RESULTS

2.5. RESULTS

2.5.1. Distribution and Profile of Acid Phosphatase Activity from the Plant Parts of Different Developmental Stages of *Sechium edule* .

It is understood from the literature review (section 2.2.) that although the enzymatic hydrolysis of phosphomonoesters by non-specific acid phosphatase has been documented from a wide range of living organisms, there is almost no information regarding the hydrolyzing machinery of phosphomonoesters by acid phosphatase in *Sechium edule* at different developmented stages. At the onset of the work an attempt has been made in this respect.

Using dialyzed low-speed supernatant as the enzyme source (section 2.4.2.3.), acid phosphatase was assayed (section 2.4.2.4.). The results presented in Figure 2.4. show that the enzyme is functional in all developmental stages of the experimental plant. However, remarkable enzymic activity was recorded in feeding root, vine of plant and mature leaf of the plant.

2.5.2. Distribution and Profile of Alkaline Phosphatase Activity from the Plant Parts of Different Developmental Stages of *Sechium edule*.

Like acid phosphatase, alkaline phosphatase was assayed (section 2.4.2.2.) in *Sechium edule* at different developmental stages using dialyzed low-speed supernatant as the enzyme source (section 2.4.2.5). The results are depicted in Figure 2.5. It has been revealed from the same figure that this enzymic activity is maximum in the feeding root part of the plant, although as considerable titre of the enzymic activity can easily be detected in other plant parts too with a less extent.

2.5.3. Distribution and Profile of L-myo-inositol-1-phosphate synthase Activity from the Plant Parts of Different Developmental Stages of *Sechium edule*.

The principal enzyme, responsible for the metabolism of *myo*-inositol in connection with various cellular events, i.e., L-*myo*-inositol-1-phosphate synthase was isolated

(section 4.2.2.5) and assayed (section 4.2.2.6) from different plant parts in *Sechium edule* using dialyzed low-speed supernatant as the enzyme source. The results of such experimetns are presented in Figure 2.6. It can be read from the same figure that this enzymic activity is principally confined within the female flower and underground tuberous root. However, a basal activity of this enzyme is existing even in the male flower and in the mature fruit.

2.5.4. L-*myo*-inositol-1-phosphate Synthase Activity from the Germinating Seeds of *Sechium edule*.

It can be recalled from the previous experiment that there is a basal activity of L-*myo*-inositol-1-phosphate synthase in the mature fruit which casts the possibility of it's function during seed germination of this experimental plant.

In order to establish the realistic status of this probability, L-*myo*-inositol-1-phosphate synthase was isolated (section 4.2.2.5) and assayed (section 4.2.2.6) from plant samples of different germination hours viz. 0-h, 24-h, 48-h, 72-h, 96-h and 120-h. The results are presented in Figure 2.7. From this figure it is evident that this enzyme operates maximally at 72-h stage of germination. Sharp decline in catalytic activity was recorded either before or after 72-h stages of germination.

2.5.5.L-*myo*-inositol-1-phosphate Synthase Activity from the Seeds During Maturation from *Sechium edule*.

Phytic acid (Ca^{2+}, Mg^{2+} salt of *myo*-inositol hexaphosphate) is considered as the principal storage phosphate in seeds. It is evident from the "Inositol phosphate cycle" or "Biswas cycle" (De and Biswas, 1979) that the synthesis and metabolic utilization of this *myo*-inositol hexaphosphate is possible through the successive operation of some enzymes namely *myo*-inositol-1-phosphate synthase, phosphoinositol kinase, IP_6-ADP-phosphotransferase, phytase and *myo*-inositol-1-phosphate dehydrogenase during maturation followed by germination of seeds where the first enzyme is the principal rate regulating one during maturation of seeds in the metabolic cycle. Therefore, an attempt has been made to look after the profile of this enzyme (MIP synthase) during maturation of *Sechium edule* seeds.

L-*myo*-inositol-1-phosphate synthase was isolated (section 4.2.2.5) and assayed (section 4.2.2.6) at the interval of 4-days i.e. 0-d, 4-d, 8-d, 12-d, 16-d and 24-d respectively from the flowering or fruiting stages with the inception of bud/fruit. Results of such experiments are depicted in Figure 2.8. It is evident that this enzyme operates maximally at 12-d stage of seed maturation. Sharp decline in enzymic activity was recorded either before or after 12-d stages of seed maturation.

2.5.6. Detection of Fructose-1, 6-bisphosphatase Activity in the Leaves of *Sechium edule*.

It will be apparent from the foregoing literature review that although enzymatic hydrolysis of fructose-1, 6-bisphosphatase in connection with the photosynthetic function (during operation of the Calvin cycle), by the specific phosphohydrolase, fructose-1, 6-bisphosphatase has been documented among varied members of the plant kingdom ranging from unicellular algae to angiosperms, detection of this enzymic activity as one of the important criteria of photosynthetic efficiency becomes obligatory while some one is interested to look after this in a specific plant grown under certain environmental condition. This is exactly what has been aimed at the onset of the experimental work with *Sechium edule*.

Using dialyzed homogenate and low-speed supernatant separately from the leaves as a whole (section 2.4.2.7) and also from the isolated chloroplasts (section 2.4.2.9), the activity of $FruP_2$ase was screened following the method as described in section 2.4.2.8.

Table 2.1. depicts the results of such a survey. As evident from Table 2.1., the activity of $FruP_2$ase was appreciable in both the sources. In case of the whole leaves, the specific activity of the homogenate fraction (total extract of leaves) was comparatively higher than the low-speed supernatant fraction (total cell-free extract of leaves) obtained from the same source. In contrast, greater $FruP_2$ase activity was recorded in low-speed supernatant (total soluble extract of chloroplasts) fraction than the homogenate fraction (total extract of chloroplasts) while isolation followed by $FruP_2$ase assay were confined on chloroplasts only as the source.

Table 2.1. Fructose-1, 6-bisphosphatase activity in juvenile leaves of *Sechium edule*. Specific activity defined as n mole P_i released per hour per milligram protein

Enzyme source extracted	Total protein activity	Specific
Total extracts of leaves	1.874 (mg/g. fresh weight)	240.20
Total cell-free extract of leaves	1.215 (mg/g fresh weight)	163.98
Total extract of chloroplasts	0.762 (mg/ml chloroplast)	316.26
Total soluble extract of chloroplasts	0.554 (mg/ml chloroplast)	384.25

2.5.7. Identification of Isolated Chloroplasts from the Leaves of *Sechium edule*.

As it had been revealed from the fundamental results of section 2.5.6. about the higher $FruP_2$ase activity in homogenate fraction of the total leaves along with the evidence of lower $FruP_2$ase activity in homogenate fraction of the isolated chloroplasts than its subsequent low-speed supernatant fraction.

In a series of experiments the Hill activity was separately carried out with the isolated chloroplast suspension, suspension of 500g pellet and 2,500g supernatant, in order to find out the reality of major confinement of such activity in isolated chloroplasts only. Such experimental results are depicted in Table 2.2. Table 2.2. also shows the total content of chlorophyll which has been expressed in terms of milligram chlorophyll per gram fresh weight of leaves and also in terms of milligram chlorophyll per 0.1 ml suspension of the experimental fraction(s).

A clear assessment can be made from Table 2.2. that notable Hill activity is only confined to isolated chloroplast preparation as evident from the reduction in dye colour

under illuminated condition only. However, the other two fractions (the suspension of 500g pellet and the 2,500g supernatant) also exhibit a little amount of Hill activity which may be contributed by some phastidial green pigments during processing of isolation as contaminant an expected idea regarding the probable existance of a membrane - bound $FruP_2$ase or more likely the occurrence of the chloroplastic $FruP_2$ase in connexion with the photosynthetic function could be anticipated. Therefore, the identification of the isolated organelles as chloroplasts was launched.

The purity of the preparation (isolated chloroplasts) was tested only on the basis of a fundamental observation under the compound light microscope. A homogenous mass of green - coloured bead-like strucures was found there (data not shown). In addition to it, the sanctity of identification of this preparation was principally relied on the efficiency of the preparation to carry out the characteristic Hill reaction along with the estimation of the total chlorophyll content from the same batch of isolated chloroplasts as described in section 2.4.2.9.2.

The variation in degree of the total chlorophyll content in all experimental fractions does not protest the aim of such experiment. Therefore, the Hill activity and the total chlorophyll content of a specific fraction is directly proportional.

2.5.8. Partial Purification of Photosynthetic Fructose-1, 6-bisphosphatase from the Isolated Chloroplasts of *Sechium edule*.

Partial purification of photosynthetic Fructose-1, 6-bisphosphatase was made from the isolated chloroplasts of *Sechium edule* by the methods as described in section 2.4.2.10.

Table 2.3 shows the outlines of the purification. As apparent, the enzyme could only be purified to about 16-fold over the homogenate. The yield or recovery of the enzyme based on total activity was about 15%, although based on protent content it was found to be 0.92% only. During the purification of *Sechium edule* photosynthetic $FruP_2$ase, it has been revealed that the enzymic activity increases almost consistantly.

Table – 2.2. Demonstration of Hill activity and the total content of chlorophylls in isolated chloroplasts, 500g pellet-suspension and 2,500g supernatant fractions of *Schium edule*.

Fraction	Tube No.	DCIP Solution (ml)	Suspension of the exprimental fraction (ml)	H_2O (ml)	Na-dithionite crystals	Experimental condition	Initial O.D. (520nm)	Final O.D. (520nm)	O.D. (520)	Total chlorophyll content	
										(mg) chl. 0.1 ml suspension)	(mg chl ./gmf.w.
	1	9.9	0.0	0.1	-	Light	0.54	0.55	0.00		
Isolated chloroplast suspension	2	9.9	0.1	0.0	-	Dark	0.68	0.64	0.04		
	3	9.9	0.1	0.0	-	Light	0.71	0.46	0.25	0.263	2.63
	4	9.9	0.1	0.0	+	Light	0.11	0.10	0.00		
500g Pellet-suspension	1	9.9	0.0	0.1	-	Light	0.52	0.50	0.02		
	2	9.9	0.1	0.0	-	Dark	0.81	0.76	0.05	0.067	0.67
	3	9.9	0.1	0.0	-	Light	0.80	0.72	0.08		
	4	9.9	0.1	0.0	+	Light	0.10	0.14	0.02		
2.500g Supernatant	1	9.9	0.0	0.1	-	Light	0.55	0.55	0.00		
	2	9.9	0.1	0.0	-	Dark	0.61	0.58	0.03	0.026	0.26
	3	9.9	0.1	0.0	-	Light	0.64	0.58	0.06		
	4	9.9	0.1	0.0	+	Light	0.08	0.10	0.00		

Table 2.3. Partial purification of photosynthetic (chloroplastidial Fructose-1, 6-bisphosphatase)from isolated chloroplasts of *Sechium edule*

Purification steps	Total volume (ml)	Protein concen-tration (mg/ml)	Total protein (mg)	Specific activity (n mole P_i released/ hr./mg protein)	Total activity (n mole P_i released/ hr.)	Yield (%)	Fold purifica-tion
1. Homogenate	16.50	0.204	3.366	328.42	1105.46	100.00	1.00
2. 10K supernatant	13.45	0.183	2.461	364.50	897.03	81.14	1.10
3. Streptomycin sulfate fraction	12.80	0.167	2.137	358.64	766.41	69.33	1.09
4. 0-80% $(NH_4)_2SO_4$ fraction	5.0	0.370	1.850	388.93	719.52	65.09	1.18
5. DEAE-cellulose fraction	6.0	0.021	0.126	3111.44	392.04	35.46	9.47
6. Sephadex G-200 fraction	2.25	0.014	0.031	5247.34	162.66	14.71	15.97

The table represents a typical partial purification obtained from isolated chloroplasts (4.5 ml) of *Sechium edule* leaves (100 g).

2.5.9.Fundamental Characteristics of Photosynthetic Fructose-1, 6-bisphosphatase from *Sechium edule* :

The active Sephadex G-200 fractions were pooled together and used for enzymatic characterization.

2.5.9.1. Stability :

The chloroplastidial form of $FruP_2$ase obtained from *Sechium edule* is appreciably stable in its catalytic activity. Stability varies with the enzyme preparation at different stages of purification. While the 10 K supernatant remains active for 10 to 15 days or so when stored at 0°C, the Sephadex G-200 fractions are found to be stable with insignificant loss of activity only upto 7 to 8 days. However, repeated freezing and thawing causes loss of activity. Addition of an enzyme stabilizer, 2-mercaptoethanol, clearly increases the stability of the enzyme.

2.5.9.2. Requirements for Photosynthetic Fructose-1, 6-bisphosphatase of *Sechium edule* :

Photosynthetic *Sechium edule* $FruP_2$ase when assayed in presence of 100 mM Tris-HCl buffer (pH 7.5), 5 mM $MgCl_2$, 0.2 mM $FruP_2$, 0.1 mM EDTA and an appropriate protein aliquot referred to as the complete set recorded optimal activity (Table 2.4). In absence of the substrate ($FruP_2$), no enzymatic activity was detected. About 50% of the activity was lost when the alkaline buffer was ommitted from the complete reaction mixture. Ommission of Mg^{++} reduced the activity to about 24%. Lack of EDTA, *in vitro*, at a very low concentration (0.1 mM) was responsible for about 16% retardation in $FruP_2$ase activity. No enzymic activity was recorded when a heat-killed enzyme replaced the active one (Table 2.4).

Table 2.4.Requirement of *Sechium edule* photosynthetic Fructose-1, 6-bisphosphatase activity.

Assay Condition	Specific activity (n mole P_i released/hr./mg protein)	Percent activity
1. Complete set	4936.52	100.00
2. Minus $FruP_2$	0.00	0.00
3. Minus buffer	2541.25	51.47
4. Minus $MgCl_2$	3773.88	76.44
5. Minus EDTA	4165.20	84.37
6. Heat killed enzyme	0.00	0.00

2.5.9.3. Effect of protein concentrations :

Using increasing concentrations of particulate enzyme protein (27 to 216 ug), $FruP_2$ase assay was carried out under optimal conditions as described in section 2.4.2.8. The results are presented in Figure 2.9. As evident, the enzyme activity increases linearly with respect to protein concentration of about 135 ug (Figure 2.9).

2.5.9.4. Progress of enzymic reaction with time :

Chloroplastic *Sechium edule* Fructose-1, 6-bisphosphatase incubation was carried out for different time period under optimal assay conditions. It is observed that the biochemical reaction proceeds linearly with time up to 90 minutes (Figure 2.10).

2.5.9.5. Temperature sensitivity :

In order to find out the relative enzyme activity as a function of incubation temperature, the particulate form of the enzyme, $FruP_2$ase, obtained from *Sechium edule* was incubated separately for one hour at temperature between the range of 0°C to 60°C at an interval of 10°C in presence of standard assay mixture. Results presented in Figure 2.11 show that the activity of the photosynthetic $FruP_2$ase was insignificant respectively at 0°C and 60°C - the two extremes. However, the enzyme was significantly active between the temperature range of 20°C to 40°C with a temperature optima at 30°C (Figure 2.11).

2.5.9.6 Substrate specificity :

The partially purified *Sechium edule* photosynthetic $FruP_2$ase has been found to specifically utilize D-fructose-1, 6-bisphosphate as the substrate for the production of D-fructose-6-phosphate and inorganic phosphate. Enzyme assay was carried out according to the procedure described in section 2.4.2.8. i.e., in all experimental sets the value of D-fructose-6-phosphate hydrolysis (if any by the presence of concomitant non-specific phosphatase) was corrected as control. In this experiment, among the other phosphate-esters of sugars, either pentose, hexose or triose, used in place of D-fructose-1, 6-bisphosphate and at the same concentration, all were insignificantly effective as substrate for the photosynthetic $FruP_2$ase (Table 2.5).

Table 2.5. Substrate specificity of photosynthetic *Sechium edule* Fructose-1, 6-bisphosphatase activity

Substrate	Concentration (mM)	Specific activity (n mole P_i released/ hr./mg protein)	Percent activity
D-fructose-1, 6-bisphosphate	0.2	5043.50	100.00
D-ribulose-1, 5-bisphosphate	0.2	103.67	2.05
D-glucose-6-phosphate	0.2	45.18	0.89
beta-glycero-phosphate	0.2	62.73	1.24

2.5.9.7. Effect of substrate concentration on chloroplastidial form of *Sechium edule* Fructose-1, 6-bisphosphatase activity and determination of K_m and V_{max} values :

Between a concentration range of 0.0 to 1.0 mM of D-fructose-1, 6-bisphosphate (along with the control sets of identical concentrations of D-fructose-6-phosphate for correction of nonspecific phosphatase activity, if any), the activity of partially purified (active Sephadex G-200 fraction) chloroplastic *Sechium edule* $FruP_2$ase was found to increase with respect to $FruP_2$ concentration up to 0.25 mM. Thereafter, the enzymic activity remained more or less unchanged up to 0.45 mM. However, the enzyme activity was considerably decreased at higher concentrations of $FruP_2$ (Figure 2.12).

The apparent K_m for D-fructose-1, 6-bisphosphate of chloroplastic *Sechium edule* was calculated to be approximately 1.11×10^{-4} M in accordance with the kinetic equation of Michaelis-Menton. The apparent V_{max} value was calculated as 57.6×10^{-8}M under experimental condition.

2.5.9.8. Photosynthetic Fructose-1, 6-bisphosphatase activity as function of pH :

The activity of chloroplastidial form of *Sechium edule* $FruP_2$ase was remarkably influenced by the pH variation even when the partially purified enzyme preparation has been used. This was determined under standard assay condition by using 100 mM Tris-HCl buffer between the pH range 6.0 to 9.5 at an pH interval of 0.5..

From the results depicted in Figure 2.13 it is evident that the chloroplastic enzyme operates maximally at an alkaline pH range between 7.5 to 9.0, having an optima at pH 8.0. Sharp decline in catalytic activity was recorded either below pH 7.5 or above pH 9.0 (Figure 2.13).

2.5.9.9. Effect of Monovalent Cations :

The activity of partially purified chloroplstidial form of $FruP_2$ase isolated from *Sechium edule* was not remarkable dependent upon addition of any monovalent cation tested at least up to 5 mM concentration. The enzyme activity as assayed in presence of three monovalent cations namely potassium, sodium and ammonium separately along with a control set. In this experiment, $MgCl_2$, one of the ingredients of the standard assay mixture was eliminated during enzymic incubation.

Using 5 mM concentration of these salts (with chloride anion), $FruP_2$ase activity exhibited minor variations. Among these three, NH_4^+ was partly inhibitory on this enzyme. The results are presented in Table 2.6.

2.5.9.10. Effect of Divalent Cations and Heavy metals :

Effect of some divalent cations and heavy metals were studied using 5 mM concentration of chloride salts of Mg^{++}, Mn^{++}, Ca^{++}, Fe^{++}, Zn^{++}, Cu^{++} and Hg^{++}. Partially purified chloroplastic form of *Sechium edule* $FruP_2$ase was incubated in presence of 5 mM concentration of individual divalent cations/heavy metals as mentioned, to the usual assay mixture components, keeping one control set without adding any divalent cation even the $MgCl_2$ as one of the assay ingredients.

Table 2.6. Effect of Monovalent cations on Chloroplastidial form of Fructose-1, 6-bisphosphatase activity obtained from *Sechium edule*.

Cation	Concentration (mM)	Specific activity (n mole P_i released/ hr./mg protein)	Percent activity
Control	0	4732.15	100.00
Na^+	5	4381.05	92.58
K^+	5	4885.00	103.23
NH_4^+	5	3962.23	83.73

Table 2.7. Effect of Divalent cations and Heavy metals on Chloroplastidial form of Fructose-1, 6-bisphosphatase activity obtained from *Sechium edule*.

Cation	Concentration (mM)	Specific activity (n mole P_i released/ hr./mg protein)	Percent activity
Control	0	4732.15	100.00
Ng^{++}	5	5402.50	114.16
Mn^{++}	5	4816.27	101.77
Ca^{++}	5	4864.92	102.80
Fe^{++}	5	4566.50	96.49
Zn^{++}	5	4109.00	86.83
Cu^{++}	5	3450.73	72.92
Hg^{++}	5	2813.46	59.45

Results presented in Table 2.7. exhibit different effects of divalent cations or heavy metals. Mn^{++}, Ca^{++}, Fe^{++} exhibited no significant effect on the enzymatic activity. However, Mg^{++} was a stimulator. Heavy metals had shown variable degrees of inhibition in enzymic

activity. Among these, Hg^{++} could be considered as the most powerful inhibitor (Table 2.7.).

2.5.9.11 Effect of EDTA :

Chelating agent like ethylenediaminetetraacetic acid (EDTA) had a very significant role on chloroplastic form of *Sechium edule* $FruP_2$ase activity.

When tried between a concentration range of 0 to 100 mM, EDTA had a very significant role of inhibition on enzymic activity in a concentration dependent manner (Figure 2.14).

2.5.10. Identification of chloroplastic Fructose-1, 6-bisphosphatase as a photosynthetic enzyme in *Sechium edule* :

In plant systems, two types of $FruP_2$ase, one with gluconeogenic function (confined in cytosol) and the other is associated with photosynthetic carbon assimilation process (confined in chloroplast) have been reported (Scala *et al.*, 1968; Buchanan *et al.*, 1967; Bianchetti and Satirana, 1967). It has been revealed from the results of Section 5.1. that the activity is appreciable in leaves of *Sechium edule* with higher titre in the homogenate fraction than the low-speed supernatant fraction while the total leaf-preparation is concerned, in contrast, notable greater $FruP_2$ase activity is recorded in the low-speed supernatant than the homogenate, just opposite, while the enzyme has been experimented only from the isolated chloroplasts of the same plant leaves. From this observation, the existence of a membrane bound $FruP_2$ase can be anticipated easily in relation with the photosynthetic function. However, conclusive identification of this enzyme as photosynthetic one has become essential. According to earlier reports (Buchanan, 1980; Lara *et al.*, 1980), photosynthetic $FruP_2$ase activity is enhanced by illumination, a condition without any effect on the gluconeogenic $FruP_2$ase. Again, another condition, gluconeogenic $FruP_2$ase activity is repressed by additional concentration of glucose which has no such significant effect on photosynthetic enzyme, established earlier by Bianchetti and Satirana (1967) etc. Thus, with these information as working hypotheses in our hand, attempts have been made to identify the plastidial enzyme with respect to its actual function

by exploiting two probes viz. using light/dark or dark growth conditions and by treatment of the plants with externally administered glucose.

2.5.10.1. Plastidial Fructose-1, 6-bisphosphatase activity of *Sechium edule* : Effect of alternate light/dark and dark growth conditions.

Two freshly collected 23-day old seedlings of *Sechium edule* were washed with distilled water thoroughly and then separately placed these in two conical flasks containing distilled water. One of these experimental sets was kept in presence of alternate light/ dark period of about 12 hours each (Temperature : 15-18°C). Sufficient fluorescent light was used to maintain the light period. The second experimental set was kept under continuous darkness (Temperature : 15-18°C). This treatment was done for 7 days.

After 7 days of treatment, plastids were isolated separately from the green leaves (plants grown under alternate light/dark condition) and also from the etiolated (plants grown under complete darkness) leaves of the respective plants according to the procedure as described in section 2.4.2.9.2. Using dialyzed low-speed supernatant fractions of the respective plastids, $FruP_2$ase assay was carried out according to the procedure described in section 2.4.2.8. The results are depicted in Table 2.8. It is observed that the $FruP_2$ase activity in plastids isolated from the leaves of dark grown plant is remarkably inhibited in comparison to the plastids isolated from the leaves of plant which has been grown under alternate light/dark condition.

Table 2.8. Chloroplastic Fructose-1, 6-bisphosphatase activity from *Sechium edule* grown under alternate light/dark and continuous dark conditions.

Source of plastids	Growth conditions	Specific activity (n mole P_i released/hr./mg protein)
Leaves of 30-day old seedling	Alternate light/dark (12 hours each) for 7 days	294.58
Leaves of 30-day old seedling	Continuous darkness for 7 days	117.92

2.5.10.2. Plastidial Fructose-1, 6-bisphosphatase activity of *Sechium edule* : Effect of externally administered glucose.

Freshly collected 23-day old seedlings of *Sechium edule* were washed thoroughly with distilled water and then placed in three different conical flasks in presence of solutions containing 0.0% (control), 2.5% and 5.0% glucose. The experimental sets were kept under normal laboratory condition (Temperature : 15-18°C) for 7 days.

Table 2.9.Chloroplastic Fructose-1, 6-bisphosphatase activity from *Sechium edule* grown under different glucose concentrations.

Source of plastids	Growth conditions (Conc. of Glucose)	Specific activity (n mole P_i released/ hr./mg protein)
Leaves of 30-day old seedlings	Alternate light/dark (12 hours each) in 0.0% glucose (control)	306.24
Leaves of 30-day old seedlings	Alternate light/dark (12 hours each) in 2.5% glucose	297.77
Leaves of 30-day old seedlings	Alternate light/dark (12 hours each) in 5% glucose	318.50

Chloroplasts from leaves of 30-day-old seedlings (under different growth conditions) were isolated as described in text. Isolated plastids were homogenized in 5 volumes of 50 mM Tris-HCl buffer (pH 7.5) containing 0.2 mM 2-mercaptoethanol and the homogenate centrifuged at 10,000g for 15 minutes. The supernatant was assayed for the enzyme activity.

After 7 days of treatment, plastids were isolated separately from the leaves of the respective plants following the method as described in section 2.4.2.9.2. Using dialyzed

low-speed supernatant fractions of the respective plastids, $FruP_2$ase assay was carried out according to the procedure as given in section 2.4.2.8. The results are presented in Table 2.9. Results reveal that no significant change in $FruP_2$ase activity is observed under the influence of increasing concentratin of glucose as the variable growth conditions for the experimental plants.

Chloroplasts from leaves of 30-day-old seedlings (under different growth conditions) were isolated as described in text. Isolated plastids were homogenized in 5 volumes of 50 mM Tris-HCl buffer (pH 7.5) containing 0.2 mM 2-mercaptoethanol and the homogenate contrifuged at 10,000g for 15 minutes. The supernatant was assayed for the enzyme activity.