

PIGMENT CONTENTS AND PHOTOSYNTHETIC ACTIVITY OF  
CHLOROPLASTS ISOLATED FROM BARLEY IRRIGATED WITH  
NUTRIENT MEDIUM PROVIDED WITH SODIUM CARBONATE OR  
SODIUM NITRATE

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ABSTRACT

Barley plants grown in sand culture and irrigated with nutrient medium provided with different concentrations of either sodium carbonate or sodium nitrate showed slight reduction in the different pigment fractions (Chlorophyll a, chlorophyll b and carotenoids), except with the high concentration of sodium carbonate (0.6%) which caused an increase in these fractions. The electron transport capacity of isolated chloroplasts was stimulated at low concentration of sodium nitrate, and inhibited at the higher concentration. On the other hand, all the sodium carbonate concentrations used induced significant stimulation in the photosynthetic capacity of isolated chloroplasts. However, the magnitude of stimulation caused by sodium carbonate was greater than that induced by sodium nitrate. The data obtained, however, suggest that these salts may have two actions on barley

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plants : an inhibitory effect on the pigment biosynthesis, which may be attributed to sodium accumulation, and stimulation of the photosynthetic electron transport capacity of the chloroplasts due to anion effect.

### INTRODUCTION

The ability of plant to carry out various photochemical processes is affected to a large extent by the type of nutrient present in the soil. Although several workers studied the influence of different cations on photosynthesis in vivo, no satisfactory studies are available concerning the effect of the anions present in the soil on the photosynthetic electron transport. However, some studies were carried out in vitro by many workers using suspensions of isolated chloroplasts. They studied the effect of adding anions to a suspension of chloroplasts on their photosynthetic capacity. In this respect several workers studied the effect of chloride on the reactions leading to oxygen evaluation in photosynthesis (Arnon and Whatley 1949 ; Gorham and Clendenning 1952 ; Izawa et al.,1969 ; Kelley and Izawa 1978) . The latter authors reported that the site of action of  $\text{Cl}^-$  involvement seemed to be on the oxidizing side of photosystem II. In addition, the effect of bicarbonate on the photosynthetic electron transport in vitro was studied by many workers (Stemler and Govindjee 1973; Govindjee et al. 1976; Metzner 1978 ) . The site of action of this

Delta J. Sci. 12 (3)1988

Anwer et al.

anion was reported to be a matter of controversy. Stemler and Govindjee (1973) and Metzner (1978) concluded that bicarbonate stimulates the charge transfer on donor side of photosystem II, while Govindjee et al. (1976) claimed the involvement of this ion in the reducing side of photosystem II.

The effect of  $\text{NO}_3^-$  on the photosynthetic  $\text{O}_2$  evolution was first observed by Warburg and Neglein (1920) and confirmed later by Osman et al. (1982), who concluded that the site of action of this ion is localized on the donor side of photosystem II. It must be reported that the aforementioned conclusions concerning the anion effect were obtained by adding the anion to a suspension of isolated chloroplasts or thylakoids.

The present study was designed to investigate the effect of addition of two anions  $\text{CO}_3^{2-}$  and  $\text{NO}_3^-$  to the basic nutrient medium of barley on the pigment contents and photosynthetic electron capacity of the isolated chloroplasts.

#### MATERIALS AND METHODS

Grains of barley (Hordeum vulgare L. cv. Giza 121) were soaked in tap water for 24 hours and then kept moistened for 2 days until germination occurred. The growing seedlings were transferred to polyethylene pots containing

Delta J. Sci. 12 (3) 1988

Pigment Contents and Photosynthetic

HCl-treated sand. The plants were irrigated every 3 days either with Hoagland solution (1950) (control) or Hoagland + 0.2 , 0.4 or 0.6% of  $\text{Na}_2\text{CO}_3$  or  $\text{NaNO}_3$ . The accumulated salts were removed by washing the sand several times with tap water every 10 days of treatment. The following measurements were carried out 40 days after cultivation.

(a) Pigment contents

The different fractions of leaf pigments (chlorophyll a, chlorophyll b and carotenoids ) were determined spectrophotometrically according to the method described by Metzner et al., (1965).

(b) Chloroplast isolation and electron transport measurements

Isolation of chloroplasts was carried out following the procedure described by Osman et al., (1982). Leaves were detached from plants, rinsed in cold tap water , wiped thoroughly and preseved for 1 hour in refrigerator at 4°C. Ten g of these leaves were shredded and homogenized for 15 seconds in a blender, using cold isolation buffer which contained 0.4 M sucrose, 20 mM HEPES-KOH (pH 7.8), 3 mM  $\text{MgCl}_2$ , 4mM sodium ascorbate and 0.1% bovine serum albumin. The mash filtered through 3 layers of gauze cloth. The suspension was centrifuged under cooling for <sup>1</sup> minute at 3000 x g. The pellet was resuspended in 20 ml of suspension buffer which contained the same components of the isolation

Delta J. Sci. 12 (3)1988

Anwer et al.

buffer without serum albumin and centrifuged again at 300 x g for 5 minutes. The supernatant was then centrifuged for 10 minutes at 1000 x g . The sediment containing the chloroplasts was resuspended in 2 ml of the suspension buffer. The centrifuge tube containing the chloroplasts was placed in an ice bath for 2 hours. The chlorophyll content (chlorophyll a + chlorophyll b ) of the chloroplasts was determined . The photosynthetic activity of the isolated chloroplasts was measured using 0.5 mM potassium ferricyanide as electron acceptor. The reaction mixture contained 0.2 ml chloroplast suspension, 3.8 ml suspension buffer (pH 7.8) and 0.5 mM potassium ferricyanide. The mixture was illuminated by means of a slide projector provided heat protection filter. The light intensity at the sample level was  $300 \text{ W m}^{-2}$ . The reduction of potassium ferricyanide, which represents the photosynthetic electron capacity, was followed spectrophotometrically at 420 nm at room temperature as described by Arnon and Shavit (1963)

### RESULTS AND DISCUSSION

The data presented in Table 1 show that addition of different concentrations of  $\text{NaNO}_3$  to the basic nutrient medium slightly reduced the amount of the different pigment fractions (chlorophyll a, chlorophyll b and carotenoids). The highest magnitude of reduction was observed at the 0.6% concentrations (it must be taken into consideration that nitrate was added in the presence of the original source

Delta J. Sci. 12 (3)1988

## Pigment Contents and Photosynthetic

of nitrogen component in the Hoagland solution). The results show no significant change in Chl a/Chl b ratio in response to  $\text{NaNO}_3$  treatments. This may indicate that the magnitude of reduction in both chlorophylls (a and b) in response to  $\text{NaNO}_3$  treatment was the same. On the other hand, low and moderate concentrations of  $\text{Na}_2\text{CO}_3$  (0.2 and 0.4%) reduced the pigment contents, whereas the higher concentration (0.6%) induced a considerable increase (about 42%) of the pigment contents. Unlike  $\text{NaNO}_3$ , only the low concentration of  $\text{Na}_2\text{CO}_3$  caused a decrease in Chl a/Chl b ratio, whereas no appreciable change was observed with the higher concentrations. However, the inhibitory effect of these salts on the pigment biosynthesis may be attributed to the toxic action of the accumulated  $\text{Na}^+$  rather than to anion effect. It has been demonstrated (data not cited) that  $\text{Na}^+$  content of shoot was increased from 422  $\mu\text{eq } 100 \text{ g}^{-1}$  dry weight to 722  $\mu\text{eq } 100 \text{ g}^{-1}$  dry weight when either 0.6%  $\text{NaNO}_3$  or 0.6%  $\text{Na}_2\text{CO}_3$  was added to the basic nutrient medium. Thus, the stimulatory effect of high concentration of  $\text{Na}_2\text{CO}_3$  on the pigment biosynthesis indicates that the toxic action of sodium was counteracted at the high concentrations of  $\text{CO}_3^{2-}$ .

The data presented in Table 2 indicate that addition of low concentration of  $\text{NaNO}_3$  (0.2%) to the basic nutrient medium increased the photosynthetic capacity of the isolated

Delta J. Sci. 12 (3) 1988

Anwer et al.

chloroplasts, whereas higher concentrations were inhibitory. On the other hand, low and moderate concentrations (0.2 and 0.4%) of  $\text{Na}_2\text{CO}_3$  induced significant stimulation in the photosynthetic capacity. Thus, 2.4- and 2.3-folds stimulations were recorded in response to treatment with 0.2% and 0.4%  $\text{Na}_2\text{CO}_3$  respectively. Higher concentration of  $\text{Na}_2\text{CO}_4$  caused a slight increase in this capacity. However, it seems surprising that the decrease in chlorophyll content induced by these salts is concomitant with the increase in the photosynthetic capacity of the isolated chloroplasts. This may be attributed to two different actions of these salts on the biochemical processes in plant : one of them is the retardation of pigment biosynthesis as a result of the toxic action of sodium while the other is stimulation of the photosynthetic electron transport due to anion effect. Concerning the effect of nitrate on the reactions leading to  $\text{O}_2$  evolution in photosynthesis, it has already been demonstrated that  $\text{NO}_3^-$  activates the charge transfer on the oxidizing side of photosystem II (Osman et al., 1982). However, Losada and Guerrero (1979) suggested that the stimulation of  $\text{O}_2$  evolution in response to  $\text{NO}_3^-$  is exclusively due to an acceleration of nitrate reduction. Thus, it could be concluded that nitrate has double function in plants : one is the stimulation of  $\text{O}_2$  evolution in photosynthesis and the other is its utilization as a nitrogen source.

Concerning the inhibitory effect of  $\text{NO}_3^-$ , Stemler and Murphy (1985) found that  $\text{O}_2$  evolution from maize chloroplasts using ferricyanide and 2,6-dimethylbenzoquinone as electron acceptors was inhibited by 80% when 20 mM nitrate was present. However, the inclusion of 5 mM  $\text{NaHCO}_3$  in the bathing medium decreased the level of inhibition produced by  $\text{NO}_3^-$  to only 27% and it was concluded that  $\text{NO}_3^-$  caused this inhibition by occupying the  $\text{HCO}_3^-$  binding site on photosystem II.

Regarding the stimulative effect of  $\text{CO}_3^{2-}$  on the electron transport capacity of the chloroplast, no literature is available to the authors to indicate the direct involvement of this ion in the photosynthetic electron transport. However, several studies have indicated the importance of  $\text{HCO}_3^-$  as effective ion in the reactions leading to  $\text{O}_2$  evolution in photosynthesis (Stemler and Govindjee 1973, Govindjee et al., 1976, Jursinic et al., 1976, Govindjee Khanna .. 1977 ). Moreover, Blubough and Govindjee (1986) reported that  $\text{HCO}_3^-$  ion not  $\text{CO}_2$ ,  $\text{H}_2\text{CO}_3$  or  $\text{CO}_3^{2-}$  is the species that stimulates electron transport of photosystem II. Since it is well known that the equilibration between carbonic species ( $\text{CO}_2 + \text{H}_2\text{O} \longleftrightarrow \text{H}_2\text{CO}_3 \longleftrightarrow 2\text{H}^+ + \text{CO}_3^{2-}$ ) is pH-dependent. Thus, the absorbed  $\text{CO}_3^{2-}$  from the basic nutrient medium may be transformed to  $\text{HCO}_3^-$  under the influence of pH value of the cell sap before the induction



Delta J. Sci. 12 (3)1988  
Anwer. et al.

of its effect.

Accordingly, the observed stimulatory effect of  $\text{CO}_3^{2-}$  in the present study may be attributed to its transformation into  $\text{HCO}_3^-$ .

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Delta J. Sci. 12 (3)1988

Anwer et al.

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Table 1 : Influence of different concentrations of  $\text{NaNO}_3$  and  $\text{Na}_2\text{CO}_3$  (added to the basic nutrient solution) on the different pigment fractions (chlorophyll a, chlorophyll b, carotenoids and total pigment) of barley. Values are means of 4 determinations and calculated as  $\text{mg g}^{-1}$  dry weight.

Treatment	Chloro- phyll a	Chloro- phyll b	Caroten- oids	Total pigment	Chl. a/b
Hoagland	9.7	4.6	2.8	17.1	2.1
" + 0.2% $\text{NaNO}_3$	9.0	4.2	2.5	15.7	2.1
" + 0.4% $\text{NaNO}_3$	9.7	4.5	2.6	16.8	2.2
" + 0.6% $\text{NaNO}_3$	8.1	4.0	2.1	14.2	2.0
" + 0.2% $\text{Na}_2\text{CO}_3$	8.0	4.4	2.0	14.4	1.8
" + 0.4% $\text{Na}_2\text{CO}_3$	9.3	4.5	2.4	16.2	2.1
" + 0.6% $\text{Na}_2\text{CO}_3$	13.8	6.7	3.7	24.2	2.1

Table 2 : Influence of different concentrations of  $\text{Na}_2\text{CO}_3$  and  $\text{NaNO}_3$  (added to the basic nutrient solution) on the photosynthetic activity of isolated barley chloroplasts. The given values are calculated as  $\mu\text{M}$  ferricyanide reduced  $\text{mg}^{-1}$  chlorophyll  $\text{hour}^{-1}$ .

Treatment	Ferricyanide reduction
Hoagland	6.1
" + 0.2% $\text{NaNO}_3$	8.3
" + 0.4% $\text{NaNO}_3$	4.1
" + 0.6% $\text{NaNO}_3$	2.0
" + 0.2% $\text{Na}_2\text{CO}_3$	14.9
" + 0.4% $\text{Na}_2\text{CO}_3$	14.2
" + 0.6% $\text{Na}_2\text{CO}_3$	6.3

المحتوى الصبغى ونشاط البناء الضوئى للبلاستيدات  
الخضراء المعزولة من نبات الشعير المرورى بوسط غذائى  
مزود بكاربونات الصوديوم أو نترات الصوديوم

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أظهرت نباتات الشعير النامية فى مزرعة رملية رويت بمحاليل غذائية مزودة بتركيزات مختلفة من أى من كاربونات الصوديوم أو نترات الصوديوم اختزالاً طفيفاً فى الاصباغ المختلفة (كلوروفيل ا ، كلوروفيل ب ، الكاروتينويدات ) ، فيما عدا تركيز ٠.٠٠٪ من كاربونات الصوديوم الذى سبب زيادة فى تلك الاصباغ . وعلى العكس من ذلك فإن إضافة تركيبات مختلفة من الاملاح الى الوسط المغذى دفع الى تنبيه قدرة البناء الضوئى للبلاستيدات المعزولة ، ولقد زادت أهمية هذا الاستحاث مع زيادة تركيز الاملاح المضافة . ومع ذلك فإن درجة الاستحاث المتسببة عن كاربونات الصوديوم كانت أكبر من تلك المتسببة عن نترات الصوديوم . وعليه فإن النتائج تقترح أن هذه الاملاح لها تأثيرين على نباتات الشعير : تأثير مباشر على البناء الحيوى للاصباغ والذى قد يعزى الى تراكم الصوديوم ، والتأثير الآخر هو استحاث القدرة على الانتقال الالكترونى الضوئى للبلاستيدات والذى يعزى الى التأثير الانيونى .