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Photosynthesis in Green and Senescent Leaves of *Portulaca oleracea* Linn.

G V JOSHI and B A KARADGE
Botany Department, Shivaji University, Kolhapur 416004

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Photosynthetic carbon assimilation and related enzymes in green and senescent leaves of *Portulaca oleracea* Linn., a C_4 succulent, have been studied. In the plant aspartate is the major initial product in green leaves while in the senescent leaves C_3-cycle dominates which is evident from increased level of C_3 intermediates, the glycine, serine, glycolate and sugar-phosphates at the early stages of photosynthesis. The analysis of long term photosynthetic products shows a marked effect on the photosynthetic pattern in the senescent leaves. The level of amino and organic acids increases while that of sugars is reduced in the older, senescent leaves. In the senescent leaves both the carboxylases, namely, RuBP-Case and PEP-Case are low and from the two, the latter is more affected during senescence. The levels of hydrolytic (protease and invertase) and respiratory enzyme (peroxidase) are more in the senescent leaves. Increased level of glycolate oxidase in the senescent leaves indicates more photorespiratory activities. The results indicate that in the senescent leaves there are low rates of carbon assimilation and C_3-cycle dominates over the C_4 one.

Key Words: **C_3-cycle, C_4-cycle, Enzymes, Green, *Portulaca oleracea*, Photosynthesis, Senescent shift**

Introduction

Portulaca oleracea, a succulent, has features typical of C_4 species indicating a low CO_2 compensation point (Downton & Tregunna 1968, Laetsch 1971) and high activities of enzymes involved in C_4 pathway of photosynthesis (Hatch & Kagawa 1974, Gutierrez et al. 1974, Hatch et al. 1975). It was found that C_4 dicarboxylic acids are the initial products of photosynthesis in this succulent (Joshi et al. 1978), and massive alanine synthesis was found within less than 10 sec exposure of the leaves to $^{14}CO_2$ in light. Kennedy and Laetsch (1973) studied changes in early photosynthetic products during leaf development in *P. oleracea*. One of the most noticeable changes according to them was a shift toward increased activity of the C_3-cycle in the senescent leaves as seen by

increased PGA production. Subsequent investigations (Kennedy 1976) have also shown increased activity of photo-respiration in the senescent leaves of the succulent.

Hatch et al. (1967) showed no differences in the distribution of ^{14}C among photosynthetic products in sugarcane leaves of different ages. Ludlow and Wilson (1971) found that the photosynthetic rate declined gradually from full leaf expansion until several days before death of the leaf at which time the ioss was apparent, in several C_4 grasses. Nimbalkar and Joshi (1975) observed a marked effect on the photosynthetic pattern in senescent sugarcane leaves. Similarly Khanna and Sinha (1973) noted drastic changes during development in the malate/PGA and RuBP/PEP-Case ratios in *Sorghum*. Recently, Raghavendra et al. (1978) reported that there is an imbalance in the development of the C_3 and C_4 photosynthetic system in the leaves of different age in *Mollugo nudicaulis*.

In the present investigation an attempt has been made to study photosynthesis with reference to initial and long term products as well as enzymes in carbon assimilation and decarboxylation, in green and senescent leaves of *P. oleracea*. Effect of senescence on the level of hydrolytic as well as respiratory enzymes namely, peroxidase, catalase, pyruvate kinase and phosphatases has also been studied.

Materials and Methods

The green leaves of *P. oleracea* grown under natural garden conditions were sampled randomly. The leaves were distinguished in green and senescent ones. The senescent leaves, irrespective of their position on the plant were used for analysis. The chlorophylls were estimated by the method by Arnon (1949).

$^{14}CO_2$ fixation studies were done following the procedure described earlier (Joshi et al. 1978).

Enzyme studies

The cell free preparations and assays of various enzymes were done by the methods given below:

Photosynthetic and photorespiratory enzymes: Phospho-enol-pyruvate carboxylase (EC 4.1.1.31) and NAD-malate dehydrogenase (EC 1.1.1.37) were assayed by the method of Weimberg (1970) while the method of Kluge and Osmond (1972) was followed for RuBP-carboxylase (EC 4.1.1.38) and NADP-malic enzyme (EC 1.1.1.40). Glycolate oxidase (EC 1.1.3.1) was assayed by the method of Hess and Tolbert (1967).

Respiratory enzymes: Peroxidase (EC 1.11.1.7) was determined following the method described by Maehly (1954); while method by Sreeramulu and Rao (1968) was employed for the assay of enzyme catalase (EC 1.11.1.6). Pyruvate kinase (EC 2.7.1.40) was assayed by the method of Weldner and Salisbury (1974).

Phosphatases: Acid phosphates (EC 3.1.3.2) was determined following the method of De Leo aud Sacher (1970) while the method of Schrieider and Szweykouska (1974) was used for the assay of alkaline phosphatase (EC 3.1.3.1).

Hydrolytic enzymes: Protease was estimated by the method of Penner and Ashton (1967) while invertase (EC 3.2.1.26) was assayed following the method by Hatch and Glasziou (1963).

Nitrate reductase (EC 1.2.6.6.): It was determined by the method of Kaufman et al. (1971).

Activities of all enzymes are expressed on protein basis. Protein was estimated by the method of Lowry et al. (1951).

Results and Discussion

From table 1 it is clear that there is no significant change in moisture content of the green and the senescent leaves. The senescent leaves have about 20% of the chlorophylls from that in the green ones. Yet they record about 58% of the photosynthetic rate (cpm/mg fresh tissue) as compared to the green ones. This indicates the ability of the senescent leaves to fix significant amounts of CO_2. However, there is a decrease in total $^{14}CO_2$ fixation rate by the senescent leaves which may be due to the effect on CO_2 assimilatory machinary of the old leaves.

Table 1 *Moisture content, chlorophyll, $^{14}CO_2$ fixation rate and distribution of radioactivity in ethanol-soluble compounds after 10 sec and 1 hr light $^{14}CO_2$ fixation by green and senescent leaves of* P. oleracea *Linn.*

	Green leaves	Senescent leaves
Moisture %	92.27	91.70
Total chlorophylls (mg/g fresh tissue)	0.97	0.192
$^{14}CO_2$ fixation rate (cpm/mg fresh tissue)	280	163

Compound	Green leaves		Senescent leaves	
	10 sec	1 hr	10 sec	1 hr
PGA	Trace	0.51	4.34	Trace
Sugar monophosphate	—	0.96	Trace	0.43
Sugar diphosphate	—	1.23	Trace	0.98
SUGAR PHOSPHATES	**Trace**	**2.70**	**4.34**	**1.41**
Aspartate	11.20	2.78	7.52	3.36
Glutamate	2.80	1.19	18.49	12.91
Glycine-serine	7.90	2.96	18.85	3.69
Alanine	30.20	6.30	10.78	6.34
Tyrosine	0.50	0.60	1.00	0.54
Valine	Trace	Trace	1.00	1.19
Leucines	—	0.60	2.00	3.90
Glutamine	—	2.60	—	Trace
AMINO ACIDS	**52.60**	**17.03**	**59.64**	**31.93**
Malate	30.20	34.28	20.11	39.04
Citrate	10.70	2.66	3.95	5.10
Glycolate	4.70	0.46	7.61	2.93
Succinate	1.80	0.68	4.35	2.49
Fumarate	Trace	0.91	Trace	—
ORGANIC ACIDS	**47.40**	**38.99**	**36.02**	**49.52**
Glucose	—	Trace	—	Trace
Furctose	—	Trace	—	3.58
Sucrose	—	41.34	—	13.45
SUGARS	—	**41.34**	—	**17.03**

Values are expresed as percentage of radioactivity counted on chromatogram

The significant observation that can be made is about the initial products of $^{14}CO_2$ photoassimilation. C_3-Cycle products (PGA, glycine, serine, and glycolate) account for 12.6% of the total radioactivity in the green, while that in the senescent leaves these increase up to 30.80%. This increase is significant and there is corresponding decrease in the radioactivity in C_4-cycle intermediate (aspartate and malate). It appears that there is a shift toward increased activity of C_3-cycle in the senescent leaves of *P. oleracea*. Similar results for the same plant have been recorded by Kennedy and Laetsch (1973, 1974b). Kennedy working with Williams (1976) further found that in *Zea mays* there is a decrease in the amount of C_4 acids labelled during leaf ontogeny but this decrease does not lead to an increase in C_3-cycle. Hatch et al. (1967) also found no major differences in distribution of ^{14}C among early labelled products of photosynthesis in sugarcane leaves of different ages.

Increased C_3-cycle activity in the senescent leaves of *P. oleracea* could be implied by the activities of photosynthetic and photorespiratory enzymes. It can be seen from table 2 that the absolute activities of both the carboxylating enzymes, PEP-Case and RuBPCase, are significantly lower in the senescent leaves. However, PEP/RuBP carboxylase ratio for the senescent leaves (10) is much below that of the green ones (43). Similar results have been obtained by Kennedy (1976) in *P. oleracea*. Declined RuBP-Case activity during senescence was also observed by Callow (1974) in cuccumber and by Peterson and Huffaker (1975) in primary barley leaves. In *P. oleracea*, PEP-Case is much more affected than RuBP-Case and hence a shift towards C_3-cycle.

Malate dehydrogenase activity decreases considerably in the sensecent leaves (table 2). Even though the activity of NADP-malic enzyme in the senescent leaves is low, it is not so much affected as that of PEP Case and NAD-malate dehydrogenase. *P. oleracea* leaves have high levels of PEP-Case and pyruvate pi-dikinase, enzymes, concerned with the primary assimilation of CO_2 in mesophyll cells (Hatch 1975). Hatch et al. (1975) have proposed that this plant transports aspartate from a mesophyll to bundle sheath cells where conversion to malate and decarboxylation via NAD-malic enzyme occurs in mitochondria. It is possible that progressive degradation of chloroplasts as well as cytoplasmic system in mesophyll cells may be more rapid than those from bundle sheath cells. This may be the reason for more damage to PEP-Case than to RuBP-Case or malic enzyme which are usually protected in the cells as they are

Table 2 *Photosynthetic and photorespiratory enzymes* in green and senescent leaves of* P. oleracea *Linn.*

Enzymes	Green leaves	Sanescent leaves
PEP-Carboxylase	0.043	0.007 (16.3)
RuBP-Carbo ylase	0.001	0.0007 (70.00)
NAD-Malate dehydrogenase	0.016	0.0028 (17.5)
NADP-Malic enzyme	0.004	0.003 (75%)
Glycolate oxidase	0.06	0.23 (383.3)

*Values for enzyme activity are expresed as μmole/min/mg protein
Values in bracket are expressed as activity in % of control (green leaves)

inside the chloroplastic membranes. This results in decreased C_4 activity thereby increasing C_3 activity in the senescent leaves of *P. oleracea*.

Photosynthetic products after 1 hr of photosynthesis (table 1) reveal that total amount of sugars synthesized is less and synthesis of amino and organic acids is more in the senescent leaves. Similar observations were made by Nimbalkar and Joshi (1975) in sugarcane. Increased amino acid synthesis is indicative of increased amino transferases in the senescent ones. As reported earlier (Joshi et al. 1978) massive synthesis of alanine at the initial stages of photosynthesis and its further utilization during 1 hr photosynthesis indicates a vital role of this amino acid in the succulent. It is evident from the (table 1) that alanine is less synthesized at the initial stages of photosynthesis in the senescent leaves and further it is not utilized. It appears that alanine metabolism is affected during senescence in *P. oleracea*.

Increased activity of glycolate oxidase in the senescent leaves (table 2) indicates increased photorespiration. Increased photorespiration in the senescent leaves of *P. oleracea* on the basis of CO_2 evolution has been demonstrated by Kennedy (1976). Kisaki et al. (1973) found that p-glycolate phosphatase, glycolate oxidase and photorespiration were maximum in the 25th leaf (largest mature) in tobacco. They also found that C_4 pathway participates to some extent in photosynthesis in young leaves while C_3-cycle is operative in mature and old leaves. The increased photorespiration in the senescent leaves of *Portulaca* is indicative of shift towards the C_3-cycle in the plant.

From table 3 it can be seen that peroxidase level in the senescent leaves increases over that in the green ones. On the other hand activity of catalase is on decrease. These results are similar to those obtained by other investigators (Kar & Mishra 1976, Debek & Hunt 1976). The first named investigators have stated that low catalase activity may be unable to remove H_2O_2 produced more due to high glycolate oxidase activity in the senescent leaves and this increased

Table 3 *Enzyme levels in green and senescent leaves at* P. oleracea *Linn.*

Enzyme	Green leaves	Senescent leaves	
Pyruvate kinase (△O.D./hr/mg protein)	1.00	0.45	(45)
Acid phosphatase (μMpNp-hydrolysed/hr/mg protein	0.73	0.42	(57.5)
Alkaline phosphatase (△O.D./hr/mg protein)	2.60	1.90	(73.1)
Peroxidase (△ O.D./min/mg protein)	0.59	0.71	(120.3)
Catalase (mg H_2O_2 broken down/min/mg protein)	0.11	0.05	(45.5)
Invertase (mg glucose liberated/hr/mg protein)	0.18	0.35	(194.4)
Protease (μgTyrosine/hr/mg protein)	2.63	6.9	(262.4)
Nitrate reductase (△ O.D./hr/mg protein)	0.84	0.35	(41.7)

Values in bracket are expressed as activity in % of control green leaves

level of H_2O_2 may be one of the reasons of onset of senescence in *P. oleracea*. Pyruvate kinase is on decrease in the senescent leaves of *P. oleracea* (table 3). Similar observation has been made by Kisaki et al. (1973) for tobacco.

Acid and alkaline phosphatases are responsible for hydrolysis of organic phosphates. De Leo and Sacher (1970) have correlated the 13-20 fold (in ppt) and 2-4 fold (in supernatent) increase in acid phosphatase during ripening of banana with respiratory climecteric. Kar and Mishra (1976) suggest total acid phosphatase associated with the catabolic process while alkaline phosphatase is associated mainly with anabolic process. They observed increased activity of acid phosphatase contrary to decreased activity of alkaline phosphatase during senescence of both attached and detached rice leaves. A considerable decrease in total phosphatase activity was observed during the maturing of tomatoes and with maturity the phosphate content decreased (Gunther & Burchkart 1968). As in *P. oleracea* level of both acid and alkaline phosphatase is on decline (table 3). It may result in low turnover of phosphate metabolism.

The levels of protease and invertase, increase considerably in the senescent leaves of *P. oleracea* (table 3). It is obvious that increased level of protease suggests increased protein degradation in the senescent levels. This is now accepted as a typical process in aging organs. Increased activity of invertase during senescence may be in response of high rate of respiration, which is reflected in decreased sucrose content during 1 hr of photosynthesis (table 1).

The role played by nitrate reductase in growth and metabolism is clear now. Teare et al. (1974) and Kohl et al. (1975) have reported decreased activity of nitrate reductase with age in *Sorghum* and cereals. Our results are on similar lines and show decreased nitrate reductase activity in the senescent leaves of *P. oleracea* (table 3).

From the foregoing discussion it can be seen that increased labelling of C_3 intermediates during photosynthesis, induction of glycolate pathway and high rate of respiration during senescence, are suggestive of a definite shift in photosynthesis and related metabolism of *Portulaca* during senescence.

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References

Arnon D I 1949 Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*; *Pl. Physiol.* **24** 1–15

Callow J A 1974 Ribosomal RNA fraction. I. protein synthesis and ribulose diphosphate carboxylase activity in developing and senescing leaves of cuccumber; *New Phytol.* **73** 13–20

Daback A J and Hunt P 1976 Biochemistry of leaf senescence in coconut lethal yellowing, a disease associated with mycoplasma like organisms; *Trop. Agri.* **53** 115–123

De Leo Pietro and Joseph A sacher 1970 Senescence: Association of synthesis of acid phosphatase with banana ripening; *Pl. Physiol.* **46** 208–211

Downton W J S and Tregunna E B 1968 Carbon dioxide compensation: its relation to photosynthetic carboxylation reactions, systematics of Gramineae and leaf anatomy; *Can. J. Bot.* **46** 207–215

Gunther Fritz and Burchkart Ottmar 1968 Investigation of plant phosphates. III. Activity

differences in the total acid phosphatase during maturing of the tomato; *Z. Libemsmittle Untersuch Forsch.* **139** 13-15

Gutierrez M, Gracen V E and Edwards G E 1974 Biochemical and cytological relationships in C_4 plants; *Planta* **119** 279–300

Hatch M D 1975 C_4 Pathway photosynthesis in *Portulaca oleracea* and the significance of alanine labelling; *Planta* **125** 273–279

—— and Glasziou K T 1963 Sugar accumulation cycle in sugarcane. II, Relationship of invertase activity to sugar content and growth rate in storage tissue of plants grown in controlled environments; *Pl. Physiol.* **38** 344–348

——, Slack C R and Johnson H S 1967 Further studies on a new pathway of photosynthetic carbon dioxide fixation in sugarcane and its occurrence in other plant species; *Biochem. J.* **102** 417–422

—— and Kagawa T 1974 Activity, location and role of NAD-malic enzyme in leaves with C_4 pathway photosynthesis; *Aust. J. Pl. Physiol.* **1** 357–369

——, —— and Craig S 1975 Subdivision of C_4 pathway species based on differing C_4 acid carboxylating systems and ultrastructural features; *Aust. J. Pl. Physiol.* **2** 111-128

Hess J L and Tolbert N E 1967 Glycolate pathway in algae; *Pl. Physiol.* **42** 371–379

Joshi G V, Karadge B A and Bartakke S P 1978 photosynthetic carbon metabolism in succulents; in *Environ. Physiol. Ecol. Pl.* pp 87–96 eds Sen David N and R P Bansal (Dehra Dun: Bishen Singh, Mahendra Pal Singh)

Kar M and Mishra D 1976 Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence; *Pl. Physiol.* **57** 315–319

Kaufman J E, Beard J W and Penner D 1971 Influence of temperature on nitrate reductase activity of *Agrostic* and *Cynodon dactylon*; *Physiol. Pl.* **25** 378–381

Kennedy R A and Laetsch W M 1973 Relationship between leaf development and primary photosynthetic products in the C_4 plant, *Portulaca oleracea* L.; *Planta* **115** 113–124

—— and ——1974b Formation of ^{14}C-labelled alanine from pyruvate during short term photosynthesis in a C_4 plant; *Pl. Physiol.* **54** 608–611

—— 1976 Relationship between leaf development, carboxylase enzyme activities and photorespiration in the C_4 plant, *Portulaca oleracea* L.; *Planta* **128** 149-154

Khanna R and Sinha S K 1973 Change in predominance from C_4 to C_3 pathway following anthesis in *Sorghum*; *Biochem. biophys. Res. Commun.* **52** 121–124

Kisaki Takuro, Seishiro Hirabayashi and Noriko Yana 1973 Effect of the age of tobacco leaves on photosynthesis and photorespiration; *Pl. Cell Physiol.* **14** 505–514

Kluge M and Osmond C B 1972 Studies on PPP, carboxylase and other enzymes of CAM of *Bryophyllum tubiliflorum* and *Sedum prealtum Z. Pflanzenphysiol.* **66** 97–105

Kohl J G, Apel M and Dudel G 1975 Enzymatic characterisation of development phases of primary leaves of cereals with respect to functional relations between nitrate reduction and other metabolic pathways; *Biochem. Physiol. Pflanzen. BPP* **167** 501–512

Laetsch W M 1971 Chloroplast structural relationships in leaves of C_4 plants; in *Photosynthesis and Photorespiration* pp 323–349 eds. M D Hatch, C B Osmond and R O Slatyer (New York: Wiley-Interscience)

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with the folin phenol reagent; *J. biol. Chem.* **193** 262–275

Ludlow M M and Wilson G L 1971 Photosynthesis of tropical pasture plants. III. Leaf age; *Aust. J. biol. Sci.* **24** 1077–1087

Maehly A C 1954 in *Methods of Biochemical Analysis* pp 385–386 ed D Gick (New York: Interscience Inc.)

Nimbalkar J D and Joshi G V 1975 Physiological studies in senescent leaves of sugarcane var. Co. 740; *Indian J. exp. Biol.* **13** 384–386

Penner D and Ashton F M 1967 Hormonal control of protease activity in squash cotyledon; *Pl. Physiol.* **42** 791–796

Peterson L W and Huffaker R C 1975 Loss of ribulose-1, 5-diphosphate carboxylase and increase in proteolytic activity during senescence of detached primary barley leaves; *Pl. Physiol.* **55** 1009–1015

Raghavendra A S, Rajendrwdu G and Das N S R 1978 Simultaneous occurrence of C_3 and C_4 photosynthesis in relation to leaf position in *Mollugo nudicaulis*; *Nature* 273 143–144

Schrieder J and Szweyakouska A cytokinin in enzyme activities accompanying cytokinin induced formation of gametophore buds in *Ceratodon purpurens*; *Z. Pflanzenphysiol.* Bd. **72S** 95–106

Sreeramulu N and Rao I M 1968 Some aspects of the method of estimation of catalase activity in germinating seeds of groundnuts; *Indian J. Pl. Physiol.* **11** 164–171

Teare I D, Radhamohanrao Manam and Kanemasu E T 1974 Diurnal and seasonal trends in nitrate reductase activity in field grown *Sorghum* plants; *Agron. J.* **66** 733–736

Weidner Manfred and Salisbury Frank B 1974 The temperature characteristics of RuDP–1, 5-carboxylase, nitrate reductase and pyruvate kinase from seedlings of two spring wheat varieties; *Z. Pflanzenphysiol.* **715** 398–412

Weimberg Ralph 1970 Enzyme levels in pea seedlings grown on highly salinized media; *Pl. Physiol.* **46** 466–470

Williams L E and Kennedy R A 1976 Relationship between early photosynthetic products, photorespiration and stage of leaf development in *Zea mays*; *Z. Pflanzenphysiol.* **81** 314–322