J. Phytol. Res. 4 (1) 1991

PRODUCTION OF ENDOGENOUS ASCORBIC ACID FROM TISSUE CULTURES OF *ABUTILON PANNOSUM* FORST. f.

VIJAY SINGH and T.N. NAG
Rajasthan Agriculture University (ARS-SGNR), Bikaner-334001 India.

The static cultures of *Abutilon pannosum* Forst. f. grown on MS medium was exploited for endogenous ascorbic acid at the growth ages of 2,4,6 and 8 weeks. The potentialities of the tissues to produce ascorbic acid increases by incorporating auxins, exogenous ascorbic acid and D-glucose in the medium.

Keywords : *Abutilon pannosum* Forst. f.; Endogenous ascorbic acid; Auxins; Growth Index; D-glucose.

Introduction

Ascorbic acid, an important biological reductant and regulator of oxidation-reduction state of protoplasm, play significant role in germination, growth, metabolism and flowering of plants. It is also well known for its property as an electron donor in photosynthetic phosphorylation (Arnon *et al.* 1954, 1956, 1958; Aberg, 1958; Mitsui and Oi, 1961; Isherwood and Mapson, 1962; Chinoy *et al.* 1967). Endogenous ascorbic acid and its exogenous effect (Nag *et al.* 1974; Jain *et al.* 1975) have been studied in plant tissue cultures of some plant species. Earlier by our group (Singh *et al.* 1990) *in vivo* ascorbic acid content of *A. pannosum* have been reported but so far there is no report on its production in tissue cultures, hence, in the present study estimation of ascorbic acid from tissue cultures of this plant species has been carried out. The effects of auxins (IAA, 2, 4-D and NAA), exogenous ascorbic acid and D-glucose has also been observed on *in vitro* growth and ascorbic acid production.

Materials and Method

The static cultures of *A. pannosum* were established from the seeds on MS medium (1962) supplemented with 5 ppm kinetin + 1 ppm 2,4-D and maintaned for ten months under aseptic uniform conditions of temperature at 26 ± 1°C, 55% relativ humidity and diffused light. During study the tissues were harvested regularly at the age of 2, 4, 6 and 8 weeks.

Further, Indole acetic acid (IAA), 2, 4 Dichloro phenoxy acetic acid (2, 4-D), Naphthalene acetic acid (NAA; 1,3,5 ppm each), ascorbic acid (250, 500, 750, 1000) mg/l and D-glucose (0.5%, 1%, 1.5%, 2%) were supplemented into the medium and the tissues were harvested. Growth indices calculated and ascorbic acid contents were estimated by photo-electric colorimeter method (Chinoy, 1962) in each tissue sample. Five replicates of each sample were taken.

Results and Discussion

Endogenous Ascorbic acid from Tissue Cultures (Control) : There is a linear increase in the growth index from two to eight weeks. Maximum GI (12.160) was found in eight week old cultures and minimum (2.602) in two week old tissues. However, the ascorbic acid content was maximum (79 50 mg/100 gfw) in two week old tissues and minimum (58.70 mg/100 gfw) in eight week old cultures (Table 1).

Effect of Exogenous ascorbic acid : The GI in general showed an increase upto the sixth week but it decreases in 8th week for each concentration of ascorbic acid fed in the medium. Maximum GI (12.60) was observed in six week old cultures grown on MS medium supplemented with 750 mg/l of exogenous ascorbic acid. This growth index is relatively higher than the maximum GI (12.160) of control tissues. The amount of endogenons ascorbic acid was maximum (167.40 mg/100 gfw) in two week old cultures grown on 750 mg/l ascorbic acid incorporated medium while minimum 72.80 mg/100 gfw) in four week old tissues grown on MS medium incorporated with 250 mg ascorbic acid/litre (Table 1).

Effect of Auxins : (a) I A A : Maximum GI (8.03) was found in 8 week old cultures fed with 5ppm. IAA while minimum (1.84) in 2 week old cultures grown on MS medium incorporated with 1 ppm IAA. The maximum amount (121.40 mg/100 gfw) of ascorbic acid was found in 2 week old tissues grown on MS medium supplemented with 5 ppm IAA and minimum (61.70) in four week old cultures fed with 1 ppm IAA (Table 2).

(b) 2, 4-D : The incorporation of 1 ppm 2, 4-D in the medium showed the maximum GI (8.60) at 8 week old age but the two week old tissues fed with 5 ppm 2, 4-D contained the maximum (138.00 mg/100 gfw) amount of ascorbic acid.

(c) N A A : The incorporation of 5 ppm NAA into the medium showed the maximum GI (6.45) and ascorbic acid concentration (115.40 mg/100 gfw) at 8 week and 2 week old age respectively.

Table 1 : Effect of exogenous ascorbic acid on growth and production of ascorbic acid (mg/100 gfw") from tissue cultures of *A. pannosum* Forst f. (Five replicates of each ± S.E.)

Age of Tissue	MS	MS+250 mg AA/l		MS+500 mg AA/l		MS+750 mg AA/l		MS+1000 mg AA/l	
	GI*	AA°	GI	AA	GI	AA	GI	AA	GI	AA
2 Week	2.602	79.50 ±.18	1.05	141.30 ±.76	1 14	153 40 ±.1.2	1.85	167.40 ±.95	1.46	162.80 ±.40
4 Week	5.294	75.30 ±.34	5.38	72.80 ±.33	6.29	84.55 ±.43	6.82	96 65 ±.47	4.57	97.50 ±.38
6 Week	12.022	69.70 ±.19	10.71	97.50 ±.56	11.52	102.60 ±.68	12.60	114.85 ±.56	10.28	122.05 ±.27
8 Week	12.160	58 70 ±.14	6.46	108.60 ±.37	7.16	113.65 ±.22	7.75	154.50 ±.42	6.94	148.25 ±.60

Table 2 : Effect of auxins on growth and production of ascorbic acid (mg/100 gfw") in tissue culture of *A. pannosum* Forst. f. (Five replicates of each ± S.E.)

Age of Tissue		IAA						2, 4-D						NAA				
		1 ppm		3 ppm		5 ppm		1 ppm		3 ppm		5 ppm		1 ppm		3 ppm		5 ppm
	GI*	AA°	GI	AA	GI	AA	GI	AA	GI	AA	GI	AA	GI	AA	GI	AA	GI	AA
2 Week	1.84	108.4 ±.21	2.06	117.3 ± 80	2.21	121.4 ±.78	1.98	112 8 ± 59	1.22	124.6 ±.38	1.86	138 0 ±1.02	1.92	104.3 ±.52	2 04	109.8 ±.63	2.14	115.4 ±.73
4 Week	4.06	61.7 ±.30	3.87	64.6 ±.49	4.46	65.0 ±.39	4.51	70.6 ±.52	2.61	85.6 ±.44	3 92	98 0 ±.88	3.81	65 0 ±.66	3 87	73.4 ±.42	4.03	78.2 ±.46
6 Week	5.98	80.2 ±.38	4.89	86.1 ±.36	6.19	87.9 ±.58	7.42	83 2 ±.60	4.89	97.0 ±.66	5 57	109.6 ±.40	4 68	77.6 ±.29	4.90	79.3 ±.66	5.26	84 3 ±.57
8 Week	7.22	94.6 ± 64	5.66	105 7 ±.42	8.03	107.1 ±.73	8 60	95.3 ± 53	5.86	112.3 ±.37	6.49	131.3 ±.58	5.56	91.5 ±.37	5.86	100.6 ±.70	6.45	103.6 ±.58

Table 3 : Effect of D-glucose on growth and production of ascorbic acid (mg/100[11] gfw) from tissue cultures of *A. pannosum* Forst. f. (Five replicates of each ± S.E.)

Age of Tissue	MS + 0.5% G		MS + 1% G		MS + 1.5% G		MS + 2.0% G	
	GI*	AA°	GI	AA	GI	AA	GI	AA
2 Wk	1.20	122.60 ±.70	1.26	147.80 ±.64	1.81	150.60 ±.88	1.47	156.20 ±.45
4 Wk	6.71	70.90 ±.58	7.25	72.85 ±.22	7.72	91.80 ±.74	4.86	94 05 ±.62
6 Wk	12 66	100.20 ±.66	13.57	102.50 ±.49	15.63	105.80 ±.37	11.87	122.60 ±.57
8 Wk	7.20	119.80 ±.47	8.60	120.40 ±.80	12.25	122.60 ±.52	8.70	125.40 ±.32

,,-gram fresh weight
*-Growth Index
°-Ascorbic acid

Effect of D-glucose : The maximum GI (15.63) was observed in 6 week old cultures grown on MS medium incorporated with 1.5% D-glucose. This GI is comparatively higher than the maximum GI (12.160) of control tissues. Maximum amount of ascorbic acid (156.20 mg/100 gfw) was found in two week old tissues fed with 2% D-glucose. However, minimum GI (1.20) and ascorbic acid content (70.90 mg/100 gfw) was found in 0.5% D-glucose fed tissue at the growth ages of 2 week and 4 week respectively (Table 3).

The present study supports that tissue cultures contain free endogenous acid, Mohan *et al.* (1974) have reported the maximum amount of free ascorbic acid in six week old callus of *Momordica charantia* where as Nag *et al.* (1974) have found it to be maximum in eight week old tissues of *Datura* spp. The effect of exogenous ascorbic acid on the production of endogenous as carbic acid *in vitro* have been studied by Shekhawat (1985) on *Z. simplex* and Grover (1984) on *L. barbarum.* They have reported the maximum amount in 8 week old culture incorporated with 1000 mg ascorbic acid/litre.

From the data presented it can be concluded that the potentialities of the tissues to produce ascorbic acid even in ten month old tissues do not decrease. The incorporation of various auxins enhance the production of ascorbic acid to a certain extent. Exogenous feeding of ascorbic acid into the medium also angments the free endogenous ascorbic acid but the supplementation of D-gulcose into the medium increases the growth of tissues as well as the production of ascorbic acid significantly. Among the auxins. 2, 4-D and the incorporation of 750 mg ascorbic acid/litre in to the medium are more potential to synthesize ascorbic acid. D-glucose increases growth and ascorbic acid content of the tissues remarkably and acts as one of the precursors of ascorbic acid (Isherwood *et al.*, 1954 and Loewus and Kelly 1961).

The marked increase in ascorbic acid content of the tissues by feeding the growth adjuvants at 2 week age may be due to their higher absorption from the media by the callus and its conversion to ascorbic acid. The amount of ascorbic acid in four and six week old tissues was low, which might be due to its utilization in growth and development and also in synthesis of some secondary metabolites.

Acknowledgement

One of us (VS) humbly acknowledge the financial assistance provided by CSIR, New Delhi as SRF.

Accepted July, 1990.

References

Aberg B 1958, *In* : Encyclopedia of Plant Physiology W. Ruhland (eds), Springer-Verlag, Berlin, 6. 479.

Arnon D I, Whatley F R and Allen M B 1954, *J. Amer. Chem. Soc.* **76** 6324

Arnon D I, Whatley F R and Allen M B 1956, *Biochem. Biophys. Acta* **20** 449

Arnon D I, Whatley F R and Allen M B 1958, *Science* **127** 1026.

Chinoy J J 1962, *Indian J. Plant Physiol.* **5** 172

Chinoy J J, Pandya R B, Saxena O P, Dave I C and Abraham P G 1967, *J Ind. Bot. Soc.* **46** 344

Grover S 1984, Ph. D. thesis, University of Rajasthan, Jaipur.

Isherwood F A and Mapson L W 1962, *Ann. Rev. Plant Physiol.* **13** 329

Isherwood F A, Chen Y T and Mapson L W 1954' *Biochem. J.* **56** 1

Jain S C., Nag T N, Mohan S and Khanna P 1975, *Science and Culture* **41** 292

Loewus F A and Kelly S 1961, *Nature* **191** 1059.

Mitsui A and Oi Y 1961, *Plant Cell Physiol.* **2** 45

Mohan S, Nag T N, Jain S C and Khanana P. 1974, *Vidya B. Science* **17** 8

Nag T N, Jain S C, Mohan S and Khanna P 1974, *Ind. J. Pharm.* 36 49

Shekhawat S S 1985, Ph D. thesis, Univ. of Rajasthan, Jaipur.

Singh V, Mathur K, Sethia M, Bhojak S and Nag T N 1990, *eobios* **17** 35