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## Changes in Photosynthesis and Respiration of *Hyoscyamus muticus* and *Datura stramonium* in Response to Salinization

By

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### Summary

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The light-fixation of  $^{14}\text{CO}_2$  by *Hyoscyamus muticus* and *Datura stramonium* decreased with the rise of salinization level. The incorporation of the radioactive carbon however into the various metabolic fractions (sugars, lipids, organic acids, amino acids and alkaloids) in both plants varied according to the plant tested as well as the level of salinization used.

The photosynthetic oxygen evolution was also followed under the various treatments. In the case of *H. muticus*, salinized for 20 days, the oxygen evolution was inhibited with the rise of salinization level, but when the plants were salinized for 40 or 60 days, oxygen evolution was markedly enhanced, especially under the moderate salinization levels. In the case of *D. stramonium* the photosynthetic oxygen evolution was generally enhanced with the rise of salinization level, whatever the salinization period applied.

Respiration of *H. muticus*, salinized for 20 or 40 days, was retarded with the rise of salinization level. When salinized for 60 days, however, oxygen consumption was highly enhanced. In *D. stramonium*, the consumption of oxygen was always enhanced with the rise of salinization level whatever the period (20, 40 or 60 days) of salinization used.

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### Zusammenfassung

AHMED A. M. ALI R. M. 1990. Änderungen der Photosynthese und der Atmung von *Hyoscyamus muticus* und *Datura stramonium* unter Salzeinwirkung. – *Phyton* (Horn, Austria) 30 (2): 273–281. – Englisch mit deutscher Zusammenfassung.

Die Fixierung von  $^{14}\text{CO}_2$  durch *Hyoscyamus muticus* und *Datura stramonium* im Licht nahm mit zunehmender Salzeinwirkung ab. Der Einbau von  $^{14}\text{C}$  in die einzelnen Stofffraktionen (Zucker, Lipide, organische Säuren, Aminosäuren und Alkaloide) war jedoch je nach Pflanze und Salzeinwirkung verschieden. Die photosynthetische  $\text{O}_2$ -Entwicklung von *H. muticus* wurde mit steigenden Salzgaben innerhalb 20 Tagen zunehmend gehemmt, nach 40 und 60 Tagen war sie, namentlich bei mäßiger Salzeinwirkung, deutlich erhöht. Bei *D. stramonium* war die  $\text{O}_2$ -Entwicklung in allen Salzkonzentrationen unabhängig von der Einwirkungsdauer erhöht. Salzeinwirkung auf *H. muticus* erniedrigte die Atmung während 20 und 40 Tagen, erhöhte sie aber nach 60 Tagen stark. Bei *D. stramonium* war der  $\text{O}_2$ -Verbrauch durch steigende Salzgaben nach 20, 40 und 60 Tagen erhöht.

### Introduction

Photosynthesis as one of the main physiological processes in plant metabolism was broadly investigated in relation to salinity conditions. It was generally reported that the ionic environment of the chloroplast membranes (thylakoids) is important in the regulation of many physico-chemical reactions involved in the primary Photosynthetic processes (BARBER 1976, NEWTON & al. 1980, KAISER & al. 1981, BLACQUIERE & LAMBER. 1981; DARAS & SHEORAN 1984).

The photosynthetic  $^{14}\text{CO}_2$ -light fixation was also found to be altered by salinity. In glycophytes, salinity exerted a general reduction in carbon fixation, which greatly varied between plant species (see e. g. GALE & POLJAKOFF-MAYBER 1970, AHMED & al. 1977, KAISER & al. 1981, ZAGDANSKA 1984, KRAMPITZ & FOCK 1984). In halophytes and succulent plants, on the other hand, low concentrations of salts did not reduce, and sometimes enhanced the photosynthetic carbon fixation (see e. g. SHOMER-ILAN & WASEL 1973, WINTER 1974, TIKU 1976). However, the distribution of the fixed carbon between the various organic components did not always exhibit the same pattern.

Respiration was repeatedly recorded to be affected by salinization treatments. The increased respiration rates under higher salinities are probably due to the increased energy expenditure in osmotic adjustments to the new environment (LOEBLICH 1982; SCHWARZ & GALE 1984).

On the other hand, the decreased respiration rates which were always associated with a decreased availability of respirable substrates could be due to a decreased rate of photosynthesis and/or increased demand of sugars for the assimilation of osmotic organic solutes (LAMBERS & al. 1983, AHMED & al. 1989).

As far as the literature available, photosynthesis and respiration of the medicinal plants were not intensively studied under salinization treatments inspite of the increasing economical importance of this group of plants. Thus, the aim of the present work was to follow the changes that might take place in photosynthesis and respiration of variously salinized *Hyoscyamus muticus* and *Datura stramonium*.

### Materials and Methods

*Hyoscyamus muticus* and *Datura stramonium* L. were used as test plants in this investigation. Seeds obtained from the University Farm were sown and treated as given by AHMED & al. 1989. Thereafter, tenfold diluted normal Pfeffer's nutrient solution containing various salinization levels (0, 1000, 3000, 5000 and 7000 ppm) was used for irrigation. Salinization was performed by a mixture of NaCl and CaCl<sub>2</sub> (1 : 1 w/w). In order to prevent the accumulation of salts, the soil in each pot was leached every two weeks with an excessive amount of irrigation water. The plants were left to grow under these treatments for 60 days.

Oxygen evolution and dark oxygen uptake were determined using the Warburg manometric technique as recommended by UMBREIT & al. 1959. Chlorophyll content was estimated according to the method recommended by METZNER & al. 1965.

The photosynthetic <sup>14</sup>CO<sub>2</sub> fixation was also followed by the determination of the radioactivity (cpm g<sup>-1</sup> leaf dry weight) after <sup>14</sup>C-light fixation using in principle the technique applied by BASSHAM & CALVIN 1957 and the apparatus described by METZNER (1968). Fresh leaf discs were left to assimilate the radioactive carbon in the light for 10 minutes. Thereafter, these discs were extracted with boiling ethanol and then subjected to fractionation.

Lipid fraction was separated by shaking the ethanol soluble extract with petroleum ether (40–60° C) three times in a separating funnel. Then, the radioactivity of an aliquot of the ether extract was determined using Tracer Lab-G M counter. After removing the ether extract, the ethanol extract was dried at 40° C under vacuum and then taken up in 15 ml distilled water.

Alkaloids were extracted by shaking with 20 ml chloroform containing 0.3 ml of 25% NH<sub>4</sub>OH. The labelling of the lower layer (CHCl<sub>3</sub>) containing the alkaloids was determined.

The upper aqueous layer containing the water soluble metabolites was considered for the separation and determination of radioactive sugars, amino acids and organic acids using ion exchange separation method. Small columns (pyrex tubes, 15–20 cm in length and 7 mm Ø) were, half-filled with ethanol and the appropriate ion exchange resins (DICKSON 1979). The aqueous fraction was pipetted directly into the cation column. The sugars (neutral fraction) were eluted slowly with 80% ethanol.

### Results and Discussion

The amount of photosynthetically evolved oxygen varied according to the level and duration of stress used as well as to the plant tested. In *Hyoscyamus muticus*, the photosynthetic oxygen evolution was generally enhanced but only in plants salinized for 40 or 60 days (Table 1 A). However, after 20 days the oxygen evolution was retarded. This enhancement of

Table 1

Effect of level and duration of salinization on photosynthetic oxygen evolution (P) and dark respiration (R) by leaves of *Hyoscyamus muticus* (A) and *Datura stramonium* (B).  
( $\mu\text{moles O}_2\text{h}^{-1}$  of leaf tissue containing 1 mg chlorophyll.)

| Salinization level (ppm)            | 20 Days   |             |      | 40 Days   |             |      | 60 Days   |             |      |
|-------------------------------------|-----------|-------------|------|-----------|-------------|------|-----------|-------------|------|
|                                     | Oxygen    |             |      | Oxygen    |             |      | Oxygen    |             |      |
|                                     | Evolution | Consumption | P/R  | Evolution | Consumption | P/R  | Evolution | Consumption | P/R  |
|                                     | P         | R           | P/R  | P         | R           | P/R  | P         | R           | P/R  |
| <b>A. <i>Hyoscyamus muticus</i></b> |           |             |      |           |             |      |           |             |      |
| 0                                   | 166.8     | 94.6        | 1.76 | 36.9      | 12.0        | 3.07 | 16.5      | 8.8         | 1.87 |
| 1000                                | 120.5**   | 72.7**      | 1.66 | 46.1**    | 9.4**       | 4.89 | 23.6**    | 13.2**      | 1.79 |
| 3000                                | 67.1**    | 38.3**      | 1.75 | 40.1*     | 5.5**       | 7.23 | 64.7**    | 23.8**      | 2.71 |
| 5000                                | 51.3**    | 23.3**      | 2.20 | 23.7**    | 4.4**       | 5.40 | 57.4**    | 25.2**      | 2.28 |
| 7000                                | 35.5**    | 8.3**       | 4.25 | 25.2**    | 4.1**       | 6.09 | 50.1      | 22.2**      | 2.26 |
| <b>L. S. D.</b>                     |           |             |      |           |             |      |           |             |      |
| 5%                                  | 11.8      | 4.7         | —    | 3.6       | 1.1         | —    | 4.3       | 2.0         | —    |
| 1%                                  | 17.8      | 7.1         | —    | 4.2       | 1.7         | —    | 6.4       | 3.0         | —    |
| <b>B. <i>Datura stramonium</i></b>  |           |             |      |           |             |      |           |             |      |
| 0                                   | 25.18     | 16.89       | 1.49 | 100.53    | 35.18       | 2.86 | 54.29     | 22.99       | 2.36 |
| 1000                                | 44.78**   | 25.06**     | 1.79 | 213.72**  | 44.87**     | 4.76 | 206.79**  | 150.18**    | 1.38 |
| 3000                                | 44.40**   | 30.49**     | 1.46 | 206.58**  | 40.53**     | 5.10 | 266.22**  | 184.30**    | 1.44 |
| 5000                                | 37.99**   | 35.91**     | 1.06 | 195.86**  | 34.53       | 5.67 | 255.06**  | 176.52**    | 1.44 |
| 7000                                | 38.70**   | 36.48**     | 1.06 | 55.72**   | 36.65       | 1.53 | 10.00**   | 4.51**      | 2.22 |
| <b>L. S. D.</b>                     |           |             |      |           |             |      |           |             |      |
| 5%                                  | 3.80      | 2.60        | —    | 4.88      | 2.77        | —    | 12.45     | 10.17       | —    |
| 1%                                  | 5.86      | 3.93        | —    | 7.38      | 4.15        | —    | 18.85     | 15.40       | —    |

oxygen evolution after salinization for 40 or 60 days indicates that after a period of salinization, *H. muticus* plants could be adapted to stress conditions. This is further supported by the observation that after 20 days of salinization, the retardation of oxygen evolution was exerted by all salinization levels; after 40 days, retardation was exerted by only the two highest levels of salinization and after 60 days, no retardation was observed.

In *Datura stramonium*, an enhancement in oxygen evolution was mostly recorded in salinized plants, whatever the duration of stress applied (Table 1 B). Therefore, it can be said that the oxygen evolving system in *D. stramonium* exhibited its adaptation to salinization earlier than in *H. muticus*.

A stimulation of oxygen evolution of some plants, after being subjected to salinization treatments was also recorded by some authors (BEN-AMOTZ &



AVRON 1973, LOEBLICH 1982). Some other authors mentioned that salinization reduced oxygen evolution but in proportion to salt concentration (see e. g. BARBER 1976; KAISER & al. 1981; BLACQUIERE & LAMBERS 1981, DARAS & al. 1984).

Respiration (dark oxygen uptake) was also followed in variously salinized plants. In *H. muticus*, when salinized for 20 or 40 days, oxygen consumption was lowered with the rise of the salinization level (Table 1 A). However, when it was salinized for 60 days, respiration was promoted with the rise of salinization level. These results are in concomitance with those obtained with photosynthesis, that *H. muticus* plants could adapt themselves to salinization after 60-day treatment.

In *D. stramonium*, an increase in respiration was generally recorded, whatever the duration of stress applied (Table 1 B). This increase in respiration could be due to energy consumption in adaptation or adjustment, which is also in accordance with the above results obtained with photosynthesis. Salinization treatments were also reported to reduce respiration (LAMBERS & al. 1983, KRAMPITZ & FOCK 1984) or to enhance it (FRANK & WEGMANN, 1974; SCHWEAR & GALE 1981, 1984, LOEBLICH 1982, DARAS & al. 1984).

Photosynthesis/respiration ratio which could be regarded as an indicator of growth and dry matter accumulation was also variably affected by salinization treatments. In *H. muticus*, the values of this ratio mostly increased with the rise of salinization level, whatever the duration of stress applied. In salinized *D. stramonium*, the values of this ratio were not markedly altered. This could be due to the combined enhancement of photosynthesis and respiration.

The photosynthetic  $^{14}\text{C}$  fixation by *H. muticus* and *D. stramonium* plants was considerably retarded with the rise of salinization level (Table 2). This retardation is in agreement with the results obtained by some other authors using other plants (GALE & POLJAKOFF-MAYBER 1970, LAPINA & BIKMMUKHAMETOVA 1972, AHMED & al. 1977, 1979, KAISER & al. 1981, ZAGDANSKA 1984, KRAMPITZ & FOCK 1984). This lowered  $^{14}\text{C}$ -fixation by the test plants, which is not in agreement with the enhanced  $\text{O}_2$  evolution, indicates that the two photosynthetic reactions (light and dark reactions) respond variably to salinization stress. While the oxygen evolving process was promoted or at least unaffected,  $\text{CO}_2$  fixation process was retarded.

The fixed radioactive carbon was fractionated, and given as absolute counts and calculated as percentage of control or as percentage of recovered radioactivity of the corresponding treatment. It can be seen that in both plants, the labelling of alkaloids, when regarded as absolute counts or as percentage control, was steadily raised with the rise of salinization level. The other fractions were differentially labelled. In salinized *H. muticus* (Tab. 2 A), the labelling of organic acids, sugars and lipid fractions exhibited relatively lower values in comparison with those of non salinized control plant. The labelling of amino acids increased with the increase of

salinity till to the level of 3000 ppm and then decreased again. In salinized *D. stramonium* (Tab. 2 B), the labelling of amino acids, organic acids and sugar fractions exhibited mostly lower values in comparison with those of control plant. The labelling of lipids increased with the increase in salinity till to the level of 3000 ppm and then decreased again.

On the other side, when the labelling values of these fractions were calculated as percentages of the corresponding total labellings recovered after fractionation, they exhibited variable results. The percentage labelling of each of lipids, organic acids and amino acids remained more or less unchanged. Those of sugars steadily decreased with the rise of salinization level, while those of alkaloids steadily increased reaching their maxima at the highest level used. In other words, the efficiency of carbon assimilation into lipoids, organic acids and amino acids was not drastically altered under salinity stress. That of carbon assimilation into sugars was considerably retarded while that of carbon assimilation into alkaloid was considerably promoted. This means that there is a differential response in the incorporation of the fixed carbon into the various organic fractions according to the level of salinity and the fraction analysed as well as the plant tested. Such differential response in labelling of the various organic compounds to salinity was also recorded by some other authros (PLAUT 1971, PLAUT & BRAVDO 1973, AHMED & al. 1977, KAISER & al. 1981, KRAMPITZ & FOCK 1984, ZAGDANSKA 1984).

Finally, it can be said that as far as the parameters tested, the two salinized plants can grow either as or better than the control plant. In addition, the alkaloid synthesis was markedly promoted. In general, *H. muticus* could adapt itself to salinization stress better than *D. stramonium* did.

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## Recensio

Naturkundliche **Arbeitsgemeinschaft** des Bezirkes Scheibbs und Akademie für Umwelt und Energie in Laxenburg. 1989. **Auf Clusius' Spuren.** Wanderungen durch die Natur des Ötscherlandes. – In: *Naturkunde des Bezirkes Scheibbs: Bildband 1.* – 4°, 399 Seiten, 561 Photos; Ln. – Verlag R. u. F. Radinger, A-3270 Scheibbs. – ISBN 3-900974-12-8.

Das Ötscherland liegt ganz im Osten der Alpen im südwestlichen Niederösterreich und ist ein Teil der Nordöstlichen Kalkalpen. Um es gleich vorwegzunehmen, ein Werk über die Reisen des CLUSIUS ist dieser Band nicht, dem berühmten Forscher ist als eine Art Vorwort nur etwa eine halbe Seite gewidmet.

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