of 72, 45, 35, 29 and 20 kDa, but overall tendancy in protein pattern was similar to *Nicotiana tabacum*. Similar result was observed in the intraspecific somatic hybrids of *Nicotiana debneyi* (Scowcroft and Larkin, 1981). The fusion rate of heterokaryocyte formation was about 30%.

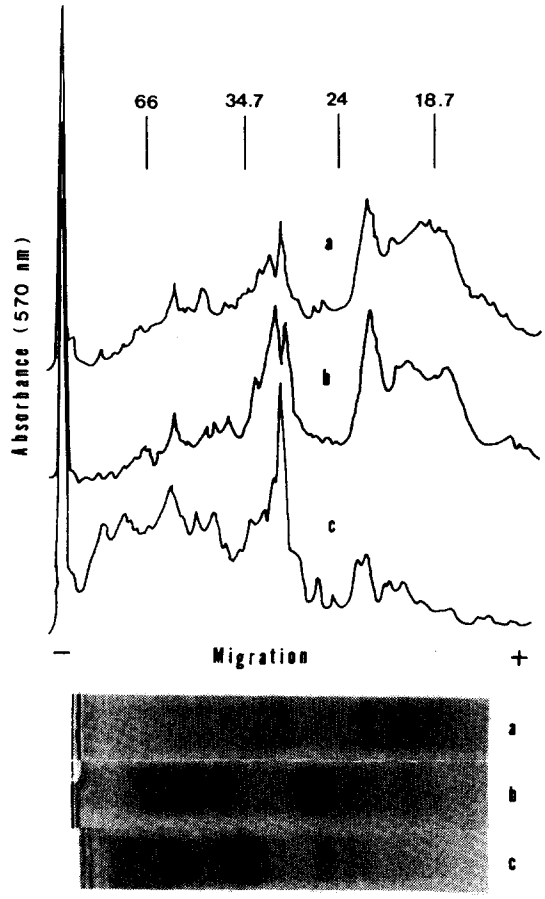

Fig. 2. Electrophoretic profiles of protein extracts from callus of two different plant sources. Numbers on the top of densitogram represent the molecular weight (kDa). a, callus from *Nicotiana tabacum*; b, callus from *Solanum nigrum*; c, callus from intergeneric protoplast fusion between *Nicotiana tabacum* and *Solanum nigrum*.

3.2. Effects of growth regulators and light on the fresh weight and protein content of callus

As shown in Table 1, blue and red light increased the fresh weight and protein accumulation of callus. Red light treatment was more effective than blue light treatment. Far-red light slightly affected fresh weight and protein

content when compared to control. Among the combinations of growth regulators, remarkable increase in fresh weight and protein content was obtained in a medium containing the combinations of NAA+BA and NAA+GA_3. The fresh weight and protein content increased to a higher level in the combination of NAA+GA_3 than that of NAA+BA group which is widely used as the best combination in plant tissue culture medium. There was no appreciable effect on the increase of fresh weight and protein content in the combinations of GA_3+BA and NAA+GA_3+BA. The results suggest that NAA in combination with GA_3 shows a synergistic effect, while NAA in combination with BA overrides the effect of GA_3 on the growth and protein synthesis. In order to investigate interactions between growth regulators and different wavelengths of light calli were applied various combinations of growth regulators and blue, red and far-red light irradiation simultaneously. The combination of NAA+GA_3 upon the irradiations of different wavelengths of light resulted in more additional growth compared to the combinations of NAA+BA, GA_3+BA and NAA+GA_3+BA. Simultaneous application of the combination of NAA+GA_3 and red light induced maximum growth and protein accumulation of callus, and resulted in 33% increase of fresh weight and 43% increase of protein content over the control.

3.3. Changes in the pattern of protein

Figs. 3-6 show the densitometer scan of protein from the callus analyzed by polyacrylamide gel electrophoresis. Proteins of callus were separated into several bands and the protein species changed quantitatively according to the combinations of growth regulators and light. Blue and red light irradiation considerably enhanced the levels of 35-29 kDa protein in the treated groups as compared to far-red light (Figs. 4-6). However, far-red light treatment induced relatively high levels of 35-29 kDa protein quantity than that of control light (Figs. 3 and 6). The levels of 29 kDa protein increased prominently in overall groups. Treatment of NAA+BA remarkably enhanced the levels of 35-29 kDa protein, and the combinative

Table 1. Fresh weight and protein content of callus subjected to various combinations of growth regulators and light

The combination of growth regulators	Light treatment (nm)							
	Control		Blue light (450)		Red light (660)		Far-red light (730)	
	Fresh weight*	Protein content**	Fresh weight	Protein content	Fresh weight	Protein content	Fresh weight	Protein content
NAA+BA	30.55±6.5	3.80±0.2	40.90±7.0	4.84±0.3	42.70±8.5	5.16±0.5	31.10±7.5	3.78±0.4
NAA+GA_3	33.05±4.0	3.72±0.3	41.65±5.5	5.12±0.7	44.10±4.0	5.30±0.7	40.30±3.5	4.98±0.2
GA_3+BA	28.55±4.5	3.48±0.4	32.05±3.0	3.78±0.2	39.80±2.0	3.98±0.5	29.30±3.5	3.48±0.5
NAA+GA_3+BA	29.50±5.5	3.50±0.2	35.10±4.0	4.04±0.7	41.50±5.5	4.06±0.5	30.05±4.5	3.96±0.3

* One gram of fused callus was cultured in MS solid medium containing the optimal concentration of growth regulators under different wavelengths of light of 2,000 lux for 7 days, and the relative percentage of fresh weight increase of callus was presented.

** Protein content was determined by the method of Lowry *et al.* (1951). Each value (mg g fr. wt^{-1}) was obtained from optical density at 750 nm using BSA as standard. Data are means ± SE (standard error) from at least three independent experiments. Each concentration of three growth regulators is that BA, 0.5 mg/*l*; NAA, 1.0 mg/*l*; GA_3, 1.0 mg/*l*.

treatment of NAA+BA under red light irradiation resulted in more additional accumulation of protein, especially in the level of 29 kDa protein compared to under the other light irradiations (Fig. 5). Further stimulative increase was observed in callus treated with NAA+GA_3. The combination of red light and NAA+GA_3 led to highest levels of 31, 29 and 19 kDa proteins in all experimental groups (Fig. 5). The protein level, especially 29 kDa was relatively low in GA_3+BA treatment as compared to NAA+BA or NAA+GA_3 treatment (Fig. 3). However, the levels of 35-29 kDa proteins in simultaneous application of red light with

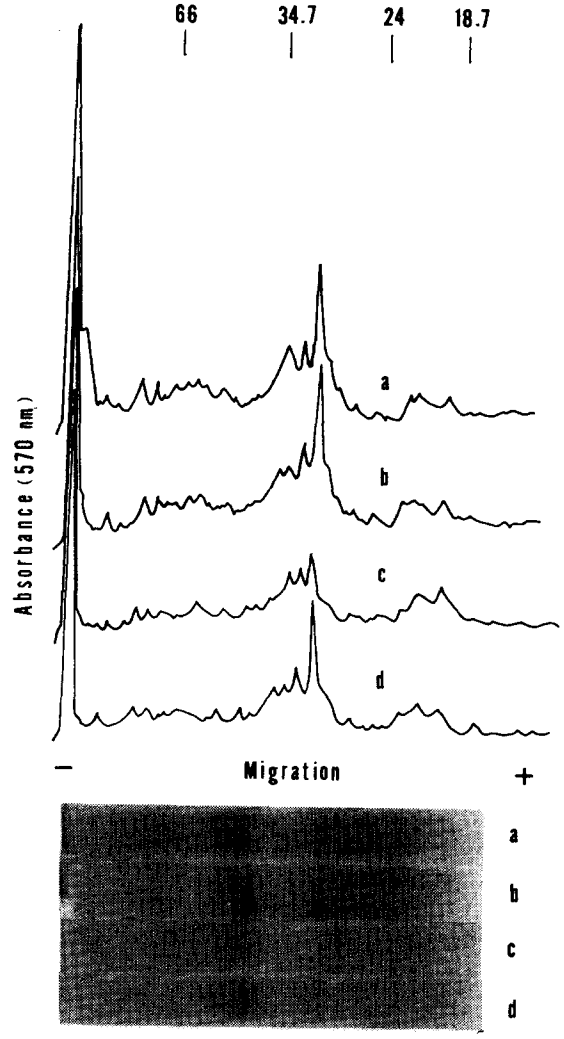

Fig. 3. Electrophoretic profiles of proteins from fused callus treated with plant hormonal combinations under control light. Numbers on the top of densitogram indicate the molecular weight (kDa). a, NAA (1 mg/*l*) +BA (0.5 mg/*l*); b, NAA+GA_3 (1 mg/*l*); c, BA+GA_3; d, NAA+BA+GA_3.

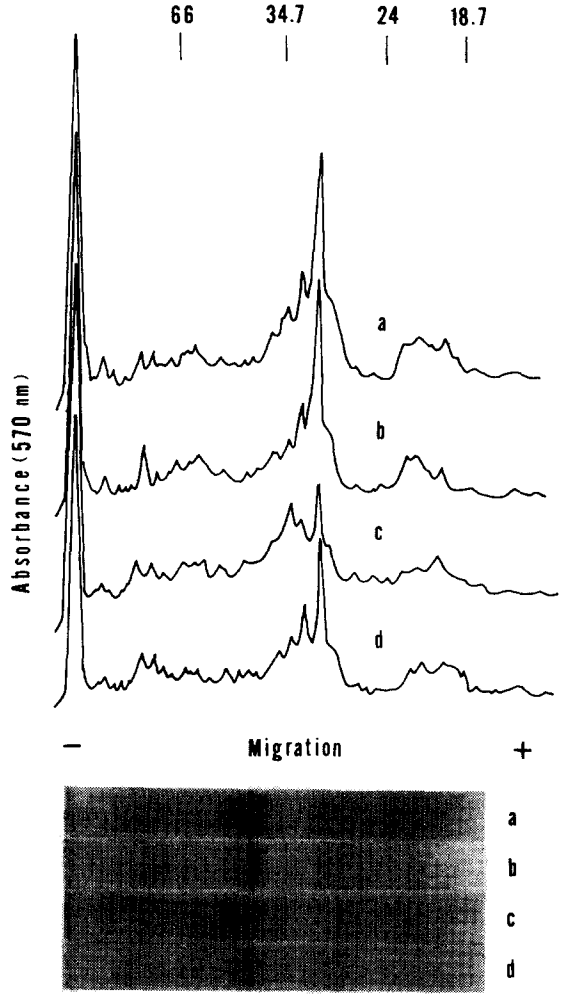

Fig. 4. Electrophoretic profiles of proteins from fused callus treated with plant hormonal combinations under blue light (450 nm).

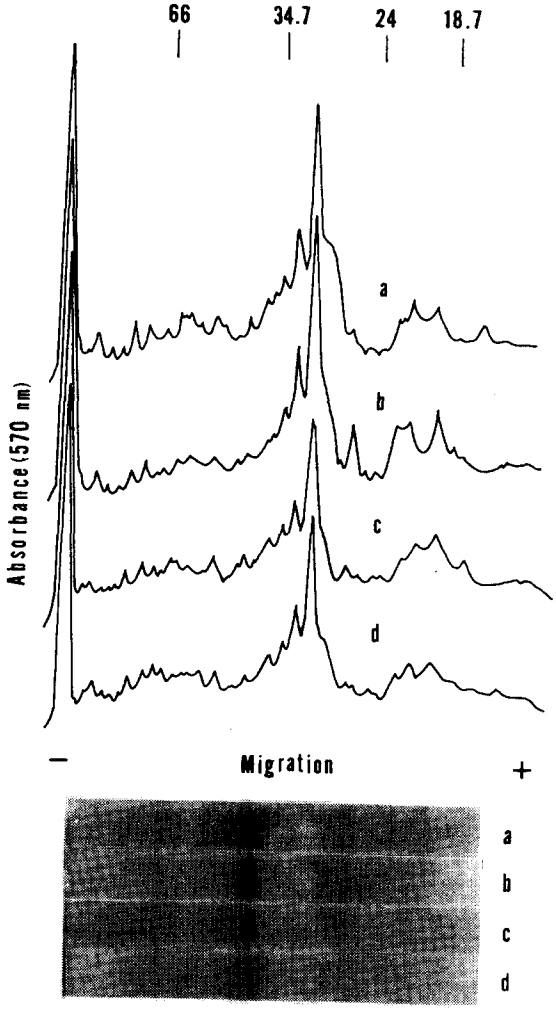

Fig. 5. Electrophoretic profiles of proteins from fused callus treated with plant hormonal combinations under red light (660 nm).

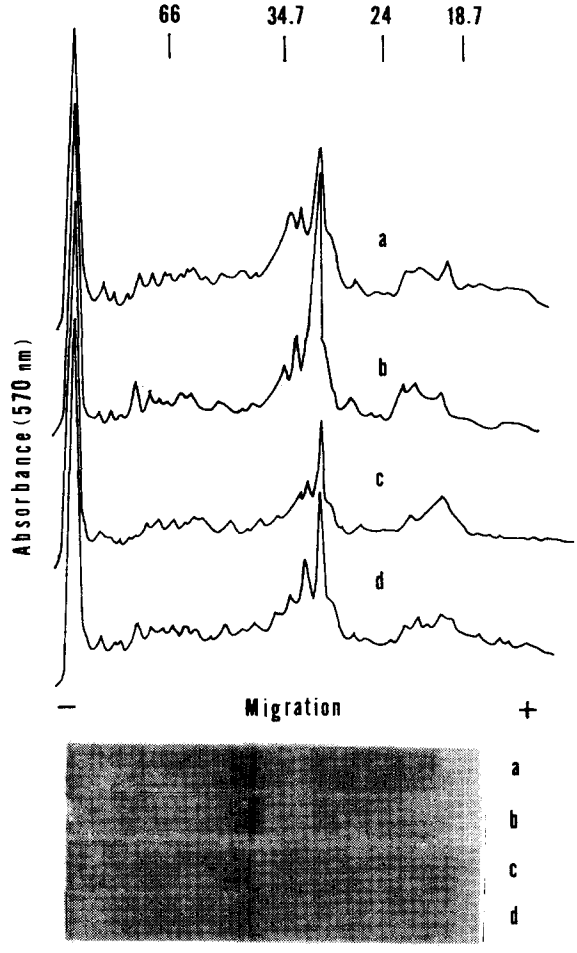

Fig. 6. Electrophoretic profiles of proteins from fused callus treated with plant hormonal combinations under far-red light (730 nm).

GA_3+BA became comparably increase to those in NAA+BA or NAA+GA_3 under control light. The protein levels in NAA+GA_3+BA treatment seemed to increase compared to GA_3+BA treatment (Figs. 3 and 5). The level of 29 kDa protein in the simultaneous application of NAA+GA_3+BA and red light was similar to the protein observed in NAA+BA treatment and control light. Therefore, the levels of 35-29 kDa proteins were highest in NAA+GA_3 treatment, moderate in NAA+BA treatment, and lowest in GA_3+BA treatment among the treatments of growth regulators.

3.4 Changes in the pattern of thylakoid membrane protein

Figs. 7-10 show the peptide composition of thylakoid membranes of callus treated with growth regulators and light qualities. There were prominent doublet at 56 kDa and minor peptides were apparent at 28 kDa in all experimental groups, and in the lower molecular weight regions the prominent peaks were found

at 24 kDa. In NAA+BA and NAA+BA+GA_3 treatments, control light influenced increasably the level of 56 kDa thylakoid protein compared to NAA+GA_3 and GA_3+BA treatments, and the combinative treatment of NAA+BA was more effective in quantitative increase in 56 kDa than that of NAA+BA+GA_3 (Fig. 7). On the whole, blue and red light stimulated the synthesis of thylakoid membrane proteins in all offered the combinations of growth regulators (Figs. 8 and 9). In NAA+BA+GA_3 treatment, control light and blue light irradiations were effective increasably higher than red and far-red light irradiation in protein changes of 56 kDa which seems to be subunit of chloroplast coupling factor 1 (CF1), however, did not affect on the increase of 33 kDa protein (Figs. 7 and 8). The proteins of 30-35 kDa (mainly 33 kDa) were

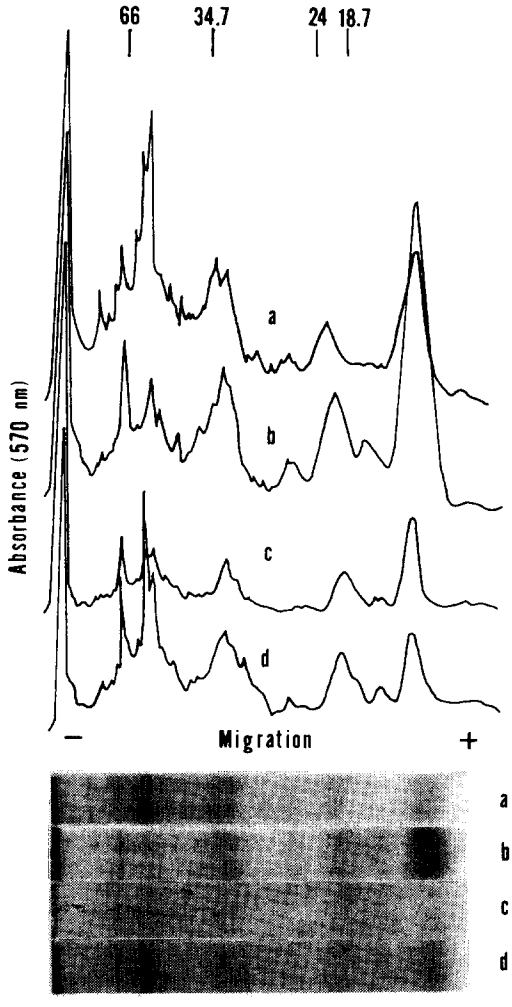

Fig. 7. Electrophoretic profiles of thylakoid proteins from chloroplast of fused callus treated with plant hormonal combinations under control light. Numbers on the top of densitogram indicate the molecular weight (kDa). a, NAA (1 mg/*l*)+BA (0.5 mg/*l*); b, NAA+GA_3 (1 mg/*l*); c, BA+GA_3; d, NAA+BA+GA_3.

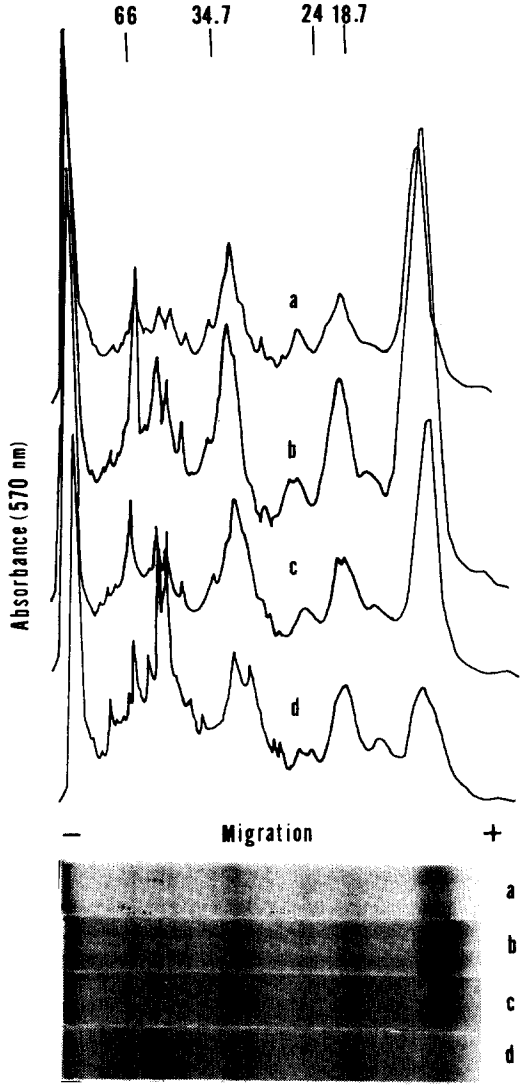

Fig. 8. Electrophoretic profiles of thylakoid proteins from chloroplast of fused callus treated with plant hormonal combinations under blue light (450 nm).

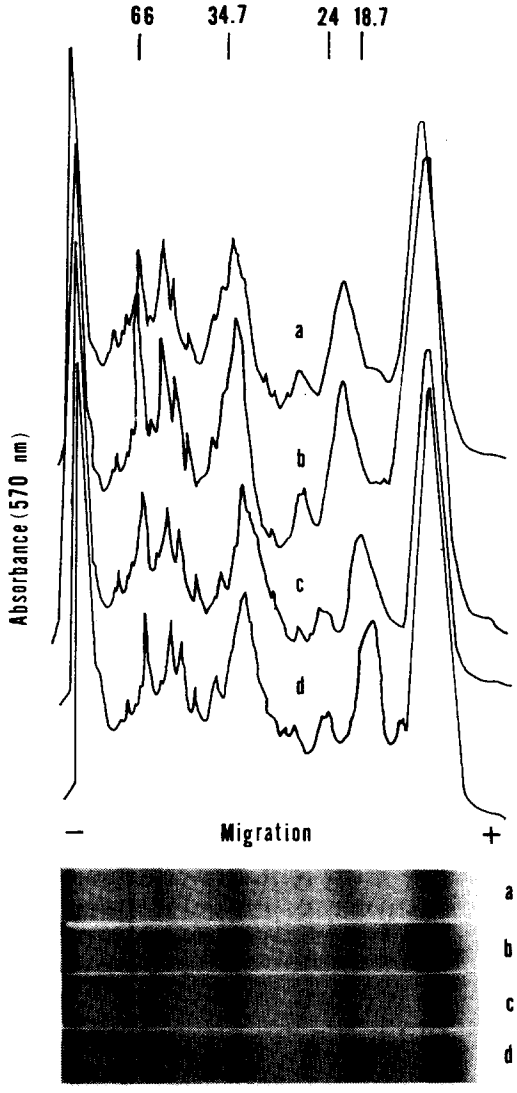

Fig. 9. Electrophoretic profiles of thylakoid proteins from chloroplast of fused callus treated with plant hormonal combinations under red light (660 nm).

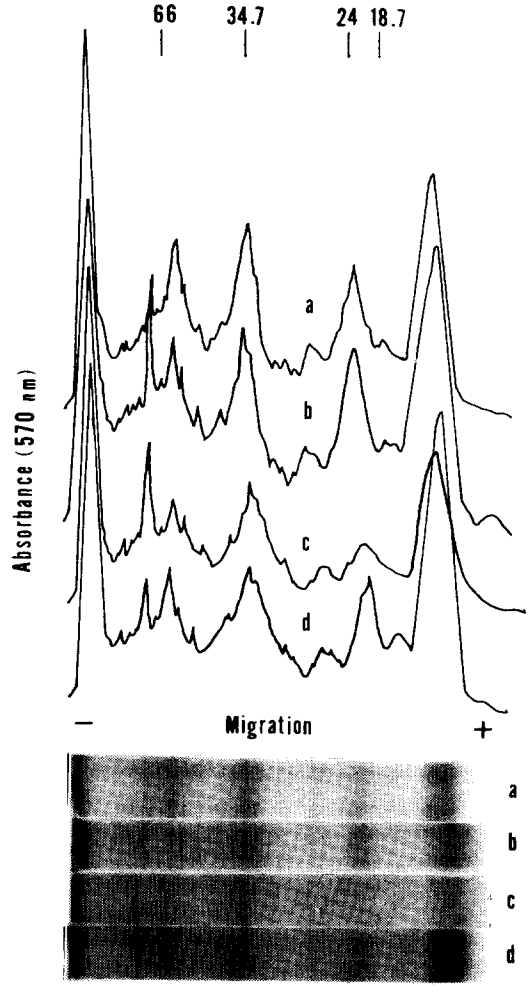

Fig. 10. Electrophoretic profiles of thylakoid proteins from chloroplast of fused callus treated with plant hormonal combinations under far-red light (730 nm).

considerably increased in the combination of NAA+GA_3 with red and blue light irradiation compared to the combination of NAA+BA (Figs. 8 and 9). In contrast, the combinations of BA+GA_3 and NAA+BA+GA_3 showed slight or little changes in the proteins when compared to the combination of NAA+BA. The proteins of 18-25 kDa (mainly 24 kDa) which seem to be light-harvesting chlorophyll proteins were a little changes in the all offered the combinations of growth regulators. Especially, the combination of NAA+GA_3 and control and red light enhanced level of protein of 24 kDa compared to the other combinations of growth regulator groups. NAA+BA and NAA+GA_3 treatments had considerable effect on the synthesis of thylakoid

membrane proteins, and NAA+GA_3 treatment prevailing than NAA+BA treatment. On the whole, the combination of NAA+GA_3 with red light irradiation was the most effective in enhancing thylakoid membrane proteins accumulation.

4. DISCUSSION

For new combinations of cytoplasmic genomes, physiological aspects of protoplast, complete fusion of cell and more ideal condition for somatic hybrid induction are on challenges in the agronomic interest (Schoenmakers *et al.*, 1994; Ratushnyak *et al.*, 1993). Plant growth regulators are required for cell proliferation and are known to stimulate the growth and synthesis of protein and RNA in protoplast (Caboche *et al.*, 1984; Walker *et al.*, 1985). Growth regulators but not the basal media, determined the morphogenetic potentiality of the callus derived from plant source (Tetu *et al.*, 1987).

NAA in combination with GA_3 upon the irradiation of blue, red and far-red light significantly enhanced the callus growth and protein accumulation than those of combination of NAA+BA. This result suggests that simultaneous application of the combination of growth regulators and light irradiation shows additive effect on the callus growth and protein accumulation. Red light irradiation resulted in protein accumulation and this observation demonstrates that there are synergistic effect between red light and NAA+GA_3 treatment.

Our finding that the combination of GA_3 and BA repressed the BA-promoted growth seems to indicate that GA_3 overrides the effect of BA on growth and protein synthesis and acts as an antagonists to BA. Simultaneous application of NAA+BA+GA_3 resulted in very little in the growth of callus and protein accumulation, indicating that there was no promotive effect between the three growth regulators. The auxin, NAA is postulated to enhance an accumulation of mRNAs and modify protein synthesis by regulating the concentration of the corresponding mRNA (Caboche *et al.*, 1984; Walker *et al.*, 1985). In cultured pea root tissue, kinetin stimulated 2- to 4-fold enhancement in the rate of RNA synthesis compared to controls (Shininger and Polly, 1977). And it was suggested that GA_3 replaced the action of red light in inducing protoplasts swelling in the presence of KCl (Keppler and Mertz, 1986), and GA_3 acted as intermediates in phytochrome-regulated responses (Garcia-Martinez *et al.*, 1987; Martinez-Garcia and Garcia-Martinez, 1992). The results of our experiments demonstrate that the combination of NAA and GA_3 significantly affects growth and increase of protein in callus, and that simultaneous application of red light and growth regulators induce a massive stimulation of callus growth and protein synthesis.

On the other hand, thylakoid protein subunits of light-harvesting chlorophyll (LHCP) have been reported to show characteristically quantitative increases during greening and to have molecular weight of 26, 27 and 27.5 kDa in maize, 26-28 kDa in pea, and 26.8-29 kDa in soybean (Grebanier *et al.*, 1979; Morrissey *et al.*, 1989; Silverstein *et al.*, 1993). Although there are a little doubtable in the molecular weight, the 24 kDa protein of callus from intergeneric protoplast fusion seems to be a subunit of LHCP in this experiment. The 56 kDa protein seems to be a subunit of chloroplast coupling factor 1 (CF1) of callus, because their electrophoretic mobilities are identical to that of spinach CF1 subunit. It has been reported that chloroplast grown under blue and red light has much total pigment, but less light-harvesting chlorophyll-protein complexes (Grebanier *et al.*, 1979; Bennet, 1981). In this experiment, LHCP was increased by the simultaneous application of blue or red light and NAA+BA and NAA+GA_3. Particularly, NAA+GA_3 treatment with red light irradiation

led to an increase in LHCP as compared to NAA+BA treatment. It suggests that the combination of NAA+GA_3 and red light may induce the increase of amount of proteins necessary for chlorophyll synthesis. However, there is also a possibility that precursor of LHC apoprotein may be stored in the cytoplasm before it penetrates into plastids, and NAA+GA_3 may enhance the LHC apoprotein accumulation in plastids levels. But it is insufficient to express the reciprocal effect of growth regulators and light quality from these results.

The investigations aimed at a correlative analysis of the influence of different physiological states of plant materials, combined with the action of growth regulators have been rather limited. It is apparent that further research efforts should be directed toward an understanding of what multiple growth regulator sequences and with active light were necessary.

REFERENCES

Bennet, J., 1981, Biosynthesis of the light-harvesting chlorophyll a/b protein: Polypeptide turnover in darkness. Eur. J. Biochem. 118: 61-70.

Bevan, M. and D. H. Northcote, 1981, Subculture-induced protein synthesis in tissue cultures of *Glycine max* and *Phaseolous vulgaris*. Planta 152: 24-31.

Bhatt, D. P. and G. Fassuliotis, 1981, Plant regeneration from mesophyll protoplasts of eggplant. Z. Pflanzenphysiol. 104: 81-89.

Blakeley, S. S., B. Thomas, J. L. Hall and D. Vince-Prue, 1983, Regulation of swelling of etiolated-wheat leaf protoplasts by phytochrome and gibberellic acid. Planta. 158: 416-421.

Bonnema, A. B. and J. M. O'Connell, 1992, Molecular analysis of the nuclear organellar genotype of somatic hybrid plants between tomato (*Lycopersicon esculentum*) and *Lycopersicon chilense*. Plant cell Rep. 10: 629-632.

Caboche, M., G. Aranda, A. M. Poll, J. C. Heut and J. J. Leguay, 1984, Auxin conjugation by tobacco mesophyll protoplasts. Plant Physiol. 75: 54-59.

Cho, S. H. and G. A. Thompson, 1989, Galactolipids of thylakoid pigment protein complexes separated electrophoretically from thylakoids of *Dunaliella salina* labeled with radioactive fatty acids. Plant Physiol. 90: 610-616.

Chung, C. H., S. U. Lim, P. S. Song, J. D. Berlin and J. R. Goodin, 1988, Swelling of etiolated oat protoplasts induced by phytochrome, cAMP and gibberellic acid: A kinetic study. Plant Cell Physiol. 29(5): 855-860.

Dalling, M. J. and A. M. Nettleton, 1986, Chloroplast senescence and proteolytic enzymes. *In* Plant Proteolytic Enzymes, M. J. Dalling ed., Vol.II, CRC Press, Boca Raton, Florida, pp. 125-153.

Derks, F. H. M., J. C. Hakkert, W. H. J. Verbeek and C. M. Colijin-Hooymans, 1992, Genome composition of asymmetric hybrids in relation to the phylogenetic distance between the parents. Nucleus-chloroplast interaction. Theor. Appl. Genet. 84: 930-940.

Frisch, C. H. and N. D. Camper, 1987, Effect of synthetic auxins on callus induction from tea stem tissue. Plant Cell, Tissue and Organ Culture 8: 207-213.

Gaba, V. and M. Black, 1987, Photoreceptor interaction in plant photomorphogenesis: The limits of experimental techniques and their interactions. Photochem. Photobiol. 45: 151-156.

Garcia-Martinez, J. L., B. Keith, B. A. Bonner, A. Stafford and L. Rappaport, 1987, Phytochrome regulation of the response to exogenous gibberellins by epicotyls of *Vigna sinensis*. Plant Physiol. 85: 212-216.

Grebanier, A. E., K. E. Steinback and L. Bogorad, 1979, Comparison of the molecular weights of proteins synthesized by isolated chloroplasts with those which appear during greening in *Zea mays*. Plant Physiol. 63: 426-439.

Kao, K. N. and M. R. Michayluk, 1975, Nutritional requirements for growth of *Vicia hajastana* cells and protoplasts at a very low population density in liquid media. Planta 126: 105-110.

Keppler, L. D. and D. Mertz, 1986, Effect of phytochrome on the uptake of gibberellin by protoplasts of *Nicotiana glutinosa* L. Plant Cell Physiol. 27: 861-865.

Kim, H. H. and C. S. An, 1990, Induction of callus and culture of protoplasts from cotyledon-originated callus in *Alnus Hirsuta*. Korean J. Bot. 33(4): 253-257.

Kim, Y. S., D. K. Moon, J. R. Goodin and P. S. Song, 1986, Swelling of etiolated oat protoplasts induced by cAMP and red light. Plant Cell Physiol. 27(2): 193-197.

Laemmli, U. K., 1970, Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227: 680-685.

Leong, T. Y. and J. M. Anderson, 1983, Changes in composition and function of thylakoid membranes as a result of photosynthetic adaptation of chloroplasts from pea plants grown under different light conditions. Biochem. Biophys. Acta 723: 391-399.

Lowry, O. H., N. J. Rosebrough, A. Lewis Farr and R. J. Randall, 1951, Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275

Martinez-Garcia, J. F. and J. L. Garcia-Martinez, 1992, Phytochrome modulation of gibberellin metabolism in cowpea epicotyls. *In* Progress in Plant Growth Regulation, C. M. Karssen, L. C. Van Loon and D. Vreugdenhil eds., Kluwer Acad. Publ. pp 585-590.

Mathis, J. M. and K. O. Burkey, 1987, Regulation of light-harvesting chlorophyll protein biosynthesis in greening seedling. Plant Physiol., 85: 971-977.

Mayfield, S. P. and A. Huff, 1986, Accumulation of chlorophyll, chloroplastic proteins, and thylakoid membranes during reversion of chromoplasts to chloroplasts in *Citrus sinensis* epicarp. Plant Physiol. 81: 30-35.

Morh, H. and W. Shrosphire, 1983, An introduction to photomorphogenesis for the general reader. 'Encyclopedia of Plant Physiology' NS 16A: 24-38.

Morrissey, P. J., R. E. Glick and A. Melis, 1989, Supramolecular assembly and fuction of subunits associated with the chlorophyll a-b light-harvesting complex II (LHCII) in soybean chloroplasts. Plant Cell Physiol. 30(3): 335-344.

Murashige, T. and F. Skoog, 1962, A revised medium for rapid growth and bioassays with tobacco tissue culture. Plant Physiol. 15: 473-497.

Murphy, D. J., 1986, The molecular organization of the photosynthetic membranes of higher plants. Biochem. Biophys. Acta. 864: 33-94.

Ovaska, J., P. Maenpaa, A. Nurmi and E. M. Aro, 1990, Distribution of chlorophyll-protein complexes during chilling in the light compared with heat-induced modifications. Plant Physiol. 93: 48-54.

Power, J. B. and J. V. Chapman, 1985, Isolation, culture and genetic manipulation of plant protoplasts. *In* Plant Cell Culture, R. A. Dixon ed., IRL Press, Oxford, pp. 37-66.

Qamaruddin, M. and E. Tillberg, 1989, Rapid effects of red light on the isopentenyladenosine content in Scots pine seeds. Plant Physiol 91: 5-8.

Ratushnyak, Y. I., N. N. Cherep, A. V. Zavgorodnyaya, S. A. Latypov, I. V. Borozenko, R. I. Rachkovskaya and Y. Y. Gleba, 1993, Fertile asymmetric

somatic hybrids between *Lycopersicon esculentum* Mill. and *Lycopersicon peruvianum* var. *dentanum* Dun. Mol. Gen. Genet. 236: 427-432.

Sauders, J. W. and M. E. Daub, 1984, Shoot regeneration from hormoneantonomes callus from shoot cultures of several sugarbeet (*Beta vulgaris* L.) genotypes. Plant Science Letters 34: 219-223.

Schoenmakers, H. C. H., J. J. M. van der Meulen-Muisers and M. Koornneef, 1994, Asymmetric fusion between protoplasts of tomato (*Lycopersicon esculentum* Mill.) and gamma-irradiated protoplasts of potato (*Solanum tuberosum* L.): the effects of gamma irradiation. Mol. Gen. Genet. 242: 313-320.

Scowcroft, W. R. and P. J. Larkin, 1981, Chloroplast DNA assorts randomly in intraspecific somatic hybrids of *Nicotiana debneyi*. Theor. Appl. Genet. 60: 179-184.

Shininger, T. L. and L. D. Polly, 1977, Biochemical responses of pea root tissue to cytokinin. Plant Physiol. 59: 831-835.

Shinkle, J. R. and W. R. Briggs, 1984, Indole-3-acetic acid sensitization of phytochrome-controlled growth of coleoptile sections. Proc. Natl. Acad. Sci. USA. 81: 3742-3746.

Silverstein, T., L. Cheng and J. F. Allen, 1993, Redox titration of multiple protein phosphorylations in pea chloroplast thylakoids. Biochim. Biophys. Acta 1183: 215-220.

Tanimoto, S. and H. Harada, 1982, Physical and hormonal factors influencing organogenesis in *Rudbeckia bicolor* explants cultured *in vitro*. Plant cell physiol. 23: 107-113.

Tetu, T., R. S. Sangwan and B. S. Sangwan-Norrel, 1987, Hormonal control of organogenesis and somatic embryogenesis in *Beta vulgaris* callus. Jour. of Exp. Bot. 38: 506-517.

Thornber, J. P. and H. Highkin, 1974, Composition of the photosynthetic apparatus of normal barley leaves and a mutant lacking chlorophyll b. Eur. J. Biochem. 41: 109-116.

Vanderhoef, L. N. and W. R. Briggs, 1978, Red light-inhibited mesocotyl elongation in maize seedlings. Plant Physiol. 61: 534-537.

Van-Geyt, L. N. and M. Jacobs, 1985, Suspension culture of sugarbeet (*Beta vulgaris* L.): Induction and habituation of dedifferentiated and self regenerating cell lines. Plant Cell Reports 4: 66-69.

Walker, J. C., J. Legocka, L. Edelman and J. L. Key, 1985, An analysis of growth regulator interactions and gene expression during auxin-induced cell elongation using cloned complementary RNAs. Plant Physiol. 77: 847-850.

Wolters, A. A., A. C. Vergunst, F. van der Werff and M. Koornneef, 1993, Analysis of nuclear and organella DNA of somatic hybrid calli and plants between *Lycopersicon* spp. and *Nicotiana* spp. Mol. Gen. Genet. 241: 707-718.

*Nicotiana tabacum*과 *Solanum nigrum*의 속간 원형질체 융합에서 유도된 캘러스의 단백질 양태변화에 미치는 생장조절제 및 광선의 효과

김 영 상 · 이 동 희

부산대학교 자연과학대학 생물학과

(1994년 5월 6일 접수)

담배(*Nicotiana tabacum*)와 까마중(*Solanum nigrum*)의 속간 원형질체 융합으로 유도된 캘러스를 재료로하여 캘러스의 전체 단백질과 틸라코이드막 단백질의 양태변화를 중심으로 식물생장 조절물질과 단색광의 생리적 상호효과를 조사하였다. 여러 단색 광선을 캘러스에 조사하였을때, 적색 및 청색광이 캘러스의 전체 단백질과 틸라코이드막 단백질의 합성을 촉진하였으며 적색광이 보다 효과적이었다. 다양한 호르몬 조합을 함유한 배지에서 캘러스를 배양하였을때, NAA+GA_3와 NAA+BA의 조합구에서 캘러스의 전체 단백질과 틸라코이드막 단백질의 축적이 활발히 일어났으며, NAA+GA_3 처리구에서 더욱 효과적 이었다. NAA+GA_3 처리구에 청색광, 적색광 및 근적외광을 제각각 처리하였을때 캘러스의 전체 단백질과 틸라코이드막 단백질의 합성은 적색광에 의하여 가장 촉진되었다. 따라서 적색광과 NAA+GA_3구의 동시처리가 캘러스의 전체 단백질 및 틸라코이드막 단백질의 합성을 상승적으로 촉진함을 보였다.