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STUDIES ON PIGMENTS AND VITAMIN E AT DIFFERENT STAGES OF GROWTH OF SOME LEGUMINOSAE PLANTS

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Abstract. Carotenoid and chlorophyll contents of some leguminous plants have been determined at various stages of growth with the view to finding out the right stages for the commercial production of these pigments. Trial A with cutting period of 20 days may be preferred over trial B with the cutting period of 40 days for this purpose. Suggestion has been made for the complete utilization of the right leguminous plant for the production of carotenoids, chlorophylls and proteins which are devoid of any colour and flavour. The vitamin E content at various stages of growth was also determined.

In earlier communications the carotenoid content of green plants,[1] citrus fruits,[2] the effect of dehydration and storage on the carotenoid content of alfalfa[3] and the preparation of carotenoid concentrate from ' berseem"[4] have been reported. These studies were made with the view to finding out the best source for the commercial production of carotenoids to be used as natural food colours. Leguminosae plants like alfalfa, "berseem" and "shaftal" among the green plants were found to be the richest sources of carotenoid pigments. These crops yield many cuttings without reseeding and are practically available for 4-6 months continuously.

It was, therefore, considered advisable to determine the carotenoid/chlorophyll of some leguminous plant content, at different stages of growth in order to determine the right time for the maximum extraction of pigments (carotenoid/chlorophylls). Other components like vitamin E have also been studied at different stages of growth.

Experimental

Reagents and apparatus. α-Tochopherol : pure α-tochopheral was obtained from Hoffmann-LaRoche. Inc.

Ferric chloride solution: 0.2% sol. in absolute ethanol was prepared daily before use in an amber flask.

Dipyridyl solution: 0.5% sol. in absolute alcohol was prepared.

The solvents were distilled before use. All the reagents used were of analytical grade. Beckman DB and Unicam spectrophotometers were used for spectrophotometric estimations.

Moisture Determination: The moisture content was determined according to the AOAC method.[5]

Estimation of Pigments (carotenoids/chlorophylls) and vitamin E : Lucerne, "berseem" and "shaftal" were grown at the laboratory campus and the plot was divided into two portions, A and B. The cuttings were collected at 20 days' interval from portion A (trial A) and at 40 days interval from portion B (trial B) for the estimation of pigments and vitamin E.

Carotenoids and chlorophylls were separated and estimated as described earlier (Shah & Elahi.)[4] The results are given in Tables 1-2. Vitamin E was estimated by the method of Emmerie and Engel[6] as modified by Vilyams and Semenova[7] which is briefly as follows :

Samples (2 g) of the stem and leaves of the plants were cut after a period of 20 and 40 days and ground with ethyl alcohol and filtered through glass wool. The residue was washed with alcohol and diethyl ether and the washings mixed with the previous alcohol extract. To this solution 4 M KOH was added for saponification of chlorophylls. Alkalis and chlorophylls were removed with water washings and the ethereal solution was dried using anhydrous sodium sulphate (Na_2SO_4). Ether was evaporated at low temperature under reduced pressure. The residue was dissolved in benzene and this solution passed through a column (15 cm × 1 cm) containing an 8-cm. thick layer of cotton. The tocopherols were absorbed by the cotton while the carotenoids passed through.

The tocopherols were eluted with ethyl alcohol and made to a definite volume (25 ml). To 1 ml of this solution was added 1 ml of 0.2% $FeCl_3$ and 1 ml of 0.5% bipyridine solution and made upto 5 ml with absolute alcohol. After 10 min. a red colour was developed. The intensity of the red colour was measured spectrophotometrically at 520 mμ and vitamin E was estimated from a standard curve. The results are included in Table 3. The contents of carotenoids and chlorophylls and vitamin E have been reported on dry weight basis in round figures.

Discussion

Table 1 includes the carotenoid content of some leguminosae plants at different stages of growth. It will be observed in Table 1 that the total carotenoid

content increases upto 4th cutting and then starts decreasing in the leaves as well as stems of trial A. It is interesting to note that the rate of increase in the carotenoid content of the plants under study is different, but at the 4th cutting the carotenoid content is almost equal. In trial B the maximum level of carotenoids reaches at the third cutting after which it begins to decline both in the leaves and stems. "Shaftal" and "berseem" leaves contain almost equal amounts of carotenoids and more than lucerne leaves at the third cutting.

Table 2 indicates the variations in chlorophyll content of some leguminous plants at different stages of growth. In both the trials it follows the same pattern as that of carotenoids, *i.e.* the chlorophyll increases up to the 4th cutting in trial A and then starts declining both in the leaves and stems. At the 4th cutting lucerne leaves and the stem of "shaftal" contain the maximum chlorophyll content. "Shaftal" leaves contain more chlorophyll at the maximum level, *i.e.* the 4th cutting than lucerne and "berseem" which contain almost equal amounts of chlorophyll. At the maximum level stems of the plants under study contain almost equal amounts of chlorophyll.

More than five cuttings were obtained and the present studies indicate that as the plants approach flowering stage the pigments content decreases.

Table 3 shows the vitamin E (tocopherols) content of some leguminous plants at different stages of growth. Vitamin E is of great physiological importance as its lack or deficiency may produce a series of symptoms, many of which are irreversible. Chemically it may act as a typical antioxidant. Alfalfa is an excellent source of vitamin E[8,9] and has therefore been used in poultry feeds[10] for the prevention of ancephalomalacia in chicks and for increasing fertility in laying hens.[11] It has been fed to dairy cows as a source of vitamin E in milk to prevent oxidized flavour.[12] In trial A, the leaves of all the plants have almost the same amount of vitamin E upto the 2nd

TABLE 1. CAROTENOID CONTENT OF SOME GREEN PLANTS AT DIFFERENT STAGES OF GROWTH (IN MG).

Name and portion of the plant	Botanical name	Trial A					Trial B				
		1	2	3	4	5	1	2	3	4	5
Lucerne leaves	*Medicago sativa*	117.0	156.2	193.0	244.5	180.0	165.4	190.0	240.0	209.0	156.0
Lucerne stalks		58.3	77.7	80.0	90.0	76.0	50.6	72.5	93.75	80.0	77.5
"Berseem" leaves	*Trifolium alexandrium*	153.3	197.0	200.0	225.7	156.2	177.5	194.1	275.0	205.6	150.0
"Berseem" stalks		53.1	58.1	77.5	84.8	70.0	55.4	60.0	86.5	80.0	72.0
"Shaftal" leaves	*Trifolium resupinatum*	125.0	147.2	240.0	250.0	166.0	161.0	223.0	280.0	192.0	143.75
"Shaftal" stalks		43.5	67.0	73.5	84.0	61.4	64.0	73.7	85.2	74.0	65.0

mg=mg. of carotenoid content per 100 g. dry weight of sample.

TABLE 2. CHLOROPHYLL CONTENT OF SOME GREEN PLANTS AT DIFFERENT STAGES OF GOWTH (IN MG).

Name and portion of the plant	Trial A					Trial B				
	1	2	3	4	5	1	2	3	4	5
Lucerne leaves	733	956	1008	1332	1217	926	1100	1203	1360	1157
Lucerne stalks	444	548	554	600	580	427	557	625	650	518
"Berseem" leaves	800	1070	1047	1284	1107	991	1079	1047	1340	1148
"Berseem" stalks	345	448	555	597	492	402	451	600	657	527
"Shaftal" leaves	773	917	1223	1253	1109	967	1112	1171	1457	1105
"Shaftal" stalks	288	463	584	636	476	500	626	623	647	456

mg=mg. of chlorophyll content per 100 g. dry weight of sample.

TABLE 3. VITAMIN E CONTENT OF SOME GREEN PLANTS AT DIFFERENT STAGES OF GROWTH (IN MG).

Name and portion of the plant	Trial A					Trial B				
	1	2	3	4	5	1	2	3	4	5
Lucerne leaves	34.0	34.0	49.0	74.2	91.7	36.7	54.0	71.5	93.0	
Lucerne stalks	13.01	16.1	25.0	40.5	71.8	19.7	24.0	13.2	45.0	
"Berseem" leaves	34.4	34.3	46.7	76.0	104.0	36.6	46.7	55.5	90.4	
"Berseem" stalks	5.4	10.2	21.9	39.0	64.0	9.5	18.5	11.5	47.7	
"Shaftal" leaves	31.0	30.0	53.0	83.1	85.7	29.6	49.7	56.4	87.3	
"Shaftal stalks"	3.5	8.4	21.1	40.0	42.1	4.6	14.9	8.1	41.7	

Mg=mg. vitamin E content per 100 g. dry weight of sample.

cutting (29-34 mg%) after which it increases upto the 5th cutting. Stems of these plants contain vitamin E which increases from the 1st to the fifth cutting. The leaves contain more vitamin E than the stems but the difference between the vitamin E content of these two parts decreases with increase in the number of cuttings.

In trial B vitamin E increases right from the first to the fourth cutting of leaves. The leaves of lucerne contain slightly more vitamin E than the leaves of the other plants. The stems show increase in vitamin E upto the second cutting, and then declines accompanied by a sudden rise in the fourth cutting in all the cases. This feature is common in stems of these plants and is different from the stems of the same plants in trial A.

Kohler *et al*[13] has reported the α-tocopherol content of fresh freeze dried meal of alfalfa grown in the USA as 18-22 mg% which is obviously the meal prepared by the whole plant consisting of both leaves and stems. In our studies the leaves contain much more quantities than this and even stems contain more quantities than this after the first cutting.

They have reported loss upto 5-33% during dehydration and storage. In the present work the effect of dehydration on the stability of vitamin E has not been studied.

The present studies show that there is not much difference in the contents of pigments and vitamin E of corresponding cuttings in trials A and B and trial A produces more carotenoids, chlorophylls and vitamin E also in all the species taking into view the time factor. Therefore, it is recommended on the basis of results of the present studies that a cutting period of 20-25 days would be more favourable for the production of these compounds.

Much work has been done on the production of leaf protein concentrates from forage crops[14-16] and the method employed is the extraction of juice by mechanical means followed by precipitation by heat. The product so obtained is dark green in colour due to the presence of chlorophylls, has a grassy taste and the high lipid content affects its keeping quality.

If the present procedure is adopted, *i.e.* the pigments are extracted first and the residue is treated for the preparation of leaf protein concentrate, the protein obtained is almost colourless and odourless due to the removal of lipids by solvent extraction and hence a better keeping quality. Moreover, it would be much cheaper as all the components could be beneficially utilized. Biological evaluation and preparation of protein concentrate from the residue so obtained are being studied.

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