NUCLEIC ACID CHANGES DURING GERMINATION AND GROWTH OF GLYCINE MAX (L) MERR.

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Several investigations on nucleic acid changes in germinating and developing seedlings have been reported [1-6]. Galitz and Howell [7] studied the RNA and total free nucleotides of developing soybean seeds by employing Ogur and Rosen method, the method is now known to be unsuitable for quantitative analysis of RNA and DNA in plants [8]. Discarlo et al.[9] reported that the mature soybean contain about 1 per cent nucleic acid. As the results obtained by Ogur and Rosen methods are unreliable, it was thought worth while to investigate the changes in nucleic acids during germination and ripening of soybean.

Experimental:

The germination was carried out in washed and sterilized sand. Sand was filled in trays and levelled properly. The sand for sowing was sterlized because the seeds of soybean are susceptible to fungus growth. For germination, soybean seeds of variety Pb. No. 1 were

kept at the depth of one inch in sand. The trays were kept in green house, under normal atmospheric conditions.

Samples of seedlings were taken, each after 0, 2, 4, 6 and 8 days of germination. Seedlings were washed with double distilled water to make them free from adhering sand particles and were then seperated into cotyledons, radicles, plumule and leaves wherever possible.

For ripening study, open flowers were tagged and samples for the present study were drawn periodically only from pods set on tagged flowers. Tagging of flowers was done during the peak flowering period. Samples of pods and leaves were collected after 17, 27, 37 & 47 days after tagging. The pods were seperated into seed covers and seeds.

The samples of seeds, seed covers, leaves and seedlings were dried at low temperature (60°C) and grinded in a grinder and stored in glass stoppered tubes for further analysis.

The following estimations were carried out in duplicate and recovery was tested in each case and results were calculated on that basis.

Determination of RNA and DNA

Extraction.

For the extraction of nucleic acids, alkali hydrolysis procedure suggested by Nieman and Poulson[10], which is essentially a modification of Schmidt - Thannhauser methods, was adopted.

200 mg of plant material was taken. The material was thoroughly ground in a pestle and mortar with a pinch of washed sea sand and about 1 ml of 80 per cent ethanol at room temperature. The homogenised material was extracted with hot 80 per cent ethanol and centrifuged. The supernatent containing the chlorophyll was discarded. The residue

left was reextracted thrice with hot ethanol each time and centrifuged. The residue was used for further extractions.

Extraction and estimation of RNA

The residue left after chlorophyll extraction was extracted with 4 mlchilled 0.2 N perchloric acid at 4^{OC} for 15 minutes and centrifuged at room temperature. The supernatent containing acid soluble phosphate was discarded. The residue was next extracted with with 4 ml cold ethanol for 15 minutes and centrifuged. The supernatent containing ethanol soluble phosphate was discarded. The residue was then treated with 5 ml ethanol: ether (3:1, v/v) at $50^{\circ}C$ for 30 minutes and centrifuged.

The residue was next suspended with 3 ml of 0.3 N sodium hydroxide and incubated at $30^{\circ}C$ for 18 hours. The sediment was removed with centrifuging and washed with 1 ml of 0.3 N sodium hydroxide. The extract and washing were combined and acidified to pH 1 with 15 per cent perchloric acid, and held at $4^{\circ}C$ for 40 minutes and centrifuged. The supernatent equals the RNA fraction. The sediment was resuspended with 1 ml of 1 N perchloric acid, held at $4^{\circ}C$ for 20 minutes and centrifuged. The fraction was made upto 10 ml with distilled water.

The estimation of RNA was carried out by the orcinol reaction outlined by Markham[11] (1955).

Extraction and estimation of DNA

The DNA protein sediment left after RNA extraction was suspended with 0.5 N perchloric acid, heated at $70^{\circ}C$ for 15 minutes and centrifuged. The supernatent was kept aside. The residue was again washed with 0.5 N perchloric acid, heated to $70^{\circ}C$ for 15 minutes, and centrifuged. The two supernatents were combined together and

volume made to 10 ml with distilled water.

The DNA content was determined by Burton's modification of diphenylamine reagent (Burton[12]).

RESULTS & DISCUSSION

The samples of cotyledons, roots, shoots, leaves and testa taken after 0, 2, 4, 6 and 8 days of germination of soybean and of seed, seed covers and leaves taken after 17, 27, 37 and 47 days after tagging of soybean flowers were analysed quantitatively for their contents of nucleic acids. The results obtained are tabulated in table I & II and shown graphically in fig. 1 to 4.

Table-I

Changes in Nucleic acid during germination of Soybean
(mg/gm on dry weight basis).

	Days after germination	Plant parts				
		Cotyledons	Testa	Roots	Shoots	Leaves
RNA	0	9.2	-	-	-	-
	2	5.7	5.2	2.5	-	-
	4	4.5	-	3.1	3.1	-
	6	2.3	-	4.2	4.2	5.6
	8	4.8	-	6.2	5.0	6.2
DNA	0	0.63	-	-	-	-
	2	2.20	0.31	0.42	-	-
	4	2x20 0.91	-	0.42	0.15	-
	6	0.52	-	0.47	0.36	4.22
	8	0.69	-	0.58	0.63	4.93

Table-II

Changes in Nucleic acid during ripening of soybean
(mg/gm on dry weight basis).

	Days after tagging of flowers.	Plant parts		
		Seed	See cover	Leaves
RNA	17	11.1	11.9	6.7
	27	8.4	10.3	8.3
	37	9.2	7.8	6.4
	47	5.0	7.3	5.3
DNA	17	6.30	2.00	1.06
	27	1.20	0.45	1.14
	37	1.14	0.38	1.27
	47	0.31	0.23	0.60

A perusal of data in Table-I reveals that during germination, RNA in case of cotyledons decreased upto 6th day and increased thereafter, while in case of roots, shoots and leaves it increased. Although there was a rapid decline in cotyledonary RNA during germination, the total seedling RNA increased. The increase in total seedling RNA is chiefly due to its increase in the roots and shoots. There are two possible explanations for the loss of RNA during germination. First some RNA may be degraded by phosphodiesterases and secondly the RNA could be transported as a macromolecule to the embryonic axis. The probability of macro molecular RNA translocation was suggested by the work of Oota et al.[13] which showed that ribosomal RNA may be transported from the cotyledons to the hypocotyl of bean (Vicia sesquipedalis) and by that of Ledoux and Huart[14], which demonstrated that in barley, exogenously labelled RNA is transported via the endosperm to the embryonic axis. Barker and Hollinshead[15] also observed decrease in RNA content in bean seed embryo and Pisuin arvense cotyledons during germination. Holdgate

and Goodwin[1] discussed the increase in RNA level in plumule and radicle of rye during few hours of imbibition, in relation to initiation of cell division. A net increase in RNA of endosperm was related to enzyme synthesis. The increase of nucleic acids in growing axis is also due to de-novo synthesis.

During germination DNA content of cotyledons increased upto 2 days of germination and then decreases, where as in roots and shoots it increased. Holdgate and Goodwin[1] also observed an increase in DNA content of the endosperm of rye during the first 16 hours of germination processes, whereas Cherry[4] has recorded an increase in the DNA content of Arachis hypograr cotyledons during the first 8 days of germination. The significance of these increases in rye, peanut and soybean is difficult to asses, but it is tempting to suggest that they may be important in controlling enzyme synthesis in storage tissue. In leaves DNA content was found to be maximum and it increased with germination. Marcus and Feeley[16] reported that during peanut germination, embryonic axis showed a sustained increase in RNA & DNA. They suggested that nucleic acids of peanut cotyledons may have a metabolic function during germination rather than serving primarily as a source of "transportable" nucleic acid. Beevers and Guernsey[17] also observed similar results in pea seeds.

During ripening of soybean seeds RNA content was found to decrease in seeds and seed covers, where as in leaves, it increased upto 27th day after tagging of flowers and decreased there after. The decrease in RNA content may be due to inactivation of enzyme involved in RNA synthesis. Vecher and Matoshka[18] reported that RNA accumulated only in the first stage of ripening of lupinus and then remained constant or decreased. Sturani and Cocucci[19] showed that RNA was maximum in

the young endosperm of easter bean and decreased at maturity. Galitz and Howell[7] also observed similar results during ripening of soybean.

DNA content of seed and seed cover during ripening decreased with the onset of maturity, while DNA content of leaves increased during early stages upto 6th day of germination and decreased afterword.

SUMMARY

A study of nucleic acid metabolism during the early stages of germination and ripening of soybean has been made. The samples of cotyledons, roots, shoots, leaves and testa were taken after 0, 2, 4, 6 and 8 days after germination and of seeds, seed covers and leaves taken after 17, 27, 37 and 47 days after tagging of soybean flowers, were quantitatively analysed for their content of nucleic acids.

The content of RNA in seeds and seed covers was found to decrease, where as in leaves, the content of RNA decreased after an initial increase. DNA content was found to decrease during ripening time in seed and seed covers, whereas in leaves DNA content increased with maturity. The RNA content was found to decrease in cotyledons, while in roots, shoots and leaves it increased. DNA content decreased after an initial increase in cotyledons, where as in roots, shoots and leaves it increased. The DNA content was found to be maximum in young leaves.

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