PRELIMINARY STUDIES FOR GENETIC TRANSFORMATION EXPERIMENTS IN *LILIUM LONGIFLORUM* THUNB.

by

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Manhattan, Kansas

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Approved by:

Robert J. Campbell
Major Professor

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INTRODUCTORY STATEMENT

This thesis has been written in manuscript form. Manuscript II is to be submitted for publication in the Plant Science Letters Journal. The research was conducted in summer 1976 and in spring 1977, in the Department of Horticulture, Kansas State University laboratories.

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EFFECTS OF EXOGENOUS DEOXYRIBONUCLEIC ACID APPLICATION ON LILIUM LONGIFLORUM THUNB. EMBRYOS

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SUMMARY: Forty-five and 50 days but not 30- 35- and 40- day post-pollination Lilium longiflorum Thunb. 'Ace' x 'Nellie White' embryos were suitable as recipients of exogenous DNA, being unaffected by various lengths of incubation or various salmon DNA concentrations and germinating after 2 weeks on Norstog medium. The 30, 35 and 40 days post-pollination embryos did not germinate on Norstog medium after 30 days of culture and turned brown about 2 weeks after transfer onto Emsweller medium.

INTRODUCTION

As a florists' pot plant, Lilium longiflorum Thunb., the Easter lily, possesses many desirable characters, shortness, long leaves, large flowers, ease of forcing and buyer acceptance. This species could be improved if flower colors other than white could be transferred into it from colored lilies. However, interspecific incompatibility and incongruity between L. longiflorum and other lily species limits the possibility of transferring desirable genetic materials by conventional crossing methods.

In recent years a kind of genetic manipulation known as transformation has been successfully carried out with plant and animal cells and with intact tissues both in vitro and in vivo. Foreign deoxyribonucleic acid

(DNA) can be taken up by higher organisms (Anker and Stroun 1968, Ledoux 1965; Ledoux and Huart 1969; Ohyama, Gamborg and Miller 1972) and integrated into the host cell genome (Holl, Gamborg and Pelcher 1974; Hess 1969, 1970 and 1972). In higher plants replication, transcription and translation, stable transmission and phenotypic expression of the transplanted genetic material have been reported (Hess 1969, 1970, 1972).

Hess (1969), in his work on the induction and stable transmission of anthocyanin synthesis in Petunia hybrida, reported the successful incorporation of genes coding for anthocyanin formation into a white-flowering mutant using DNA from the red-flowering wildtype. Two experiments were carried out, one using the donor DNA from the leaves of the mutant petunia and the other using the DNA from the leaves of the wildtype. When white-flowering mutants were treated with DNA extracted from the leaves of their own genotype he obtained about 9% of the flowers showing traces of anthocyanin production while 95% remained white. This slight tendency to anthocyanin production was unstable in both sexually and vegetatively-propagated offspring. However, when the white-flowering mutants were treated with DNA from the red-flowering wildtype by floating the seedlings in the DNA for 15 min, planting them for 48 hr in quartz sand soaked with the same DNA, and then planting them in normal growing-medium, 27% of the plants produced colored flowers indicating synthesis of anthocyanin. On selfing the 'transformed' mutants, he obtained F_1 individuals with red-colored flowers. DNA from the wildtype plant was probably responsible for the transformation of the white-flowering plant into an anthocyanin-producing plant (red flower).

Following a procedure similar to the anthocyanin experiment, Hess (1970) obtained some transformation of the leaf shape in Petunia from the mutant type to that of wildtype in the F_1 generation.

Plant cells take up exogenously applied DNA. Ledoux and Huart (1969), on barley seeds, cut off the end of the seed furthest away from the embryo, and placed the cut seed in a solution of radioactively labelled DNA for 6 hr. The developing seedlings had traces of the labelled DNA in the shoots and roots.

Stroun and Anker (1968) studied the different factors affecting the quantity of uptake of bacterial DNA applied to the tomato (Lycopersicon lycopersicum L.) plant. Tomato plants incubated in light took up more foreign bacterial DNA through the roots than those incubated in the dark. Soaking the roots in 0.15 M sodium citrate solution for 2 hr before DNA treatment increased the DNA uptake.

Nothing is known about the effect of exogenous DNA application on lily embryos. Being the smallest organized plant, an embryo might have least resistance to DNA uptake. Based on this and an embryo's ability to develop into a whole plant, the embryo is a promising recipient of exogenous DNA for a transformation experiment. With the use of embryo culture it is possible to grow a developing lily embryo into a mature plant. Stimart and Ascher (1974) successfully cultured embryos from reciprocal intercultivar crosses of L. longiflorum Thunb. 'Ace' and 'Nellie White' using Norstog (1973) and Emsweller et al (1962) media.

The objectives of this study were to determine the effect of exogenous

DNA application on lily embryos and to determine the best stage of embryo to use. Since the best procedure for the extraction of lily DNA had not been determined, we used salmon DNA.

MATERIALS AND METHODS

Reciprocal intercultivar pollinations of Lilium longiflorum Thunb. 'Ace' and 'Nellie White' (NW) were made in the greenhouse, and the developing capsules were harvested at 30, 35, 40, 45 or 50 days after pollination and sterilized in 10% household bleach for 30 min in a sterile laminar flow hood. The hood space and the equipments brought into the hood were either sterilized with 95% ethanol or autoclaved and the hood area kept sterile by a continous flow of sterile air. Sterile capsules were cut open, the seeds taken out of the capsules, placed in a drop of water on a dissecting scope and the embryos excised using a spear-shaped needle with a single edge and a straight sharp needle. Both needles were dipped in 95% ethanol and flamed before each use. Ten embryos from 1 of the 5 development stages were placed on sterile solidified agar (6 g agar (Difco) / 1 water) in a petri plate; 2 drops (enough to submerge the embryo) of salmon DNA (Sigma) (filter-sterilized by injection through a syringe fitted with a 0.22 um filter (Millipore) and a 26 gauge needle) of 0, 1, 10, 100 or 1000 ug/ml applied one concentration level to a plate; and the whole incubated for 2, 4, or 8 hr at 24°C. For the control sterile distilled water was used. After incubation, the treated embryos were transferred onto the slanted surface of Norstog medium (Norstog, 1973) in 1/3-filled 25mm x 95mm 8 dram vials. Two embryos were placed in each vial and 5 vials were used for each of the 75 treatments. The vials were

closed with Dispo-plugs (Scientific Products), and were labelled and placed in a 24°C incubator in the dark.

The embryos were transferred under a sterile laminar flow hood using a sterile spear-shaped single edge needle to Emsweller medium (Emsweller et al 1962) after 30 days on Norstog medium for the 30- and 40- day embryos and after 15 days for the 50-day embryos. The 35- and 45- day embryos were not transferred to the Emsweller medium since all the 35-day embryos were dead after 30 days on Norstog medium and all the 45-day embryos germinated after 15 days on Norstog medium. The embryos were placed under room light either after transferring to Emsweller medium or after germination on Norstog when the greenish coloration of the shoot was observed.

RESULTS

The DNA apparently retarded the growth of 30- 35- and 40- day post-pollination embryos on Norstog medium, but not the 45- and 50- day post-pollination embryos.

Thirty-day embryos: During the first 30 days on the Norstog medium, the embryos remained dormant and white; however, when transferred to the Emsweller medium, all the embryos turned brown after 15 days.

Thirty-five day embryos: Except for the 2 ug/ml DNA treatment all embryos incubated for 2 hr turned brown after 15 days on Norstog medium. The remaining treatments were dead after 30 days on Norstog medium.

Forty-day embryos: The embryos remained dormant and white for all treatments on Norstog medium, but all turned brown after 11 days on

Emsweller medium.

Forty-five day embryos: All embryos germinated after 15 days on Norstog medium, the radical growing upward, and the green plumule corkscrewing downward into the agar. The plumule turned darker green when the vials were placed under room light. By the third week 50% of the germinated embryos had a brown plumule tip and the medium seemed to be drying out. After 35 days on Norstog medium, all embryos had tip dieback.

Fifty-day embryos: All embryos germinated on Norstog medium after 2 weeks culture. As in the 45-day embryos the plumules were mostly twisted and growing downward into the agar. Browning of the plumule tip occurred after 30 days on Emsweller medium.

DISCUSSION

Stimart and Ascher found that 35- and 40- day embryos reached the size of mature embryos on Norstog medium; since this did not occur in the DNA-treated and control embryos in our experiments, other factors may have influenced the embryo development. One possibility was that the medium seemed to be drying out faster than expected even though a tray of distilled water was placed in the incubator; another possibility would be that the applied DNA retarded the development of immature embryos (35- and 40- day embryos).

Contrary to the findings of Stimart and Ascher that the 55-day embryos did not germinate on Norstog medium, we found that the DNA-treated and control- 50-day-embryos germinated 2 weeks after culture on Norstog. The

tip die-back that occurred on the 45-day embryos could possibly be due to the drying out of the Norstog medium since the 50-day embryos transferred to the newly prepared Emsweller medium 15 days sooner did not show tip die-back.

Clearly, 30-, 35- and 40- day embryos should not have DNA applied to them. Since both the DNA concentration as well as the incubation period have no effect on the development of the 45-day embryos, it can be concluded that this is the best stage of embryos to be used for DNA-treatment in transformation experiments.

We now know that the rate of drying out and the toxic substances that have been reported to be released from sterilized Dispo-plugs could be reduced and eliminated if the vials openings are closed or covered with aluminum foil.

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HIGH MOLECULAR WEIGHT DNA FROM *LILIUM LONGIFLORUM* THUNB. STIGMA-STYLES[1]

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SUMMARY: A spectrophotometrically pure and high molecular weight DNA (greater or equal to 2×10^6 daltons) was extracted from *Lilium longiflorum* Thunb. stigma-styles following a modified procedure of Heyn et al. Modification of the Laulhere and Rozier's procedure does not yield spectrophotometrically pure nucleic acid although we do obtain an elution profile from the Sepharose 4-B gel column which is similar to those of other workers. The modified procedure of Heyn et al yielded 1.083 mg DNA/g dry weight of stigma-style, while that of Laulhere and Rozier yielded 65.45 ug/g dry weight.

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INTRODUCTION

Several procedures have been described for the isolation of nucleic acids from plant materials (Ohyama et al 1972; Heyn et al 1974, Laulhere and Rozier 1970; Stern 1968). Heyn et al (1974) developed a fast, easy procedure for the extraction of highly polymerized plant deoxyribonucleic acid (DNA) from normal- and crown-gall- tissues of tobacco (Nicotiana tabacum L. cv. White Burley), yielding high molecular weight DNA (12-30 x 10^6 daltons) within 5 hr with an isolation efficiency of 90-95%. The procedure specifically extracts DNA and not the other nucleic acid species; since, with the extraction medium at pH of 9.4, only DNA is extracted. Ohyama et al (1972) used a similar procedure on Ammi visnaga L. and Daucus carota L. and obtained highly pure and high molecular weight DNA (3.04 x 10^7 daltons for A. visnaga and 1.92 x 10^7 for D. carota DNA), the qualities desirable for genetic transformation experiment. The Heyn et al procedure is preferred to that of Ohyama et al because of the ease and speed of manipulation as well as its ability to be used for isolation of DNA from both small and large samples. In both, DNA was separated from other compounds in the extraction solution by filtration through a Sepharose 4-B gel column that excludes compounds with molecular weights equal to or greater than 2 x 10^6 daltons. The Sepharose 4-B gel column is much used to separate nucleic acids from lower molecular weight compounds (Loeb and Chauveau 1969; Charles 1977)

Laulhere and Rozier (1976) developed a one-step procedure to extract DNA and RNA from leaves of spinach (Spinacia oleracea L.), potato (Solanum tuberosum L.), thali of liverwort (Marchantia polymorpha L.) and suspensions of isolated chloroplasts, using an extraction medium with pH 6.65 and

precipitation of total nucleic acid with cold ethanol. Yield of DNA and rRNA from spinach ranged from 100-120 ug and 960-1200 ug/g fresh weight. DNA was separated from the tRNA on a Sepharose 4-B column.

No detailed procedure is available for the rapid extraction of high molecular weight nucleic acids from lily stigma-styles. Campbell (1975) extracted nucleic acids from lily stigma-styles using phenol- 1 x SSC buffer pH 7.6, and separated the nucleic acid species using methylated albumin kiesulghur (MAK) column into approximately 59% RNA and 30% DNA. The nucleic acids obtained are contaminated with phenol and are not suitable for use on living tissue. The phenol extraction procedure is time consuming and the preparation of the MAK column tedious.

The abundance of endomitotic stylar canal cells in L. longiflorum Thunb. (U. Kumar and P.D. Ascher, unpublished) is the main reason for choosing the lily stigma-style as the source of nucleic acids. Rosen and Thomas (1970) found that canal cells of L. longiflorum Thunb. have large nuclei (25-30 u).

The purpose of this study was to determine the nucleic acid profile from lily stigma-style nucleic acids applied to and eluted from Sepharose 4-B gel columns, to obtain DNA with molecular weight equal to or greater than 2×10^6 daltons, and to determine the quantity and quality of nucleic acids extracted by the modified procedures of Heyn et al (1974) and by that of Laulhere and Rozier (1976).

MATERIALS AND METHODS

Flowers of greenhouse-grown Lilium longiflorum Thunb. 'Ace' were cut early on the morning of the day of anthesis, placed in water, and kept at room temperature for one day. Stigma-styles were removed from the flowers by cutting through the ovary using a triangular needle, the ovary snapped off, and the stigma-style placed on moist filter paper in a petri plate and cooled in ice.

A. Modified procedure of Heyn et al (1974)

Lily stigma-styles were frozen in liquid nitrogen, lyophilized in a Virtis Automatic Freeze-Dryer (Model 10-010) and stored at -20°C until used. Five hundred and ninety mg of the lyophilized stigma-styles were cut into small pieces using an ice-cold razor blade and place in a 10 cm porcelain mortar in ice. Liquid nitrogen was added and the brittle sample ground into a powder using a pestle. Liquid-nitrogen-frozen lily stigma-styles could not be pulverized on a vortex mixer using glass beads as specified by Heyn et al. Six ml pronase solution (Pronase E Merck : 8 mg/ml, traces of DNase inactivated following the procedure of Hotta and Bassel (1964) and the solution made up to 1 M NaCl), 3.8 ml of isolation buffer (3.033 ml Sarkosyl NL 30 (Ciba Geigy) / 100 ml buffer, 5mM EDTA, 100 mM Tris-HCl (Sigma), 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, and 250 mM NaCl, pH 9.4), and 0.1 mM DTT (1,4-dithiotheitol, Sigma) were added to the ground tissue. (Sarkosyl NL 30 was used instead of NL 97 because the latter was not available.) The whole was gently stirred, rapidly brought to 56°C in almost boiling water while stirring with a thermometer, and incubated in 56°C water bath for 3 hr. Debris was removed by centri-

fugation at 20,000 g for 10 min (5°C) in a Sorvall RC2-B centrifuge using an SS-34 rotor, the supernatant saved, the pellet washed twice with 10 ml wash buffer (similar to the isolation buffer except 1 M NaCl was used), and the wash solution combined with the supernatant. Heyn et al found DNA and other lower molecular weight compounds in the supernatant.

Four ml of supernatant placed on a Sepharose 4-B gel (Sigma) column (17 cm x 2.5 cm diameter) was eluted with 0.1 x SSC buffer (0.15 M NaCl, 0.015 M sodium citrate, pH 7.5). Two ml fractions were collected using an Isco Golden Retriever fraction collector. Absorbance was read at 230, 260, and 280nm on a Beckman Spectrophotometer (Model 25). From each of the 2 peaks obtained, the fraction with the highest absorbance at 260nm was subjected to spectrophotometric scanning to determine the absorption spectrum.

The DNA concentration in the exclusion volume was estimated using a salmon sperm DNA (Sigma) standard curve.

B. Modified procedure of Laulhere and Rozier

Four gr fresh weight of ice-cooled stigma-styles were cut into small pieces using an ice-cooled razor blade and transferred to a 10 cm porcelain mortar in ice. Liquid nitrogen was poured into the mortar and the frozen tissue gently ground with a pestle to a fine powder. We used this grinding procedure instead of a Waring blender or mortar and sand as specified by Laulhere and Rozier to reduce fragmentation and shearing of the nucleic acids. Ten ml of the extraction medium (0.5 M NaCl, 100 mM Tris-HCl (Sigma) pH 7.6, 10 mM $MgCl_2$, 20% ethanol (v/v), 3% sodium lauryl sulfate (Sigma)

(w/v), 20 mM EDTA), and 200 ul of DEP (diethylpyrocarbonate, Sigma) was added to the powdered tissue, the slurry transferred to a glass centrifuge tube, stirred in a 37°C water bath for 5 min, 10 ml of chloroform added and the whole gently stirred to homogenize the solution and then cooled in ice. The cooled sample was centrifuged at 2,000 g at 5°C for 10 min, the aqueous phase transferred to a polycarbonate centrifuge tube, 2 volumes of cold (-20°C) 95% ethanol added, the tubes stored at -20°C for 2 hr, and the precipitate containing total nucleic acids pelleted by centrifugation at 15,000 g for 10 min. In another modification the supernatant, instead of being discarded, was poured into a cold beaker in ice and after 5 min the remaining nucleic acid precipitate was spooled up on a glass rod. The spooled nucleic acid precipitate and the pellet were washed with cold 95% ethanol before suspension in 2 ml of 3 M sodium acetate (pH 6.0) and centrifugation at 13,000 g for 10 min. The supernatant was saved and the pellet again suspended in 2 ml of 3 M sodium acetate and centrifuged. The supernatants were combined; Laulhere and Rozier reported that the supernatant contained DNA and tRNA while the pellet contained rRNA.

DNA was separated from tRNA on a Sepharose 4-B gel column as in procedure A. The fractions with the highest absorbance at 260nm from the 2 peaks obtained were subjected to spectrophotometric scanning to determine the absorption spectrum. DNA concentration was estimated as in procedure A.

RESULTS

Procedure A: Elution of the supernatant from the Sepharose 4-B gel column gave 2 peaks at 260nm (Fig. 1), the first one probably containing DNA in the exclusion volume (fractions 8-14) and the second consisting of lower molecular weight compounds. The 260/280 and 260/230 ratios of the fraction in the exclusion volume with the greatest absorbance at 260nm was 1.77 and 2, and the UV absorption spectrum of this fraction gave rise to a typical nucleic acid spectrum with maximum at 257nm and a minimum at 233nm (Fig. 2). The yield of DNA estimated using salmon DNA as a standard was 1.083 mg/g dry weight. The 260nm absorbance of the second peak reached a maximum of 9.614 and the 260/280 ratio was about 1.2.

Procedure B: Elution of the supernatant from the Sepharose 4-B gel column revealed 2 peaks at 260nm (Fig. 3), the first (Peak a) probably containing DNA in the exclusion volume and the second (peak b) consisting probably of tRNA. The 260/280 and 260/230 ratios of the fraction from the first peak with the greatest absorbance at 260nm was 1.28 and 0.407. UV scanning of this fraction, however, did not produce a prominent peak at 258 and 233nm, but a shoulder developed between 260 and 243nm and the maximum absorption occurred at 225nm (Fig. 4). Estimation of the total DNA from the exclusion volume was 20.1 ug/g fresh weight (equivalent to 65.4 ug/g dry weight).

Fraction 34 in the second peak (Fig. 3, peak b), with the greatest absorbance at 260nm, had 260/280 and 260/230 ratios of 1.5 and 0.098. Estimation of the molecular weight from the exclusion volume standard curve suggested that the compound in the second peak had a molecular weight

less than 10^4 daltons.

DISCUSSION

For both procedures the elution of crude nucleic acid extracts from the Sepharose 4-B gel column gave rise to 2 peaks, the whole profile resembling that obtained by other workers (Charles 1977; Laulhere and Rozier 1976; Heyn et al 1974). The Sepharose 4-B gel column separates compounds on the basis of molecular weight. The nucleic acid profile obtained is separated into DNA and RNA or DNA and lower molecular weight compounds. Although there exist some shoulders on the 2 peaks in the profile, it is not possible to determine if this is due to further species separation. The 260/280 and 260/230 ratios, (using the value of 2 as an indication of pure nucleic acids for both ratios) suggest that procedure A yields a higher purity DNA than does procedure B. The low 260/280 ratio in the exclusion volume in procedure B is an indication of protein contamination. Since the Sepharose 4-B gel column separates by molecular weight (exclusion limit 2×10^6 daltons) no free protein would be coming off in the exclusion volume, hence the presence of protein could be in the form of a protein-DNA complex. The contamination, coupled with low amount of nucleic acids extracted, could account for the shoulder in the 233 and 258nm region in the UV absorption spectrum instead of the expected minima and a maxima at those wavelengths.

The modifications made in both procedures are necessary for use with lily stigma-styles. Heyn et al separated DNA from RNA by precipitation of the RNA on the column using 2M NaCl and removed it later from the column by eluting it with a low-salt buffer. There was no need to precipitate

the RNA for its separation from DNA on the Sepharose 4-B gel column since we were only interested in the exclusion volume, and RNA which has molecular weights of less than 2×10^6 daltons could not be in the exclusion volume. In Laulhere and Rozier's procedure, the spooling of the precipate resulted in a higher exclusion volume peak with 0.12 maximum absorbance at 260nm as opposed to a small peak with 0.007 maximum absorbance at 260nm when spooling was not done.

More research is necessary to characterize the nucleic acids extracted and to determine the reasons for low quality nucleic acids extracted by the modified procedure of Laulhere and Rozier. For the transformation experiment to be carried out, we will use the modified procedure of Heyn et al since high molecular weight and high purity DNA were obtained. Laulhere and Rozier's procedure for nucleic acids extraction from lily stigma-styles can still be used if contamination of the nucleic acids can be avoided.

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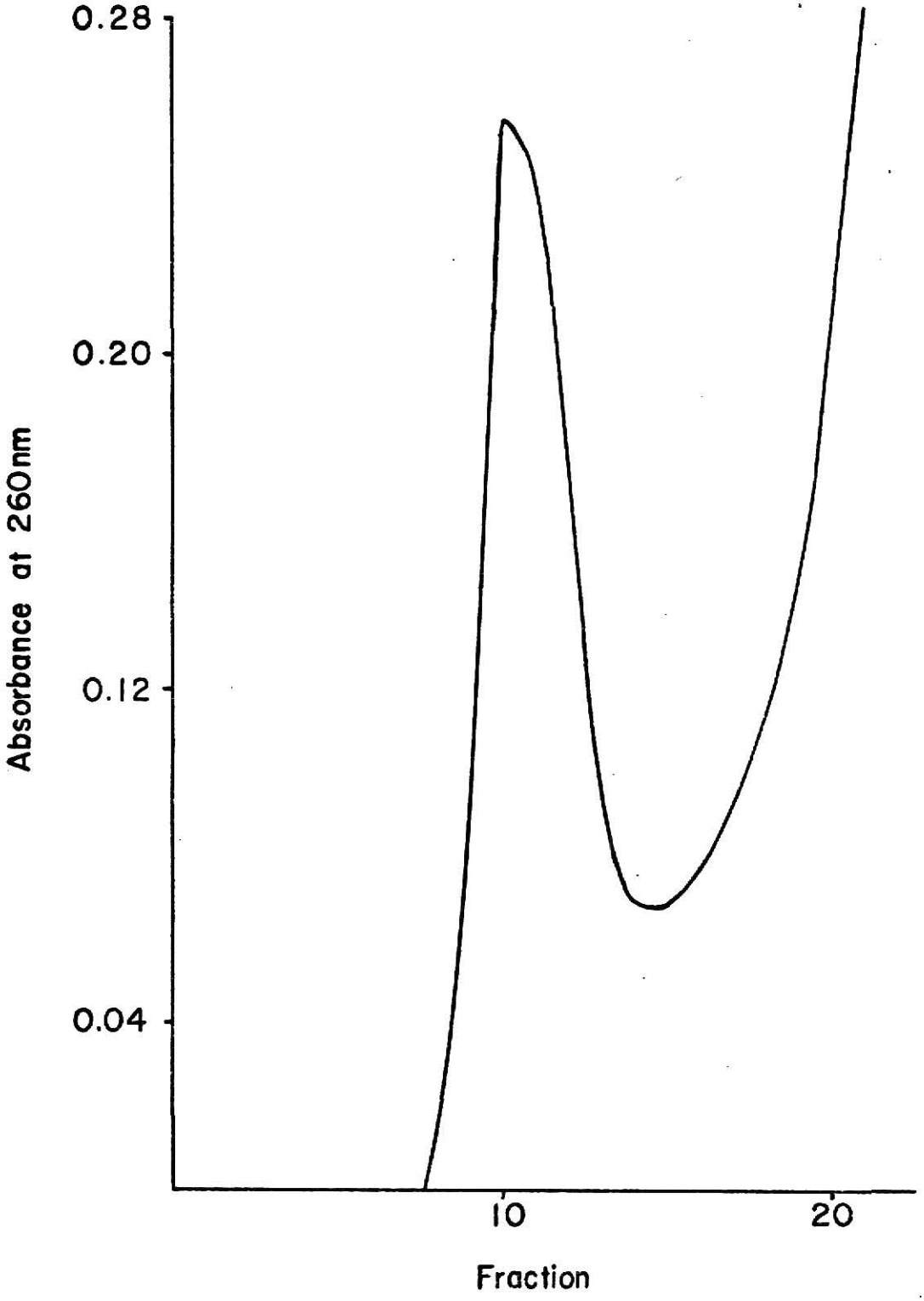


Fig. 1

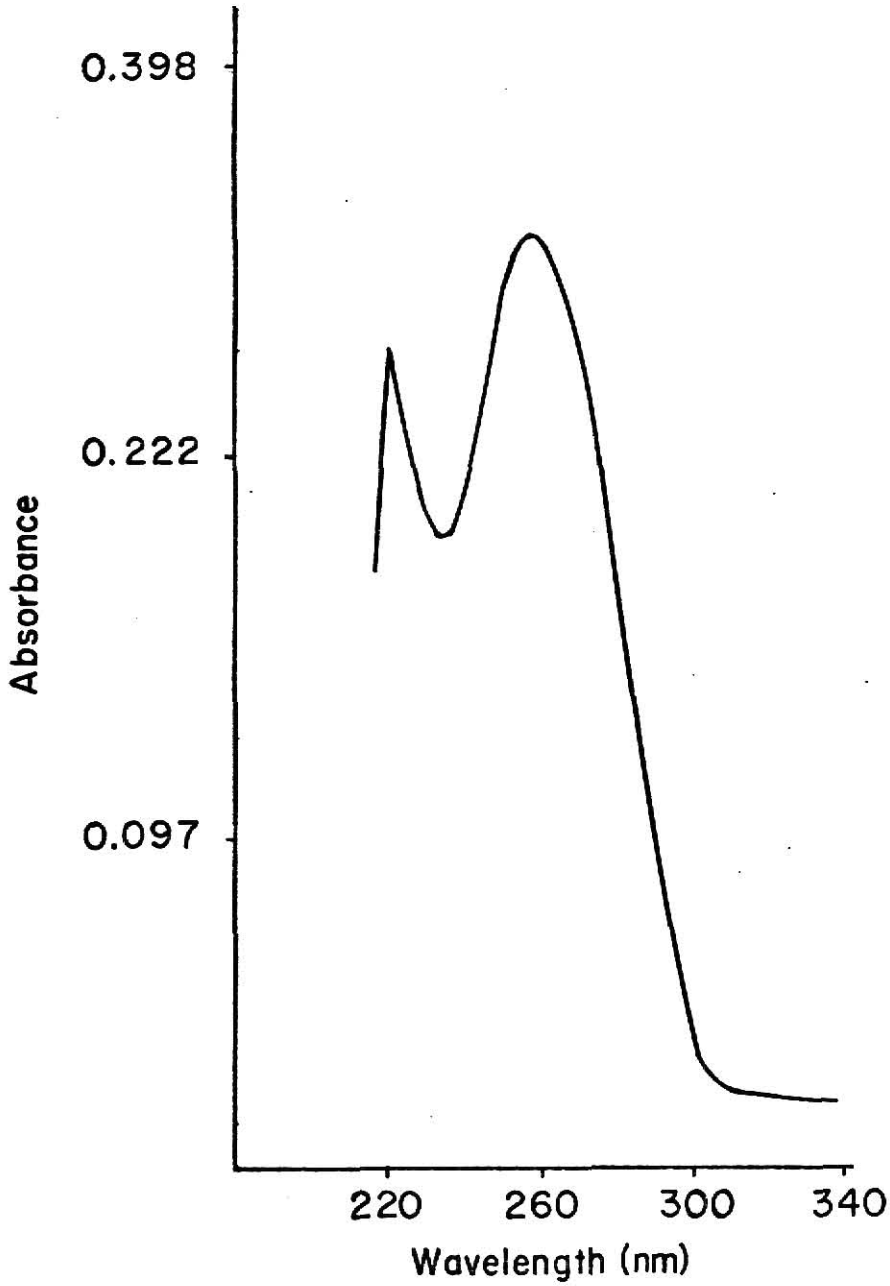


Fig. 2

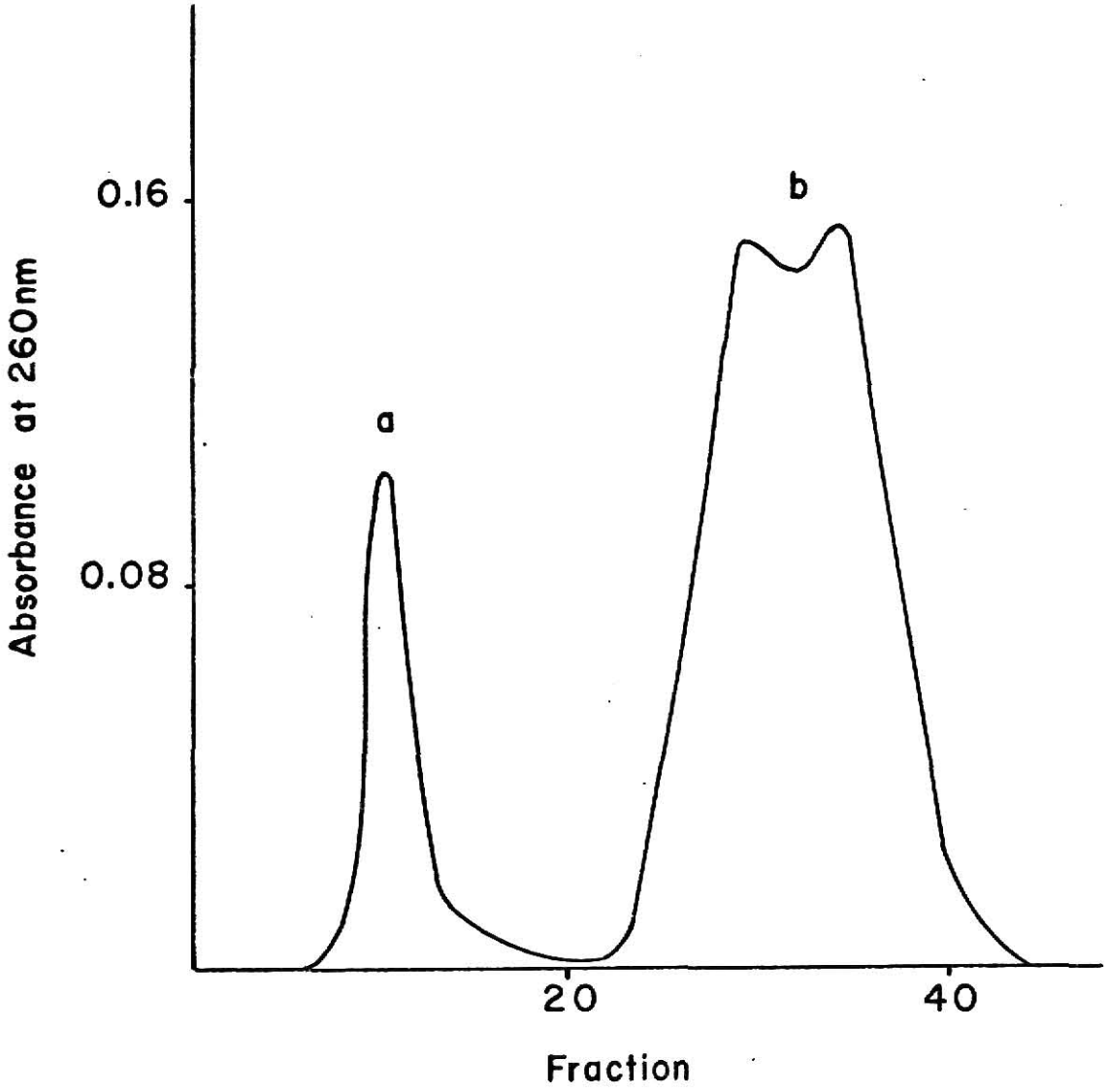
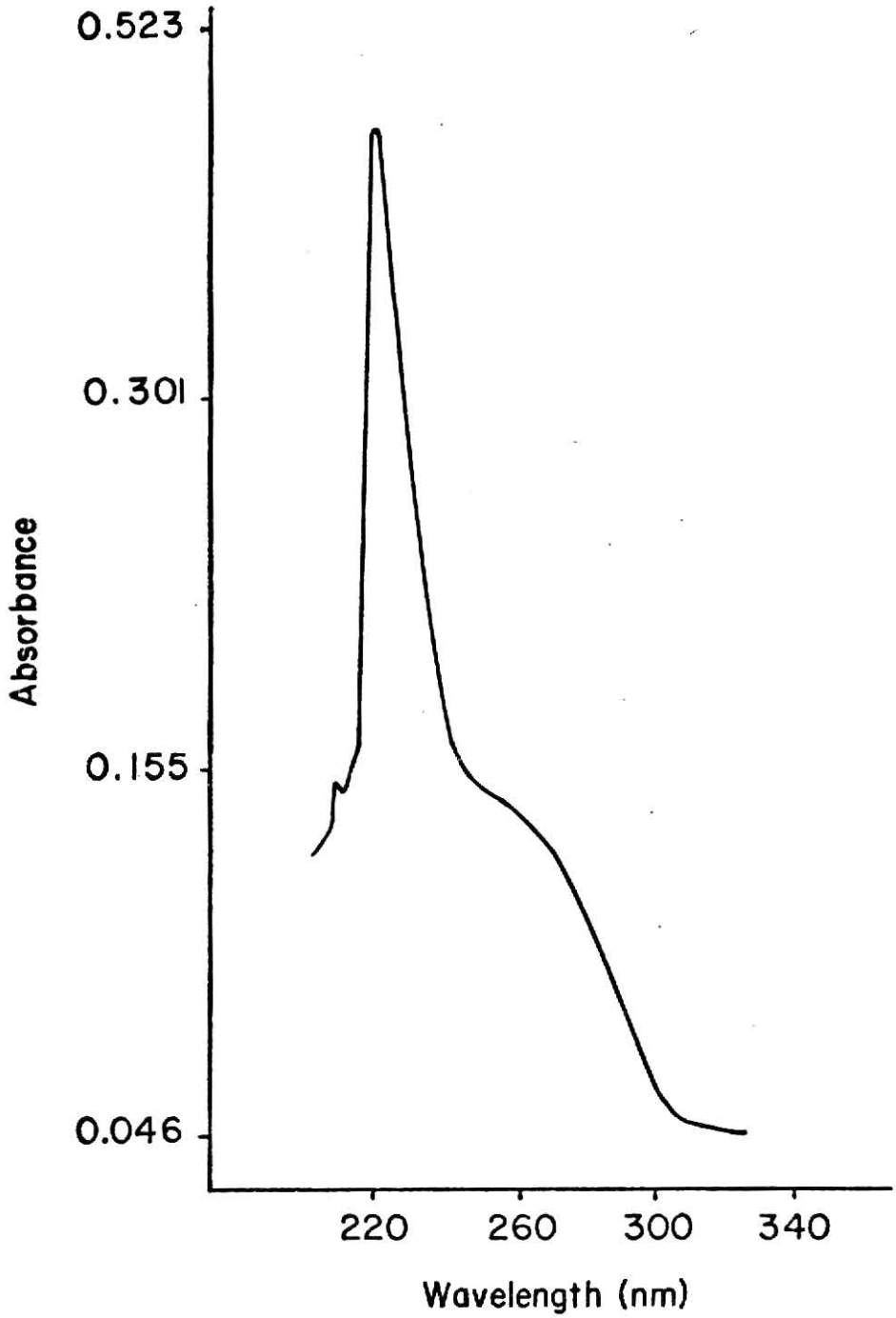


Fig.3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0.301
0.155
0.046
Absorbance
220
260
300
340
Wavelength (nm)

Fig. 4

APPENDIX

Experimental Procedures

Column preparation: The Sepharose 4-B gel column was prepared using a 20cm x 2.5cm diameter glass column which tapers to 10cm x 0.5cm diameter tubing. Glass wool was placed at the base of the 2.5cm diameter column and the elution buffer poured until 10% of the column was filled. The Sepharose 4-B gel was slowly poured along the side of the column to fill the column and eluted with the buffer to settle the gel. Additional gel was added to the desired height and the column eluted overnight before being used. The column was repacked when bubbles were detected in the gel.

A 500 ml separatory funnel was used as a reservoir for the elution buffer and the base of the funnel fitted to the top of the gel column using a rubber stopper. The flow rate was regulated by controlling the lower opening of the separatory funnel.

Column calibration : The Sepharose 4-B gel column was calibrated using blue dextran (Sigma) (2×10^6 daltons) at 0.4 mg/ml, Bovine albumin fraction V (Sigma) (6.7×10^4 daltons) at 2 mg/ml and salmon DNA (Sigma) at 0.25 mg/ml. The exclusion volume was obtained using the elution of these standard compounds (Fig. A1).

Earlier, we did attempt to use a Pharmacia adjustable-height column (2.5 cm diameter) with the Sepharose 4-B gel filled up to 57 cm., with the idea that the longer column would give a better separation. However, after allowing the gel to settle overnight it was difficult to increase the flow rate to greater than 0.15 ml/min without the use of a peristaltic

pump (we had none). When crude nucleic acids extracted by the procedure of Laulhere and Rozier were placed on the gel, no peak at 260 nm absorbance was observed in the exclusion volume (Fig. A2). Since it took 5 days to elute everything out of the gel, the problem could be that adsorption could have occurred since the flow rate was only 0.15 ml/min. Continued elution freed the molecules from the gel. Therefore we resorted to the use of the small gel-filtration column.

With the Laulhere and Rozier's procedure, a number of experiments were carried out before a high peak was obtained in the exclusion volume. Fig. A3 is a typical profile obtained when no additional spooling of the nucleic acids was done, and this differs from Fig. 3, where additional spooling was carried out, in that the 260nm absorbance peak in the exclusion volume was much higher in the latter. We did not attempt however to centrifuge at a higher rpm or at a longer time since the pellet obtained at the speed normally used was already difficult to dissolve in 3M sodium acetate.

Laulhere and Rozier cited the work of Solymosy et al[1] that DEP induced modifications in the structure of nucleic acids if the concentration or the contact period was too high or too long. Laulhere and Rozier indicated that the DEP concentration of 200 ul and a contact period of 5 min. had no effect on the nucleic acids extracted. Since in our procedure DEP is in the homogenized extraction medium for about 2 min and is present for another 5 min during incubation period at 37°C, there was a possibility that a 7 min contact period between the DEP and the nucleic acid extracted

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[1]F. Solymosy et al, Biochim. Biophys. Acta, 238 (1971) 406.

might cause a modification in the structure of the nucleic acid. We then carried out an experiment with a DEP-DNA contact period of only 25 seconds using the grinding as specified by Laulhere and Rozier. We homogenized the tissue in a Sorvall Omni mixer homogenizer using a stainless steel leak-proof chamber (50 ml maximum capacity) with 4 inches rotor-knife blade assembly. The remaining procedure was similar to that described in the text. The fraction with the highest 260 nm absorbance in the exclusion volume was subjected to the UV scanning and the spectrum was found to be similar to that obtained in the modified procedure (Fig. A4) except that the profile gave the highest absorbance at 260 nm lower than the modified procedure.

We also carried out a similar experiment but without using DEP. The spectrum obtained was similar to the previous experiment (Fig. A5) but the highest absorbance at 260nm in the exclusion volume was lower indicating that the extraction gave lower amount of DNA.

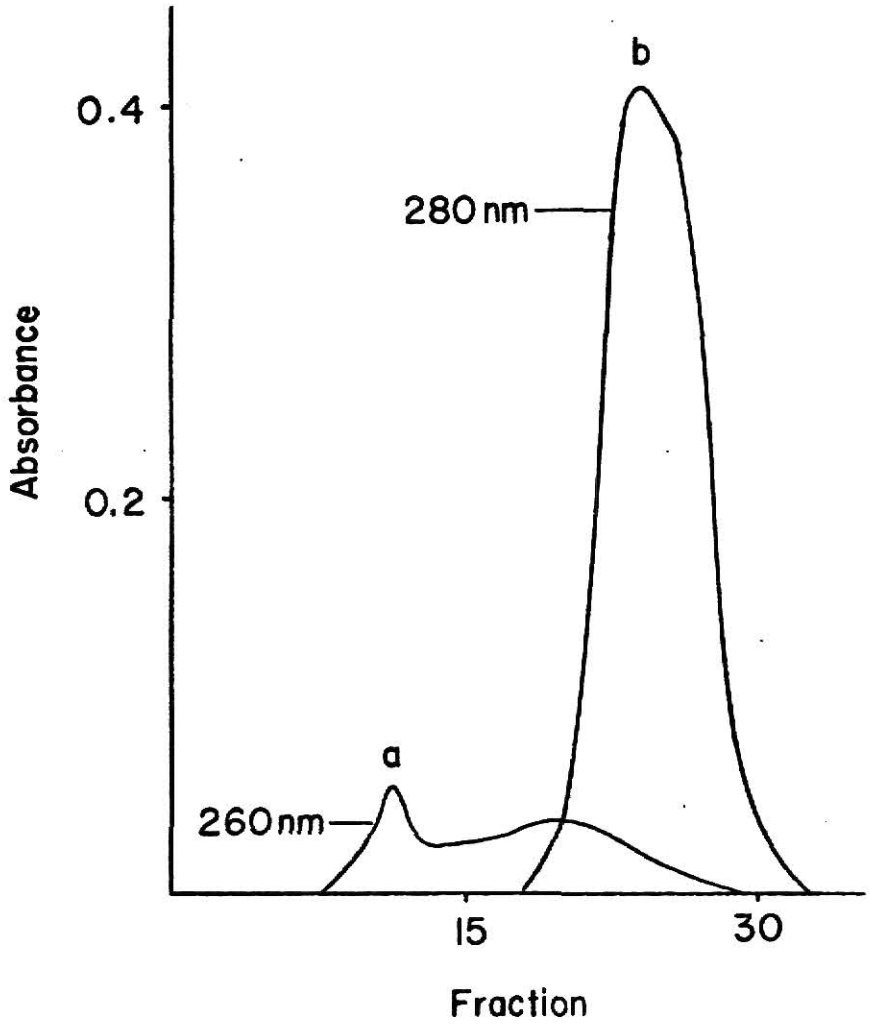

Fig. AI

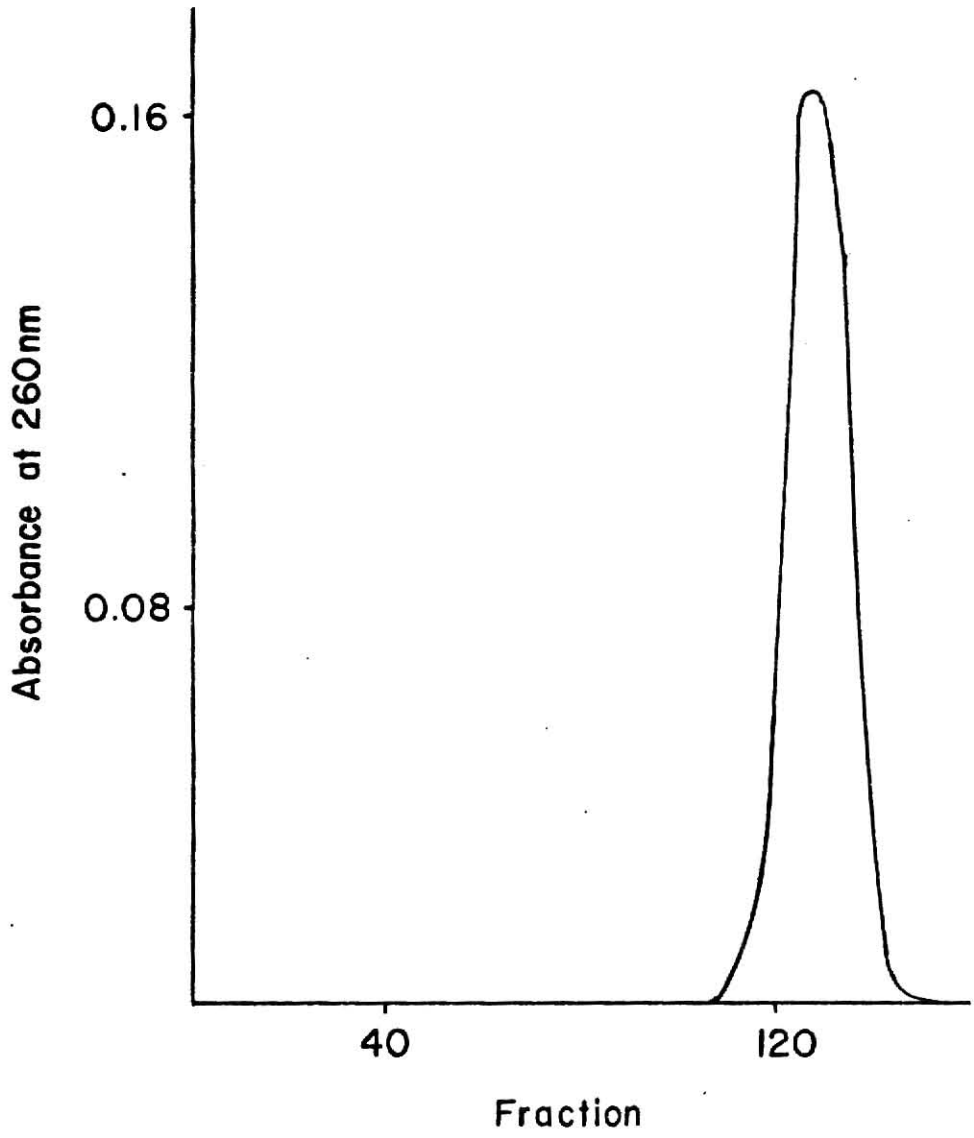

Fig. A2

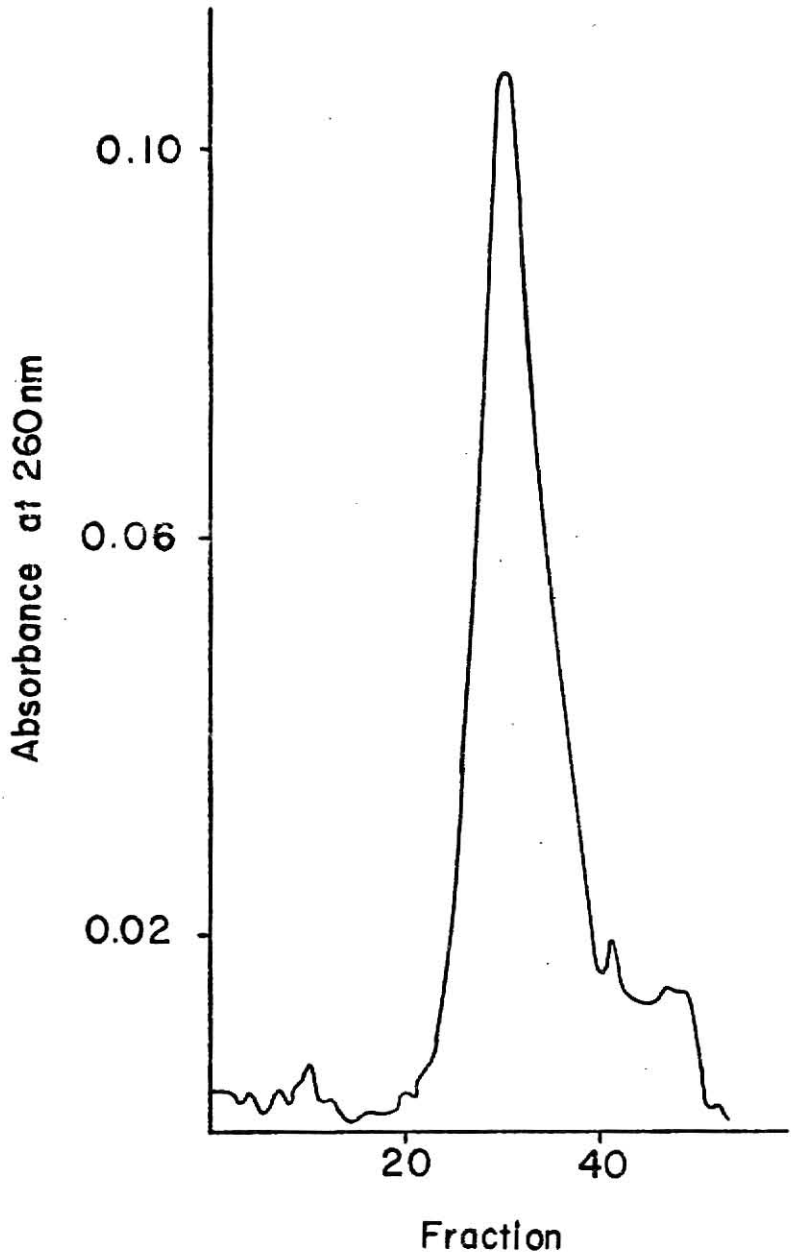

Fig. A3

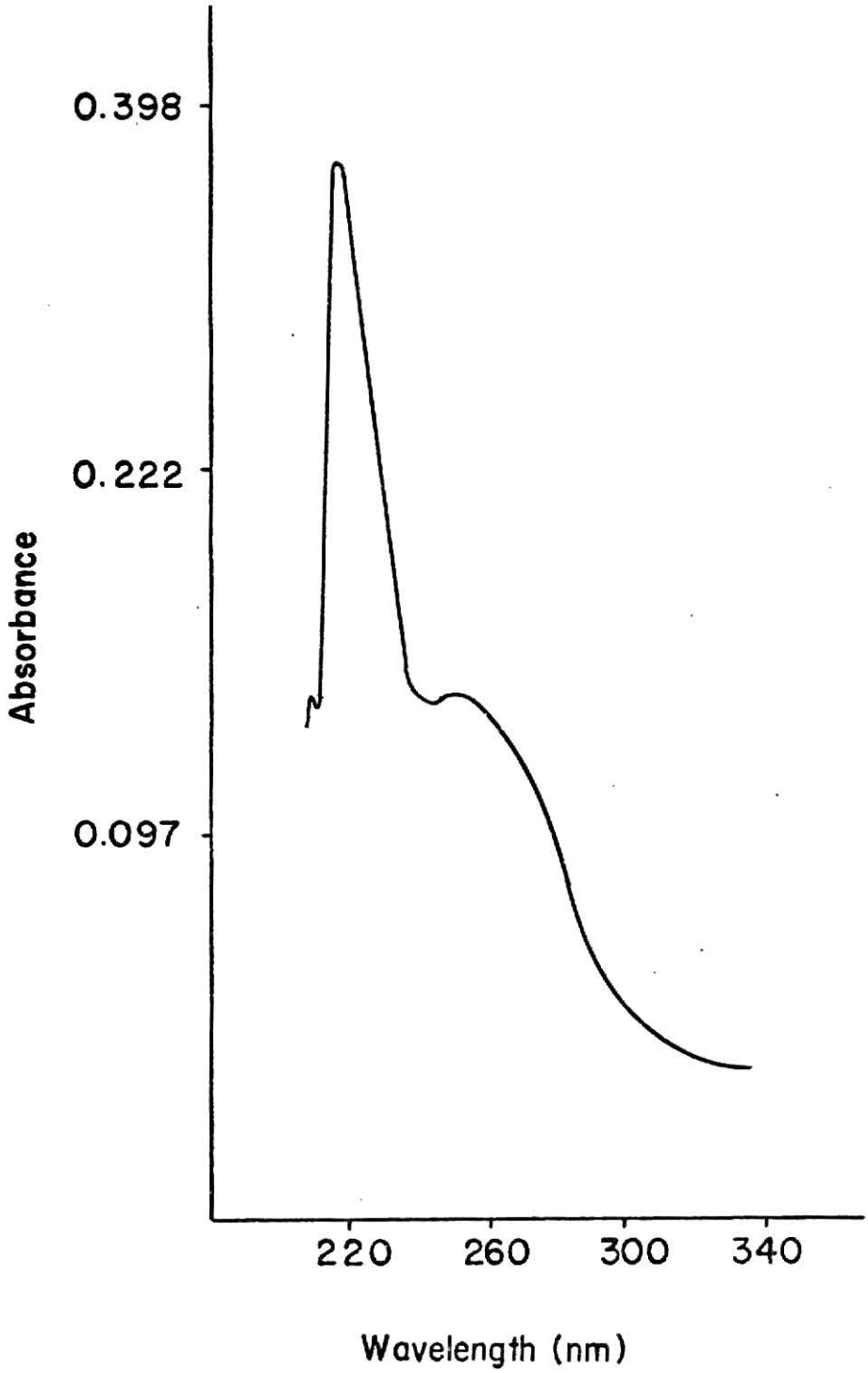

Fig. A 4

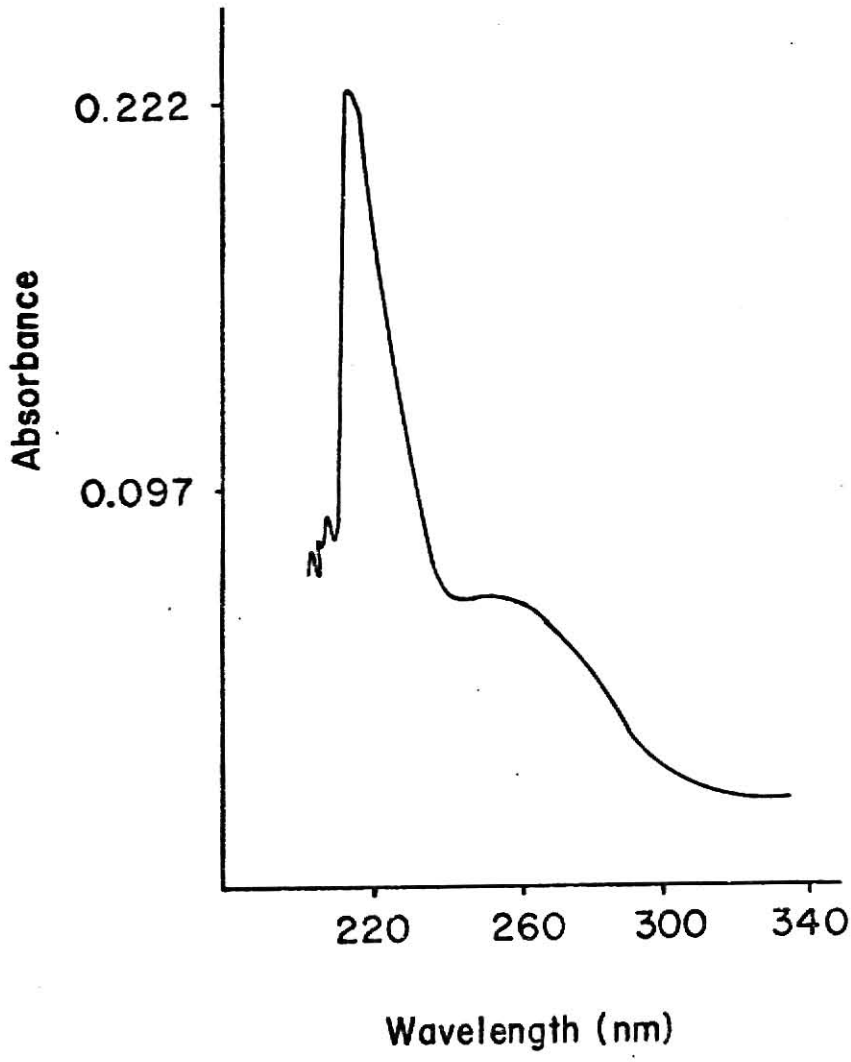

Fig. A 5

PRELIMINARY STUDIES FOR GENETIC TRANSFORMATION EXPERIMENTS IN *LILIUM LONGIFLORUM* THUNB.

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Forty-five and 50 days but not 30-, 35- and 40- day post-pollination Lilium longiflorum Thunb. 'Ace' x 'Nellie White' embryos were suitable as recipients of exogenous DNA, being unaffected by various lengths of incubation or various salmon DNA concentrations and germinating after 2 weeks on Norstog medium. The 30-, 35- and 40- day post-pollination embryos did not germinate on Norstog medium after 30 days of culture and turned brown about 2 weeks after transfer onto Emsweller medium.

A spectrophotometrically pure and high molecular weight DNA (greater or equal to 2×10^6 daltons) was extracted from Lilium longiflorum Thunb. stigma-styles following a modified procedure of Heyn et al. Modification of Laulhere and Rozier's procedure does not yield spectrophotometrically pure nucleic acid although we do obtain an elution profile from the Sepharose 4-B gel column which is similar to those of other workers. The modified procedure of Heyn et al yields 1.083 mg DNA/g dry weight of stigma-styles, while that of Laulhere and Rozier yields 65.45 ug/g dry weight.