Ovarian cholesterol, glycogen and ascorbic acid concentration during non-breeding and breeding periods

| Biochemical constituents | Non-breeding | Breeding | p < values |
|---|---|---|---|
| Cholesterol (mg/g wet wt) | 3.25 ± 0.472 | 5.35 ± 0.465 | 0.01 |
| Glycogen (mg/g wet wt) | 41.08 ± 3.74 | 74.16 ± 4.40 | 0.005 |
| Ascorbic acid (mg/100 g wet wt) | 20.3 ± 1.75 | 24.8 ± 2.13 | 0.01 |

Values are mean ± SD, n = 10.

cholesterol level in the ovary may be due to less thyroid activity during the breeding period. Cholesterol accumulates under the stimulation of FSH, a hormone required for the development of ova (Lofts[13]). Therefore, it is possible to state that both ovarian development and ovarian cholesterol may be under pituitary control.

The thyroid hormone accelerates the rate of absorption of monosaccharides, and therefore glycogen store of different tissues diminishes as a consequence of glycogenolysis. Hypothyroidism, on the other hand, increases the glycogen content of the liver and muscle (Thapliyal et al.[14]). In the bird under investigation, the thyroid activity is at the lowest during the breeding period (May to July) and the thyroid activity is high during the non-breeding period which is from August to April (Sinha[12]). Therefore, the increase in ovarian glycogen may be due to less glycogenolysis as a consequence of reduced thyroid activity during breeding period.

It is generally accepted that ascorbic acid has a role in the conversion of cholesterol to corticosteroids (Sayers et al.[15]). The concurrent increase of ascorbic acid in the ovary may be due to the increased demand of ascorbic acid for the conversion of cholesterol to steroids and also for the production of mature ova.

The thyroids and ovary are both controlled by hormones of the anterior pituitary. It is possible that thyrotrophic and gonadotrophic functions of the pituitary are antagonistic. The pituitary is affected by temperature; low temperature in winter induces thyrotrophic activity and high temperature during summer induces gonadotrophic activity. In this bird, the high level of thyroxin in winter by itself could have a depressant effect on oogenetic activity.


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## Changes in photoelectron transport of chloroplasts isolated from dark stressed leaves of maize seedlings[1]


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*Summary.* Transfer of light-grown maize seedlings to dark causes a loss in the contents of chlorophyll, protein and RNA of leaves, and a decline in DCPIP photoreduction by isolated chloroplasts. The loss in DCPIP-Hill reaction is attributed to the dark stress-induced damage of $O_2$ evolving system of thylakoid membranes.


Leaf senescence induced by dark stress causes a decline in the contents of total chlorophyll, protein and nucleic acids of the leaves[2-6]. The process brings about sequential loss and ultrastructural modifications of various cellular organelles[5]. Chloroplasts are shown to be the first organelle damaged by leaf aging[5]. The structural changes of chloroplasts induced by dark aging begin with a gradual decay of stroma lamellae followed by unstacking and destruction of grana thylakoids with concomitant appearance of osmiophilic globules[5]. These ultrastructural changes are accompanied by the changes in the various photochemical functions of plastid membranes[5,7,8]. Reports from various laboratories are available on the losses in the efficiency of photoelectron transport[3,7], photophosphorylation[8,9] and carbon dioxide fixation[9] of chloroplasts isolated from dark-induced senescing leaves. Leaf senescence has also been found to cause alteration in optical properties of chloroplasts[10]. However, these age-related changes of chloroplasts have mostly been studied during dark-induced senescence of leaves in detached condition. Literature on the photosynthetic changes during dark-induced senescence of attached leaves is meagre[11]. Secondly, little is known about the precise mechanism of loss in electron transport efficiency of plastid membranes, even though reports on aging induced loss in Hill reactions are well documented[3,7,11]. In the present investigation, an attempt is made to study the mechanism of loss in the efficiency of photosynthetic electron transport of chloroplasts isolated from attached senescing leaves of maize seedlings incubated in continuous dark. The data are compared with the previous results on dark-induced senescence of detached leaves.

*Materials and methods.* Maize seeds (*Zea mays* L. hybrid, Ganga 5) were germinated in petri dishes fitted with water-soaked cotton, and seedlings were kept in beakers with 10 ml of distilled water at 25 °C under continuous illumination (~ 2500 lx). A set of seedlings after 4 days of germina-

tion was transferred to continuous dark. Pigments were extracted in 80% cold acetone and estimated according to Arnon[12]. All experiments were conducted under safe dim green light. The macromolecules of the leaves were extracted as described by Schneider[13], RNA was estimated by orcinol[14] and DNA by diphenylamine[15] methods. Total trichloro-acetic acid insoluble protein was measured by the method of Lowry et al.[16]. Chloroplasts were isolated from the leaves of light- and dark-grown seedlings by the procedure as described earlier[3]. The DCPIP (2,6-dichlorophenol indophenol) reduction by isolated chloroplasts was monitored by the procedure as reported by Biswal and Mohanty[3]. The concentration of $MnCl_2$ and diphenyl carbazide (DPC) in the reaction mixture for DCPIP reduction were 1.6 mM and 0.5 mM respectively. The bovine serum albumin (BSA) was used at the concentration of 0.2%. DPC was prepared freshly everyday in methanol and the concentration of methanol in final volume of 3 ml reaction mixture was 1.6%. Methanol at this concentration had no effect on the DCPIP-Hill reaction. The reaction mixture contained 5 mM phosphate buffer instead of 10 mM when DPC was used as electron donor.

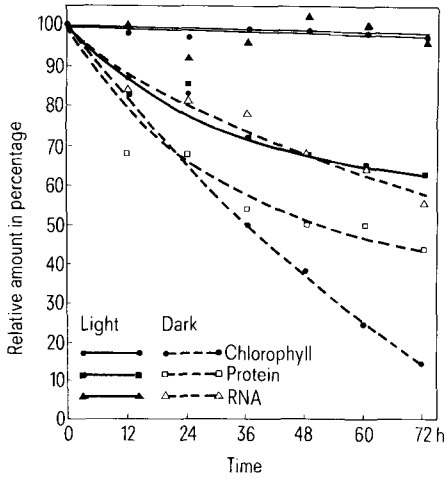


Fig. 1. Changes in the contents of chlorophyll, protein and RNA of the leaves of the maize seedlings incubated in light or dark. The initial value (100%) at zero time is equivalent to 2.1 mg/g fresh wt for chlorophyll, 22 mg/g fresh wt for protein and 12.5 mg/g fresh wt for RNA. Each value in the figure is the average of 4 experiments.

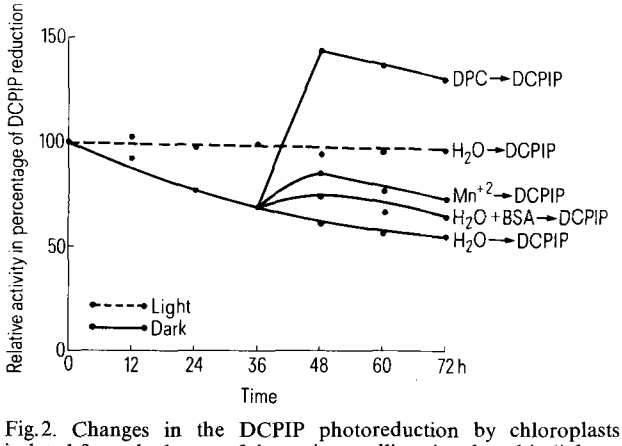


Fig. 2. Changes in the DCPIP photoreduction by chloroplasts isolated from the leaves of the maize seedlings incubated in light or dark. The initial value (100%) at zero time for DCPIP (2,6-dichlorophenol indophenol) photo reduction is equivalent to 55.23 µmoles/mg chlorophyll/h. The concentration of $MnCl_2$ and diphenyl carbazide (DPC) used were 1.6 mM and 0.5 mM respectively. Bovine serum albumin (BSA) was used at the concentration of 0.2%. Each value in the figure is the average of 4 experiments.

*Results and discussion.* Senescence in detached leaves is normally characterized by the loss of pigments, proteins and nucleic acids[2-5]. Figure 1 shows similar results with attached leaves of 4-day-old maize seedlings incubated in continuous dark for 72 h. On dark-incubation, the leaves show considerable loss in the contents of chlorophyll, protein and RNA (figure 1). In contrast, the seedlings incubated in light do not show any loss in the contents of chlorophyll and RNA of the leaves during the same period (figure 1). The loss in protein is relatively less in light compared with the loss in case of seedlings incubated in dark. The loss in the contents of chlorophyll, protein and RNA is accompanied with a considerable loss in the DCPIP photoreduction by the chloroplasts isolated from the leaves of dark-stressed seedlings (figure 2). This would suggest that the dark-induced loss in proteins and pigments of leaves may affect the electron transport system of chloroplasts. On the other hand, chloroplasts isolated from the leaves of light-treated seedlings do not show any loss in the ability of chloroplasts to reduce the Hill oxident (dashed line, figure 2). However, the dark-induced loss in the DCPIP photoreduction is restored fully by DPC, and partially by $Mn^{2+}$, on addition of these 2 exogenous electron donors to the isolated chloroplasts (figure 2). The loss in DCPIP-Hill reaction could either be due to dark-stress induced damage of photosystem II (PS II) reaction centre or disintegration of $O_2$ evolving system. Since addition of exogenous electron donors which donate electrons before PS II reaction centre restores the loss, it would obviously mean that the $O_2$ evolving system is affected by aging which results in loss of electron transport from $H_2O$ to DCPIP. Similar loss in photooxidation of water was previously observed by one of the authors during dark-induced senescence of detached barley leaves[3]. The loss in photooxidation of water may be attributed to the dark-induced liberation of unsaturated fatty acids[17] which are normally present in considerable amount in chloroplast membranes[17]. The fatty acids are reported to be progressively released upon aging[17]. It is plausible that during dark-stress, the fatty acids may change the conformation of thylakoid membranes that results in a decline in the efficiency of electron transport system. This assumption is supported by our data on the partial restoration of DCPIP-Hill reaction when BSA was added to the chloroplasts isolated from stressed leaves (figure 2). BSA forms complexes with these fatty acids, resulting in the reduction fo their pool in the membrane environment, and consequently protects the membranes from their toxic action.

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