BIOLOGIA PLANTARUM (PRAHA)

16 (4) : 301—307, 1974

Biosynthesis of Ascorbic Acid and Mobilization Pattern of Macromolecules During Water Stress in Germinating Cicer Seedlings

J. J. CHINOY, Y. D. SINGH and K. GURUMURTI

Department of Botany, University School of Sciences, Gujarat University, Ahmedabad-9, India

Received March 30, 1973

Abstract. Ascorbic acid (AA) turnover, and levels of RNA and protein were determined during germination of *Cicer arietinum* cv. Chafa under — (i) normal watering; (ii) water stress of six days; and (iii) revival upto next stage of seedling growth after water stress. Water stress lowered significantly AA, ascorbigen, ascorbic acid — macromolecule complex, RNA and protein content in embryo axis while a reverse trend was seen after revival. In the cotyledon, AA, RNA and protein contents were higher during water stress. However on revival only AA and protein contents decreased, whereas the RNA content showed further enhancement. It is suggested that increased synthesis of AA in the cotyledon during water stress may trigger enhanced synthesis of RNA and consequently of enzymic proteins, thus bringing about rapid mobilization of reserve materials during revival.

Some workers have shown that the RNA of the cotyledon is transported as an intact macromolecule to the embryo axis (OOTA and TAKATA 1961; LEDOUX and HUART 1962). On the other hand there is evidence to suggest that RNA is degraded in the cotyledon before being transported to the embryo axis (MATSUSHITA 1959; VAN PARIJS 1965; MITRA *et al.* 1966; OLSON and BOULTER 1968). Further, it has also been pointed out that even if the RNA of the cotyledon is translocated to the embryo axis it does not constitute a major part of the RNA required for growth by the embryo axis (INGLE 1961; INGLE and HAGEMAN 1965).

The present investigation was, therefore, undertaken to throw some light on the question of translocation of RNA and proteins. An attempt has been made to impede the transíocation of these macromolecules by germinating seeds under water-stress and then reviving them. Besides RNA and protein, AA turnover was also studied because it has an inductive effect on the biosynthesis of RNA (CHINOY and SAXENA 1971; PRICE 1966; FELLENBERG 1969).

Abbreviations: AA — ascorbic acid; RNA — ribonucleic acid; ASG — ascorbigen.

Material and Methods

Growth and Treatment of Plants

Seeds of *Cicer arietinum* cv. Chafa were germinated in sterilized sand and various determinations were carried out at the following five stages of seedling growth: (i) Radicle upto 1 cm in length; (ii) Plumule just emerging; (iii) 1st internode upto 2 cm in length; (iv) Appearance of second internode; and (v) Appearance of the third internode. Ascorbic acid turnover as well as RNA and protein contents of the embryo axis and the cotyledon were determined: (*a*) before the initiation of desiccation treatment (undesiccated) (*b*) at the end of 6-day period of water-stress (desiccated); and (*c*) after revival of the seedling upto the next stage of seedling growth (revived). The seedlings were subjected to water stress over concentrated sulphuric acid, at all the five growth stages, in chambers maintained at very low humidity for 6 days. They were then revived by keeping them on moist sterilized sand. The seedling was considered as revived when it reached the next stage of germination to the one at which it was given desiccation treatment.

Analytical Methods

Ascorbic Acid Turnover

The sample was homogenised with ice cold CO_2-saturated deionised water and the following determinations were carried out:

a) **Ascorbic Acid**

A part of the aliquot was fortified with 3% HPO_3 (Buffered wih Citric acid — NaOH at pH 3.6) in a ratio of 1 : 1. The AA content was determined by a photoelectric colorimeter using 2,6-dichlorophenol indophenol (CHINOY *et al.*, 1969).

b) **Ascorbigen (ASG)**

The 2nd part of the aliquot was mixed with 15% W/V metaphosphoric acid solution in proportion of 2 : 1 and hydrolysed in a waterbath at 75 °C for 15 minutes to release the bound ascorbic acid, which was estimated after addition of citric acid — NaOH buffer to pH 3.6 with 2.6-dichlorophenol indophenol dye. The total ascorbic acid after hydrolysis minus free ascorbic acid gave the value of ascorbigen in the system.

c) **Ascorbic Acid Utilization (AAU)**

To the remaining aliquot, AA solution of known concentration (0.1 mg ml^{-1}) made in CO_2 — saturated glass distilled water was added in the ratio of 1 : 1 and the mixture was incubated at 30 °C for 3 h. At the end of incubation period the unutilized AA was determined by the method already described.

AA-Macromolecule Complex (AA-MM Coxplex)

After incubation of plant extract with externally added AA for determination of AAU, an aliquot of the remaining part of the extract was hydrolysed using the same procedure as described for ascorbigen, and ascorbic acid in the hydrolysate was determined as shown earlier. Deducting from this quantity the total amount of ascorbic acid unutilized gave the value of the AA released from the bound state.

RNA

The plant sample was boiled in 80% ethanol for 2 minutes, homogenized and then left overnight in a refrigerator for complete extraction. It was further extracted repeatedly until a pigment free residue was obtained. This residue was washed twice with 5% perchloric acid at 0 °C to remove all acid soluble compounds. The residue was washed twice with an ethanol-ether-chloroform mixture (2 : 1 : 1) and then hydrolysed with 0.3N KOH for 24 h at 30 °C. The supernatant containing RNA was adjusted to pH 3.0 and RNA was estimated colorimetrically employing orcinol method (MARKHAM 1955). Protein was estimated using Lowry's method (LOWRY *et al.* 1951). All determinations were carried out in duplicate and are presented as mg per gram dry weight of the sample. Analysis of variance was carried out to test the significance of the various data (FISHER 1954).

Results

Data on ascorbic acid turnover and levels of RNA and proteins presented in Fig. 1, 2 and 3. It will be seen from these figures that water stress causes a reduction in AA, ASG, AA-MM Complex, RNA and Protein levels and an increase in the utilization of AA, in the embryo axis. On revival however, a reverse trend is seen. In the case of protein the increase is even higher than that in the controlled seedlings, whereas an increase in AA, and RNA content is discernible only after the 4th stage of germination. In the cotyledon on the other hand, during water stress a rise in the levels of AA, AAU, AA-MM Complex, RNA and protein is seen while the level of ASG shows a significant fall compared with that in the control. Even after revival AA, AAU, AA-MM Complex and protein contents remain at lower levels compared with those in the control series as well as the desiccated one. On the other hand RNA content of the cotyledon of revived seedlings far surpasses that in the other two series.

Statistical Significance of Data

Results of analysis of variance of data on ascorbic acid turnover, RNA and protein are presented in Tables 1 and 2. Germination stages, desiccation treatment and their interaction are highly significant in embryo axis as well as in cotyledon indicating that the differences observed were real.

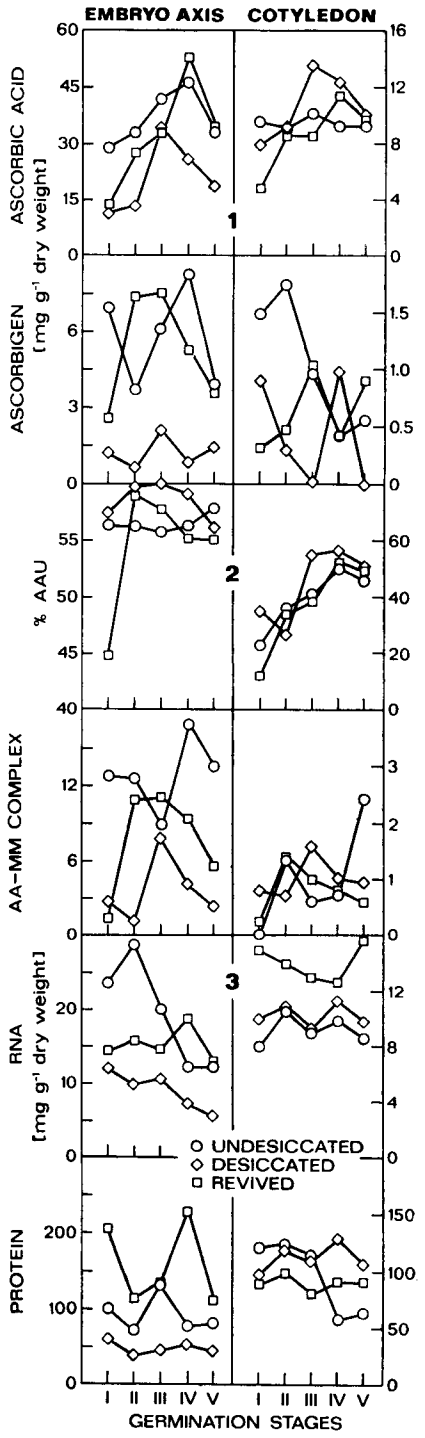

Fig. 1 to 3. The effect of water stress on ascorbic acid and ascorbigen contents, ascorbic acid utilization (AAU), AA-MM complex, and RNA and protein contents of embryo axis and cotyledon during germination.

TABLE 1
ANALYSIS OF VARIANCE

Factors	DF	F Value		
		Ascorbic acid	Ascorbigen	AAU
		Embryo axis		
Stage (s)	4	23.10*	0.84	9.17*
Desiccation Treatment (DT)	2	25.21*	10.85*	13.94*
Replicate	1	0.11	0.19	0.45
S × DT	8	3.71*	1.16	6.58*
Error	14			
Total	29			
		Cotyledon		
Stage (s)	4	5.47*	1.21	12.73*
Desiccation Treatment (DT)	2	4.52*	6.07*	1.83
Replicate	1	0.76	0.05	0.05
S × DT	8	2.20	2.52*	1.29
Error	14			
Total	29			

* = Significant at 1% level.
** = Significant at 5% level.

Discussion

Reductions, in AA, RNA and protein contents under water stress can be regarded as due to an acceleration in hydrolytic process and the impediment in the translocation of the reserve nutrient from the cotyledon. A number of workers have shown that hydrolytic process is accelerated as a result of desiccation (KESSLER 1961; DOVE 1967; MARANVILLE 1967; JANI 1969; JOHN 1969). There is a close correspondence between the reduction in the concentration of macromolecules (RNA and protein) of the embryo axis and its ascorbic acid turnover during desiccation. Free ascorbic acid, ascorbigen and AA-MM complex of the embryo axis are all reduced during desiccation, whereas utilization of AA increases. On the other hand there is an enhancement in the concentration of AA, RNA and protein in the cotyledon under water stress. An increase in AA concentration of the cotyledon may be due to the increased concentration of sugar (CHINOY *et al.* 1967) produced due to the acceleration in the amylase activity caused by water-stress (VAADIA *et al.* 1961; DIMITRIJEVIĆ 1968; ACHARYA 1968; JANI 1969; JOHN 1969) as a result of increased hydrolysis of starch. Increase in AA content of cotyledons, appears to be also partly due to its release from the bound state as shown by the depleted contents of ASG and AA-MM complex under water stress. This concomittant increase in the AA turnover as well as in RNA and protein contents of the cotyledon strongly suggests that the biosynthesis of AA and macromolecules are somehow related. It has been shown

TABLE 2
ANALYSIS OF VARIANCE

Factors	DF	F Value		
		AA-MM Complex	RNA	Protein
		Embryo axis		
Stage (s)	4	0.42	34.80*	96.27*
Desiccation Treatment (DT)	2	4.98*	158.13*	96.27*
Replicate	1	0.00	0.65	0.09
S × DT	8	0.50	20.58*	68.24*
Error	14			
Total	29			
		Cotyledon		
Stage (s)	4	0.71	3.14**	41.45*
Desiccation Treatment (DT)	2	0.21	123.42*	60.83*
Replicate	1	0.00	0.22	0.17
S × DT	8	0.61	5.47*	37.41*
Error	14			
Total	29			

* = Significant at 1% level.
** = Significant at 5% level.

earlier that AA itself can trigger enhanced synthesis of nucleic acid and protein (PRICE 1966; CHINOY and SAXENA 1971). Induction may be brought about by the removal of histone molecules from the DNA by AA (SCHOPFER 1966), or by AA combining with histone thus opening additional template sites on the DNA molecule for accelerated synthesis of RNA (FALLENBERG 1969). There is also a possibility that part of the enhancement in the RNA content of the cotyledon during water-stress may be due to an impediment in its translocation to the embryo axis during desiccation.

During revival the trends are reversed. The content of AA in the embryo axis increases and parallel to it RNA, protein and the ASG also increase. The increase in AA level may be due to the resumption of translocation of sugars and AA from the cotyledon to the embryo axis and a fall in the level of AAU; whereas RNA and protein contents increase due to inductive effect of AA on RNA synthesis which leads to enhanced biosynthesis of protein. The AA-MM complex also shows a parallel trend with AA, RNA and protein contents.

In the cotyledon on the other hand, the level of AA and protein decrease due to the resumption of translocation of these metabolites to the embryo axis while the RNA content records further increase. If the RNA of the cotyledon had been translocated either as an intact macromolecule as suggested by OOTA and TAKATA (1961) and LEDOUX and HUART (1962) or in the form of nucleotides as observed by VAN PARIJS (1965) and OLSEN and BOULTER

(1968) its level would have gone down in the cotyledon after revival of seedlings and resumption of translocation. This leads us to the conclusion that it (RNA) is retained at a high level in the cotyledon for performing an important metabolic function, namely, tha rapid synthesis of such enzymes which would bring about rapid mobilization of reserve materials within the cotyledon and to the growing axis.

This conclusion of ours is supported by the work of a number of investigators who have shown that the nucleic acid stored in storage organs of the seed can hardly cope with the actual requirement of the embryo axis for its metabolism (INGLE 1961, MATSUSHITA 1958, INGLE and HAGEMAN 1965).

Further, it is interesting to note that ASG and AA-MM complex show a parallelism with the higher biosynthetic activity in both the embryo axis as well as the cotyledon during revival. It is possible that the energy requirement to support the high rate of metabolic activity is in some way derived from AA as suggested earlier (CHINOY 1967, 1969; CHINOY *et al.*, 1969).

References

ACHARYA, U. H.: Resistance physiology of some plants of Gujarat: Effect of presowing treatment and wilting on growth, metabolism and yield of *Triticum*, *Eleusine* and *Sesamum* species. — Ph. D. Thesis, Gujarat University, Ahmedabad, India 1968.

CHINOY, J. J.: Studies in cellular and molecular aspects of floral induction and differentiation. A review. — Indian J. Plant Physiol. **10** : 202—220, 1967.

CHINOY, J. J.: A new concept of flowering on the basis of molecular and submolecular events occuring in the shoot apex and the leaf of wheat. — Indian J. Plant Physiol. **12** : 67—80, 1969.

CHINOY, J. J., SHAH, C. K., PATEL, H. T. and SUTHAR, H. K.: Role of auxin and gibberellin in the synthesis of ascorbic acid and growth of tissue explants. — Biol. Plant. **9** : 182—194, 1967.

CHINOY, J. J., SAXENA, O. P., ABRAHAM, P. G., PANDYA, R. B., DAVE, I. C., GURUMURTI, K., SASTRI, K.: Physiology of seed germination in relation to early production of ascorbic acid and ascorbic acid oxidase in wheat (*Triticum*) and peanut (*Arachis*). — P. L. 480 Final Res. Progress Report, Project No. FG-in-182 (A7-CR-87). Pp. 1—350. 1969.

CHINOY, J. J., SAXENA, O. P.: Inductive effect of ascorbic acid on RNA, amylase, protease and RNase. — 16th Int. Seed Testing Congr. Washington. Preprint No. 13, 1971.

DIMITRIJEVIĆ, D.: Productivity of sugar beet in condition of reduced soil moisture during period critical to water. — Savr. Poljopr. **16** : 419—426, 1968.

DOVE L. P.: Ribonuclease activity of stressed tomato leafleds. — Plant Physiol. **42** : 1166--1178, 1967.

FELLENBERG, G.: Changes in the nucleoprotein influenced by auxin and ascorbic acid in the course of root formation in pea epicotyls. — Planta **84** : 324—338, 1969.

FISHER, R. A.: Statistical Methods for Research Workers (12th Ed.). — Oliver and Boyd, London, 1954.

INGLE, J.: The metabolism of nucleotides and nucleic acids in food yeast and germinating barley. — Ph. D. Thesis. Bristol Univ., England 1961.

INGLE, J., HAGEMAN, R. H.: Metabolic changes associated with germination of corn II. Nucleic acid metabolism. — Plant Physiol. **40** : 48—53, 1965.

JANI, B. M.: Influence of desiccation on germination and drought resistance of some crop plants. — Ph. D. Thesis. Gujarat University, Ahmedabad, India 1969.

JOHN, D.: A comparative study of drought and salt resistance in some varieties of barley. — Ph. D. Thesis. Gujarat University, Ahmedabad, India 1969.

KESSLER, B.: Nucleic acids as factors in drought resistance of plants. — In: Advances in Botany. Vol. 2. Pp. 1153—1159. Toronto Univ. Press, Toronto 1961.

LEDOUX, L., HUART, R.: Nucleic acid and protein metabolism in barley seedlings. IV Translocation of ribonucleic acids. Biochim. — Biophys. Acta. **61** : 185—196, 1962.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J.: Protein measurement with the folin. Phenol reagent. — J. biol. Chem. **193** : 265—275, 1951.

MARANVILLE, J. W.: Effects of moisture stress on protein and nucleic acid metabolism in corn (*Zea mays* L.). — Diss. Abstr. **28** : 1762, 1967.

MARKHAM, R.: Nucleic acids, their components and related compounds. — In: PAECH, K., TRACEY, M. V. (ed.): Modern Methods of Plant Analysis. Vol. 4. Pp. 246—304. Springer-Verlag, Berlin 1955.

MATSUSHITA, S.: Studies on the nucleic acids in plants. III Changes of the nucleic acid contents during germination stage of the rice plant. — Mem. Res. Inst. Food Sci., Kyoto Univ. **14** : : 30—32, 1958.

MATSUSHITA, S.: On the ribonuclease during the germination of wheat. — Mem. Res. Inst. Food Sci. Kyoto Univ., **17** : 23—28, 1959.

MITRA, S., CHAKRABARTY, A. M., SIRCAR, S. M.: Studies of an unstable functional ribonucleic acid in germinating wheat. — Indian J. Biochem. **3** : 26—29, 1966.

OLSON, R., BOULTER, K. D.: Nucleic acid metabolism of *Vicia faba* during germination and growth. — Physiol. Plant. **21** : 428—434, 1968.

OOTA, Y., TAKATA, K.: Cytoplasmic ribonucleic acids in bean germ tissues. — In: Recent Advances in Botany. Vol. 2. Pp. 1059—1063. Toronto Univ. Press, Toronto 1961.

PRICE, C. E.: Ascorbate stimulation of RNA synthesis. — Nature **212** : 1481, 1966.

SCHOPFER, P.: Der Einfluss von Phytochrom auf die stationnaren Konzentrationen von Ascorbinsäure und Dehydroascorbinsäure beim Senfkeimling (*Sinapis alba* L.). — Planta **74** : 210 — 227, 1966.

VAADIA, Y., RANEY, F. C., HAGAN, R. M.: Plant water deficits and physiological processes. — Annu. Rev. Plant Physiol. **12** : 265—292, 1961.

VAN PARIJS, R.: Quantitative changes of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), protein and dry matter in different organs of pea seedlings during germination and cell elongation. — Arch. int. Physiol. Biochem. **75** : 125—138, 1965.

J. J. CHINOY, Y. D. SINGH, K. GURUMUTI, Botanické oddělení university, Ahmedabad, Indie: **Biosyntéza kyseliny askorbové a způsob mobilizace makromolekul při nedostatku vody během klíčení cizrny beraní.** — Biol. Plant. **16** : 301—307, 1974.

Přeměny kyseliny askorbové (AA) a hladina RNA a bílkovin byla sledována během klíčení semen ***Cicer arientinum,*** cv. Chafa a to 1) při normálním zavlažování, 2) při přerušení závlahy po dobu šesti dnů a 3) při opětném zavlažování po předchozím deficitu. Při vodním deficitu byl významně snížen obsah AA, akorbigenu, makromolekulárního komplexu s AA, RNA a proteinů v ose embrya; opačný trend byl pozorován při opětném dosycování. V dělohách byl obsah AA, RNA a proteinů během vodního deficitu vyšší. Při dosycování se snižoval pouze obsah AA a bílkovin, zatímco obsah RNA se dále zvyšoval. Dá se předpokládat, že zvýšená syntéza AA v dělohách při vodním deficitu působí zvýšení syntézy RNA a následkem toho i proteinů; tím je způsobena rychlá mobilizace zásobních látek během zpětného dosycování a dalšího růstu.

BOOK REVIEW

EHRENDORFER, F. (ed.): **Liste der Gefässpflanzen Mitteleuropas. 2., erweiterte Auflage.** — Gustav Fischer Verlag, Stuttgart 1973. 318 S.

Vorliegende Liste ist ein Katalog der Gattungen, Arten und Unterarten der einheimischen und eingebürgerten Gefässpflanzen (Farn- und Samenpflanzen) Mitteleuropas. Diese zweite, erweiterte Auflage (erste Auflage im Verlag des Notringes der wissenschaftlichen Verbände Österreichs, Wien 1967 erschienen), an der über 17 Wissenschaftler aus verschiedenen Ländern mitgearbeitet haben, fasst in gedrängter Form den derzeitigen Stand unserer Kenntnisse über die systematische Gruppierung und Verbreitung der Gefässpflanzen in Mitteleuropa zusammen. Die Daten sind sehr übersichtlich in vier durchlaufenden Kolonnen angeordnet und enthalten: eine Numerierung, standardisierte Abkürzungen, wissenschaftliche Namen (mit Synonymen) und Verbreitungsangaben. Ausser einer Karte des erfassten Gebietes, Erklärung der Abkürzungen und Zeichen sind ein systematisches Register und Literaturhinweise hinzugefügt. Das Buch ist als Grundlage für floristische Kartierung und allgemein als systematisch-nomenklatorisches sowie floristisches Nachschlagwerk gedacht und erfüllt diese Forderungen bestens.

INGRID TICHÁ (*Praha*)