STUDIES ON THE CHEMICAL COMPOSITION AND PHYSICAL PROPERTIES OF PLANT TISSUE FLUIDS. PART II.

EFFECT OF MINERAL FERTILISERS ON THE TISSUE FLUIDS OF RAGI (*ELEUCINE CORACANA*, LINN.).

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The desirability of examining the properties of saps from agricultural plants has long been established. Harris and co-workers (*J. Agr. Res.*, 1924, **27**, 267) have compared the physico-chemical properties of tissue fluids from two types of cotton grown under a variety of conditions including irrigation. Gilbert and Hardine (*J. Agr. Res.*, 1927, **35**, 185) have indicated the possibility of using sap concentration as a guide to fertiliser requirements of plants by working out critical concentrations for nitrogen, phosphorus and potassium. When the composition fell below these values, a deficiency was assumed. McCool and others (*J. Amer. Soc. Agron.*, 1928, **20**, 778; 1930, **22**, 434) have shown that fertiliser treatment altered the composition of tissue fluids. This is further supported by Pettinger (*J. Agr. Res.*, 1931, **43**, 95) who investigated the desirability of employing expressed sap as indicator for nutrient needs of corn plants. In these laboratories, studies have been made on saps of sandal in relation to health and disease by Varadaraja Iyengar (*This Journal*, 1928, **11A**, 103; 1929, **12A**, 295) of sandal, as influenced by different host plants, by Sreenivasa Rau (*This Journal*, 1934, **16A**, 146) and of beans as affected by age, season, etc., by Narasimhacharya and Sastri (*This Journal*, 1931, **14A**, 1) in Part I of this series.

Ragi is a dry cultivated crop generally raised in many parts of South India. The grain, however, forms the staple food of the masses in the Mysore State and is considered to be highly nutritious. It was considered desirable therefore to investigate the effect of added mineral fertilisers and age on the physical properties of tissue fluids derived from the different parts of this plant.

Materials and methods.—The present study was carried out with H22, a high yielding seasonal variety evolved by the Mysore Agricultural Department. The plants were grown in pots each containing 20 lbs. of surface soil well mixed with 10 lbs. of washed sand. At the rate of 80 pots for each set, 320 pots were used. The following treatments were given:—

Table showing the distribution of fertilisers.

Treatment	Nitrogen as sodium nitrate	Phosphorus as superphosphate	Potash as potassium sulphate
	Quantities added in grams per pot		
Complete minerals ($NaNO_3 + K_2SO_4 +$ Super) ..	1·44	2·72	1·36
One mineral less ($NaNO_3 +$ Super)	1·44	2·72	Nil
Two minerals less ($NaNO_3$ only)	1·44	Nil	Nil
Control (No mineral)	Nil	Nil	Nil

Sowings were done on alternate days beginning with sodium nitrate followed by nitrate and superphosphate, then control, and finally the three fertilisers added together. This arrangement facilitated the collection of samples of the same ages on different days. Addition of water was regulated after a few preliminary trials. On rainy days, watering was suspended. Earliest germination was noticed in about 70 hours and the same was complete in a week's time.

Growth was poor in the control and in the nitrate-treated pots, while in the others, it was quite satisfactory. Thus, phosphorus availability appeared to be one of the possible limiting factors. No apparent difference in growth could be noticed between the control plants and those receiving sodium nitrate. Analysis of the soils showed that in both cases, nitrate nitrogen content was almost the same. In view of this and a general deficiency of nitrogen observed in all the cases, a further dose of nitrate was added to all the pots except the controls.

Collection of samples.—Pots showing representative growths were chosen at each stage. The samples were usually collected between 8 and 9 a.m. on the appointed days. After emptying pots carefully, the plants were washed free from adhering soil and subsequently transferred in paraffined bottles to the laboratory where the roots were further washed in running water. The wet roots were gently pressed between folds of blotting paper to remove adhering moisture.

A portion of the material was utilised for moisture determination, and another for the extraction of tissue fluids. Biometric measurements of both the above-ground parts and the roots were made along with their dry weights.

The following determinations were made on the expressed sap:—freezing point, electrical conductivity, pH, total solids, total nitrogen, mineral matter, phosphorus and potassium.

Analytical methods.—(1) *Moisture*: Representative plants were selected and weighed and divided into tops and roots. Moisture was determined by drying the specimens in an oven at 105° C. for 24 hours and expressed as per cent. on fresh weight.

(2) Since the root system of ragi is normally large and curled up within the pots, the average root length of a number of plants was obtained and is considered here. The ratios top/root have also been reported.

(3) *Sap extraction.*—Known quantities of top and root portions were employed for the extraction of sap according to an earlier method detailed by us (*This Journal,* 1935, **18A**, 7). In the course of this study, the several factors involved in the extraction of the juice, such as pressure and time of extraction were kept uniform so that the difference, if any, could be traced to the related treatments only.

(4) *Total solids.*—Total solids were determined by drying known volume of the sap first on water-bath and then in oven at 105° C. for half hour.

The other physical constants were determined according to methods detailed in a previous paper (Varadaraja Iyengar, *This Journal*, 1928, **12A**, 295).

(5) *Mineral content and phosphorus.*—The dry matter resulting from total solids was ashed as usual at 500° C. for 6 hours, and weighed after cooling in a desiccator. The resultant ash was taken up with warm dilute acid and filtered. The filtrate was made up to 100 c.c. An aliquot of this was used for estimating phosphorus.

(6) *Total nitrogen.*—In another portion of the sap, total nitrogen was estimated according to Kjeldahl-Gunning's modification to include nitrate nitrogen (*Official methods, A.O.A.C.*, 1925).

(7) *Potassium.*—A third fraction of the sap was charred with concentrated sulphuric acid and subsequently ignited in a muffle. In the residue, potassium was estimated as perchlorate (Varadaraja Iyengar, *loc. cit.*).

RESULTS.

In view of the very poor growth of the control plants as also those that received sodium nitrate, the results have been considered separately in two different series.

SERIES A. (Controls and nitrate treated plants).—All the plants in this series presented a sickly, yellowish appearance and had restricted root development. Tillering was weak and production of earheads also comparatively poor. Sufficient experimental material was not available in the early stages, so only the results obtained towards the closing stages are presented in Table I.

TABLE I.

Physical and chemical data for tissue fluids of ragi plants.

Determinations	Number of days from start							
	113		122		131		140	
	Control	Nitrate	Control	Nitrate	Control	Nitrate	Control	Nitrate
Moisture content of whole plant as % ..	69·3	73·5	66·1	69·7	64·7	65·4	57·3	63·6
Top length / Root length ..	1·28	1·16	1·18	0·92	0·76	1·12	0·93	1·14
Sap yield in grams per 100 grams of tissue—								
(a) Root ..	46·6	41·8	45·4	39·4	39·8	43·6	31·3	38·7
(b) Top ..	49·4	43·8	51·0	48·3	53·8	51·2	39·7	43·4
Sap composition—								
(1) By evaporation as total solids (in grams) per 100 cc.								
(a) Root ..	1·94	1·45	1·07	1·59	1·16	1·39	1·04	1·67
(b) Top ..	5·85	5·44	4·34	5·17	4·71	4·61	3·99	4·18
(2) By lowering of the freezing point in Degree Centigrade								
(a) Root ..	0·73	0·71	0·67	0·62	0·64	0·61	0·59	0·65
(b) Top ..	1·44	1·27	1·25	1·16	1·19	1·13	1·18	1·21
pH of sap—								
(a) Root ..	6·15	6·10	6·17	6·15	6·33	6·05	6·23	6·13
(b) Top ..	5·85	5·80	5·90	5·81	5·80	5·75	5·77	5·33
Electrical conductivity $K \times 10^5$—								
(a) Root ..	399	408	433	467	465	394	479	472
(b) Top ..	1386	1381	1143	1332	1217	1149	1360	1079
Total ash as per cent. of total solids—								
(a) Root ..	1·98	1·28	2·03	1·27	2·30	1·30	2·59	1·93
(b) Top ..	3·62	3·52	5·89	3·81	5·27	4·63	5·14	5·46
Total nitrogen as per cent. of total solids—								
(a) Root ..	1·82	2·55	2·30	2·75	4·11	3·71	3·69	2·08
(b) Top ..	1·35	1·18	2·06	1·53	2·23	1·94	2·31	2·46
Phosphorus as per cent. of total soids (P_2O_5)—								
(a) Root ..	0·29	0·30	0·59	0·38	0·54	0·43	0·61	0·37
(b) Top ..	0·82	0·75	0·93	0·79	0·82	0·85	0·95	0·89
Potassium as per cent. K_2O of total solids—								
(a) Root ..	0·80	0·71	1·11	0·81	1·09	0·84	1·05	0·73
(b) Top ..	0·82	0·83	1·07	0·85	1·01	0·96	1·03	1·00

The control plants generally contained more dry matter and ash and showed a higher pH than those receiving nitrate. The sap yielded by the former was slightly greater in quantity and richer in composition than that from the latter. In both cases, however, top portions gave more concentrated juices with larger mineral contents, determined as total ash or as conductivity units. The controls had more of phosphorus, potash and also nitrogen than the nitrate receiving ones. While the tops revealed a high phosphorus content, the distribution of potash in both top and root did not show such great variations between the two parts.

SERIES B.—The growth and development of plants in this series were good. Both root development and tillering were quite satisfactory. Flowering commenced within about 3 months after sowing. Grain formation began after 110 days and the plants were harvested a month after, *i.e.*, 140 days from the beginning.

TABLE II.

Moisture, biometric data and yield of sap of ragi plants.
(Data expressed on fresh weight basis)

Number of days from start	Moisture content of entire plants as per cent.		Top length / Root length		Yield of sap (grams) per 100 grams of tissues			
					N + P		N + P + K	
	N + P	N + P + K	N + P	N + P + K	Root	Top	Root	Top
32	81·3	80·5	0·42	0·83	39·8	61·6	44·2	65·3
41	79·0	76·0	0·71	0·75	49·4	68·6	54·6	66·6
50	82 6	78·1	0·71	0·87	59·1	68·0	55·1	63·0
59	81·7	79·2	0·73	0·98	61·6	63·9	61·0	72·1
77	76·9	79·9	1·27	1·01	57·8	62·6	62·3	66·6
86	78·9	74·4	1·69	1·36	50·1	58·6	48·2	67·0
95	71·9	76·7	1·27	1·11	59·7	70·0	47·4	65·7
104	70·6	72·9	1·13	0·96	65·6	63·7	46·3	62·2
113	73·5	71·2	1·04	1·58	43·7	43·1	47·3	47·1
122	76·2	72·3	1·17	1·53	42·7	48·0	45·5	61·1
131	70·2	69·9	1·03	1·42	48·6	54·7	50·8	54·0

Generally, a greater dry weight is noticeable in the fully manured plants. The higher value for top/root ratio in them tends to decrease towards the grain formation stage. The yield of sap is erratic and cannot be directly correlated with the treatments.

TABLE III.

Sap concentration of plants treated with different fertilisers.

Number of days from start	Total solids in the expressed juice of ragi plants							
	By evaporation of sap				By lowering of the freezing point			
	N + P		N + P + K		N + P		N + P + K	
	Root	Top	Root	Top	Root	Top	Root	Top
	In grams per 100 c.c.				In Degree Centigrade			
32	2·42	4·66	2·51	4·84	0·66	1·27	0·69	1·30
41	2 05	4·84	2·68	4 85	0·68	1·25	0·72	1·29
50	2·26	4·63	2·42	4 72	0·70	1·39	0·67	1·26
59	2·06	4·94	2·53	4·79	0 64	1·21	0·70	1·32
77	2·32	4·89	2·45	5 43	0 63	1·31	0·72	1·35
86	2 60	5·09	2·44	5 80	0·73	1·38	0·69	1·34
95	2·43	5·62	2·37	5·69	0·72	1·39	0·73	1·43
104	1·78	5·16	2·69	5·91	0·70	1 36	0·66	1·33
113	2·00	6·44	2·23	5·56	0·62	1·15	0·62	1·31
122	1·95	4·49	2·21	5·40	0·72	1·41	0·83	1·55
131	2·14	4·13	2·20	5·17	0·80	1·49	0 84	1·57

Addition of potash appears to have led to a slight increase in the average solid content of the juice, obtained from the root as well as the top.

TABLE IV.

Reaction and mineral content of the juices in manured ragi plants.

Number of days from start	pH of expressed sap of plants treated with				Electrical conductivity of sap expressed as $K \times 10^5$				Ash content expressed as per cent.	
	N + P		N + P + K		N + P		N + P + K		N + P	N + P + K
	Root	Top	Root	Top	Root	Top	Root	Top	Top	Top
32	5·42	5·40	5·50	4·90	441	1158	493	1317	5·07	4·61
41	6·20	5·61	5·90	5·40	413	1421	514	1396	4·95	4·69
50	5·80	5·45	5·85	5·50	468	1298	457	1632	4·89	4·71
59	5·60	5·30	5·75	5·10	397	1186	541	1141	5·01	4·62
77	5·46	5·09	5·72	4·98	495	1277	530	1082	4·86	3·49
86	5·35	4·93	5·30	4·91	525	1365	497	1453	4·67	3·42
95	5·59	5·41	5·27	5·06	573	1284	483	1097	4·21	3 52
104	5·62	5·45	5·25	5·12	514	1208	429	1174	4·43	2·79
113	5·97	5·65	6·10	5·85	377	1029	394	1367	5·16	3·18
122	6·15	5·75	6·25	5·82	533	1129	462	1245	5·21	3·53
131	6·10	5·70	6·20	5·80	796	1129	871	1233	5·57	3·93

The sap from top portions of plants receiving potash is generally slightly more acid than that of the others without this element. The high pH value for the roots of potash-lacking plants on 41st day is indeed inexplicable. Potash also appears to have increased the electrical conductivity, though during the period of flowering and grain formation there has been a sudden drop to a lower value than in the N + P series. The ash content of sap from potash treated plants was found to be invariably lower than that from the others. This observation would at least partially account for the slight acidity of the former. Application of potash obviously leads to greater movement of minerals thus slightly reducing the buffering capacity of the medium. The nature of the moving minerals as also the direction of movement requires further study. It may be added that there does not appear to be any direct correlation between the ash content and the electrical conductivity figures. A more detailed study is under way to explain this discrepancy.

Table V.

Chemical composition of the tissue fluids.
(Expressed as per cent. on total solids in sap)

Number of days from start	Total nitrogen as N				Phosphorus as P_2O_5				Potassium content as K_2O			
	N + P		N + P + K		N + P		N + P + K		N + P		N + P + K	
	Root	Top	Root	Top	Root	Top	Root	Top	Root	Top	Root	Top
32	0·98	1·40	1·52	1·33	0·22	1·30	0·23	1·29	0·41	0·71	0·59	0·76
41	1·95	1·37	1·49	1·39	0·24	1·25	0·20	1·27	0·47	0·70	0·66	0·79
50	2·40	1·64	1·86	1·52	0·23	1·56	0·21	1·54	0·41	0·72	0·63	0·78
59	2·32	1·56	1·85	1·49	0·25	1·51	0·19	1·55	0·57	0·74	0·54	0·79
77	2·22	1·74	1·98	1·66	0·23	1·78	0·21	1·42	0·57	0·69	0·82	0·76
86	1·62	1·40	1·48	1·10	0·19	0·90	0·22	1·26	0·59	0·68	0·90	0·74
95	2·05	1·47	1·57	1·19	0·18	0·83	0·19	1·38	0·76	0·67	0·82	0·75
104	2·32	1·67	1·82	0·93	0·16	1·82	0·18	0·89	0·68	0·63	0·71	0·76
113	1·82	1·73	1·35	1·58	0·21	1·07	0·19	0·82	0·76	0·78	0·78	0·79
122	2·43	3·05	2·02	2·00	0·22	1·23	0·25	1·04	0·53	0·76	0·54	1·03
131	2·19	2·94	1·95	2·18	0·25	1·29	0·24	0·98	0·51	0·86	0·51	1·07

It may be noted that the sap from roots has generally a higher nitrogen content than the corresponding specimen from tops. (The sudden rise in the value for the top after the 122nd day is traceable to the extra dose of nitrate given a few days previously.) The sap from potash treated plants is generally poorer in nitrogen than that of the corresponding specimens without potash. Phosphorus content of sap from the top is generally more than that from the root. Here again, potash fertilised specimens give slightly lower values than those without this treatment. The potash values of roots and tops are somewhat erratic, but generally the sap from the latter has a higher value. Potash treated plants have naturally more potash in the sap than the untreated ones, but the difference is not considerable. It may be added that on the 86th day, the movement of nitrogen and phosphorus is quite striking. As pointed out earlier, the flowering of the plants set in at about this period.

DISCUSSION.

Attention has already been drawn to the fact that of the salts employed in the present study, phosphate was a limiting factor. This observation is invariably true of most South Indian soils which have been shown by Norris (*Agri. J. India*, 1920, **15**, 433) to be quite deficient in available nitrogen and phosphorus. Unless this deficit is made good, it will be extremely difficult to obtain any marked rise in the level of crop yield.

Mention has also been made of the poorer response of the crop to the first dosage of nitrate even from phosphate treated plants. This may be traceable to the fact that the applied nitrate was washed down (in part by rain and partly by good watering) to a substrate below the requirements of the young roots. The dosage of nitrate applied in drier and more available condition effected a distinctly better response, as indicated by a uniform increase in dry matter. Among the various treatments adopted, it is interesting to observe that the controls (unmanured) showed a higher dry matter content than those receiving mineral fertilisers which generally contained more moisture. Application of intrate leads to still greater increase in moisture content but there is no evidence to show that phosphate had any lowering effect. On the contrary, phosphate treated plants had less moisture than others. These findings support those of Frear (*J. Agr. Res.*, 1931, **42**, 53), but do not agree with those of McGillivray (*J. Agr. Res.*, 1927, **34**, 97). The observations of the latter would more appropriately apply to field crops which either depend on rainfall or receive only limited irrigation.

The ratio of top to root is rather erratic but it may be observed that potash treated plants have generally higher ratios towards the end than the others.

The yield of sap is presumably more influenced by season than by fertiliser treatment. As may be naturally expected, the yield of sap decreases with the approach of harvest.

There is no striking parallelism between evaporation and freezing point methods of determining sap concentration, and this is in conformity with the earlier studies on sandal (Varadaraja Iyengar, *This Journal*, 1929, **12A**, 299). Further work is in progress to determine the factors involved. The juice from top portions was invariably more concentrated than that from the corresponding root portions.

Ragi juice generally reacts on the acid side. Comparing specimens collected after the 113th day, it will be found that the sap becomes less acidic with the increasing supply of minerals. The difference, however, is not very pronounced. These observations will apply to the root as well as the top from the corresponding specimens.

Attention has already been drawn to the fact that application of nitrate leads to an increase in the total nitrogen content of the sap. It is interesting at the same time to find that the percentage of nitrogen diminishes appreciably when the nitrate is supplemented by phosphorus, and still more so when potash was also added. This diminution is more attributable to better growth and greater intake of water in the two latter cases, thus yielding a more dilute sap than to an actually reduced intake of nitrogen. Indeed, Reed (*J. Agr. Res.*, 1921, 21, 81) has correlated rapid growth in plants with low sap concentration, while higher concentrations were generally found associated with slow growth and poorer fruit-bud development. It is also generally recognised that crop lodging and other unfavourable symptoms are associated with higher nitrogen content in sap.

Application of phosphate does not produce any marked change in the phosphorus content of the root sap but leads to distinct increase in that from the top. This would show that under normal conditions, there is very little accumulation of phosphorus in the roots but that the major part of the intake is passed on to the top portions. When phosphorus is supplemented by potash, there is some slight reduction in the phosphorus content especially at the time of flowering and seeding. This would show that potash facilitates translocation and subsequent storage of phosphorus in those parts.

The potash content of the sap from the tops is always slightly more than that from the roots. Fertilising with potassium sulphate has led only to a slight increase in potash content over the others. This is in accordance with the general finding that Indian soils are not ordinarily deficient in potash and can supply the average crop requirements without any further addition. This observation does not however preclude the fact that the extra dosage of potassium will further facilitate translocation changes, improve the general growth of the plant and impart resistance to disease.

SUMMARY.

1. An examination has been made of the tissue fluids of ragi (*Eleucine coracana*) a useful crop of South India, as affected by mineral fertilisers, age, etc.

2. It was found that the growth of this species was limited by the supply of phosphorus, a feature which is common to all South Indian soils. As there was some difference in growth between the control plants and those receiving sodium nitrate, thus limiting the

availability of experimental material, a second dose of nitrate was applied at a later stage, to all the pots except the unmanured ones.

3. The tissue fluid from the controls was richer in composition, besides being slightly greater in volume, than that from plants receiving sodium nitrate. In the former case, the juice was found to contain more dry matter, ash, phosphorus and potash, in addition to its being slightly more alkaline. As would naturally be expected, the top portions of the nitrogen treated plants contained more of this element. In both instances, the top portions yielded more juice with increased mineral content, determined as either total ash or as conductivity units.

4. Among the phosphorus treated plants, the potash receiving ones had greater dry weight, with a high top/root ratio towards the harvesting stage.

5. The plants treated with potassium were found to yield a juice generally richer in composition both from the roots as well as the top portions. The influence of added potassium was not very marked on the quantity of tissue fluids derived from such plants.

6. The juice from fully manured plants was slightly more acidic and had less mineral content than that derived from plants without potash. A greater movement of minerals was indicated in the former.

7. The roots were found to store a greater per cent. of nitrogen than the tops. In the completely manured plants, nitrogen content was less than in those lacking potassium alone. Where growth was poor as in the nitrogen applied cases, the composition of the juice with reference to this component was fairly high.

8. Phosphorus content of the juice increases with the application of phosphate. and there is a rapid mobilisation of the same to the growing points.

9. Striking results were not secured with potassium treated plants. although a general increase in its concentration was apparent.

10. During the flowering period, *i.e.*, about 3 months following sowing, significant changes in the composition of the juice were observed.

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