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RESEARCH ARTICLE

Dark Incubation Induced Alterations in the Primary Reactions of Photosynthesis in Maize Primary Leaves.

Srikanth Burra*, and S Suma.

Department of Biochemistry, Chaitanya (Deemed to be University), Warangal, Telangana, India.

ABSTRACT

Dark incubation of primary leaves of maize exhibited loss mainly in the chlorophyll *a* content when compared to that of chlorophyll *b* content. 96 h of dark incubation caused change in the chlorophyll-*a*/ratio from 2.7 to 3.4 indicating the chlorophyll *a* is primary target. Similarly there is a 52% loss in carotenoid content after 96 h of incubation. The total protein content measurement clearly demonstrated that there is a loss of 64% during dark incubation. Electrons transport activity exhibited maximum loss in photosystem II activity only after 96th of dark incubation and between the photosystem I and II, PS I catalyzed electron transport is less sensitive to aging process.

Keywords: Chlorophyll, Electron transport, Maize plants, Senescence

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Corresponding author

INTRODUCTION

Senescence is a complex and highly regulated developmental phase in the life of leaf where a co-ordinated degradation of macromolecules and subsequent mobilization of components to the other parts of the plants [1,2]. In green leaves chloroplasts are usually the first cell organelles which show the changes in qualitative as well as quantitative [3,1,4]. During foliar senescence, the loss in photosynthetic pigments is one of the most visible most visible phenomena [5]. Photochemical activities limit photosynthesis during senescence [6,7,8] reported that whole chain, PS II, PS I electron transport activi- ties decline drastically during senescence [9,10]. The greater decline in whole chain elec- tron transport activity was observed and reported due to the changes in two important electron mobile carriers, PQ and PC [11]. Bricker and Newman [12] reported that the rapid decrease of P700 chlorophyll a protein complex during senescence is responsible for the loss of PS I activity in soyabean cotyledons. The loss in PS II activity during leaf senescence has been attributed due to the alterations in oxidizing side [13] or reaction centre [9] or reducing side of PS II [14]. Up to now the studies related to the maize primary leaves are scanty related to the senescence. Therefore in this investigation an attempt has been made to correlate the pigment protein contents with electron transport activities during dark incubation in maize primary leaves.

MATERIALS AND METHODS

Healthy seeds of Maize (Zea mays) were obtained from Acharya N.G. Ranga Agricultural College, Tirupati. The seedlings were grown in dark in 2 days and they were shifted to light (15 Wm^{-2}) and kept at 25 ± 1ºC. 8th day expanded leaf segments were used for experimental purpose. Leaf segments were kept for 96 h. in dark at 25 ± 1ºC in distilled water to Induce senescence. After induction of senescence the experimental parameters were measured for every 24h to analyze both structural and functional aspects. Chlorophyll was estimated with 80% Acetone by following the procedure of Arnon [15]. The total protein content of leaf segments was estimated by following the method of Lowry et al. [16]. Thylakoid membranes were isolated from control and as well as dark incubated samples ad described by Swamy et al. [13]. PS II, PS I mediated and whole chain electron transport activities assays were carried out through polarographycally with clark type O_2 electrode under saturating light intensity of 415 Wm^{-2} at 25 ± 1ºC. According to the Sabat et al. [10] the whole chain electron transport assay mixture contains 2 mL reaction buffer (pH 7.4), 0.5mM MV and 1.0 mM sodium azide and thylakoid membranes equivalent to 40 µg of chlorophyll a. PS II catalyzed electron transport assay mixture contains 2 mL reaction buffer (pH 7.4), 0.5 mM freshly prepared pBQ and thylakoid membranes. PS I catalyzed electron transport assay mixture contained 2 mL of reaction buffer (pH 7.4), 5mM ascorbate, 1mM sodium azide, 0.5 mM MV, 0.1 mM DCPIP, 10µM DCMU and thylakoid membranes.

Table 1: Effect of dark aging on total chlorophyll (Chl a+b), chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid (Car) protein contents in maize primary leaf segments. All parameters were expressed as [mg/g (f.w)]

Dark Incubation (h)	Parameter (mgf.w^{-1}) Chl (a+b)	Chl a	Chl b	Chl a/b	Car	Total Protein
0	2.59±0.10	1.90±0.80	0.69±0.30	2.7	0.060±0.002	25.66±2.00
24	1.99±0.05	1.47±0.04	0.52±0.01	2.8	0.059±0.002	23.70±1.00
48	1.25±0.08	0.94±0.05	0.31±0.02	3.0	0.046±0.001	16.14±0.80
72	0.89±0.08	0.67±0.04	0.20±02	3.3	0.031.002	13.28±0.30
96	0.62±0.04	0.48±0.02	0.14±0.01	3.4	0.028±0.001	8.50±0.50

Table 2: Activities of whole chain electron transport (WCE), [µ moles (O consumed) mg^{-1} (Chl) h^{-1}], PS II [µ moles (O evolved) mg^{-1} (Chl) h^{-1}] and PS I [µ moles (O_2 consumed) mg (Chl) h] at 24 h intervals in maize primary leaf segments during dark incubation

Dark Incubation (h)	Parameter WCE ($H_2O \rightarrow MV$)	PS II activity ($H_2O \rightarrow pBQ$)	PS I activity (DCPIP+Asc→MV)
0	104 ± 7	181 ± 10	493 ± 29
24	80 ± 7	163 ±14	461 ± 25
48	62 ± 4	140 ± 8	430 ± 25
72	39 ± 2	84 ± 7	407 ± 18
96	-	63 ± 4	385 ± 15

RESULTS AND DISCUSSION

After dark incubation of maize primary leaves exhibited loss in various pigment contents. In control (0 h) the total chlorophyll content found to be 2.59 mg/g (f.w). The incubation of the leaves in dark induced the gradual loss in total chlorophyll content up to 74% (Table 1). The loss in chlorophyll during dark incubation could be due to senescence mediated enhancement in the activities of peroxidase and chlorophyllase [17]. The other possible reason could be decreased activities of chlorophyll biosynthetic enzymes as reported by Huckmani and Tripathy [18]. To establish whether chlorophyll a (or) chlorophyll b is prone to the dark incubation, the individual contains chlorophyll a as well as chlorophyll b was made (Table 1). This dark incubation of maize primary leaves caused preferential loss of chlorophyll a (by 74%) chlorophyll b (by 62%). This is also evident from the ratio of chlorophyll a/b. The ratio has been changes from 2.7 to 3.4 indicating that chlorophyll a is more prone for degradation than chlorophyll b. Nevertheless, the level of chlorophyll a content in the PS II complex is for greater than that of PS I complex [19]. Hence the decreasing chlorophyll a during dark aging might affect the PS I chlorophyll a complex. The estimation of carotenoids are showed only 52% loss in the content after 96 h of Incubation (Table 1). Carotenoids absorb light to transfer to neighboring chlorophyll and play an important role in energy dissipation as heat and protect the chlorophylls from free radical damage [20]. This loss in the carotenoid content could be due to enzymatic degradation [21]. After analyzing the pigment contents an attempt has been made to estimate the total protein content in control and dark incubated leaf segments. After 96h of dark incubation there is a 64% decline in total protein content (Table 1). This decline in total protein content could be due to the free radical mediated induction of proteases during dark incubation and disorganization of protein components of thylakoid membrane [22]. According to Roberts et al. [23] differential degradation of thylakoid proteins is the responsible for the loss of photochemical activities during senescence. Therefore, an attempt has been made to measure the whole chain electron transport by using MV as electron acceptor (Table 2). There was a gradual loss in whole chain electron transport activity during dark incubation. 62% loss was noticed in the whole chain electron transport activity after 72 h of dark incubation. This loss in the whole chain electron transport activity could be due to impairment of PS II catalyzed electron trans- port activity was measured (Table 2). There is a progressive loss in the PS II activity. The susceptibility of PS II could arise due to alterations at oxidizing side [13] or reducing side [9]. To establish the relationship between PS II and PS I susceptibility PS I activity has been measured using DCPIP+ Ascorbate as donor. Only 17 loss in PS I activity was noticed after 72 h of dark incubation. Thus, there is a preferential decline in PS II activity when compared to that of PS I activity. The reason for the loss of PS II activity could be partial inactivation of water oxidation complex and depletion of 33 kDa polypeptide.

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