Effect of Nitrogen Level and Water Deficit on Nitrate Reductase Activity (NRA) from Leaves of Two Sugarcane Cultivars – Co 957 1001[1]

Y.A. Abayomi*; E.O. Etejere**; O. Fadayomi*

ABSTRACT

Two commercial sugarcane cultivars grown in Nigeria (Co 957 and Co 1001) were given three levels of nitrogen fertilizer equivalent to O, 100 and 200 kg N/ha and subjected to water stress lasting seven days at two ages during growth in a pot experiment. The objective was to investigate the effects of nitrogen levels and water deficit on nitrate reductase activity (NRA) and water status in sugarcane leaf. The recovery rates of the plants were also investigated by measuring NRA and water status after re-watering the plants. Nitrate reductase activity (NRA) augmented with increasing nitrogen levels when moisture was adequate. However, during the stress period, NR activity disappeared faster at higher nitrogen levels with advancement of the stress period. The relative water content of leaf was also found to increase with N levels and decrease with stress period advancement. Similarly, NR activity was found to decrease with age of the plant. Furthermore, the variations in NR activity caused by water deficit increased with age. At all nitrogen levels, Cv. Co 957 had higher NR activity than Cv. Co 1001. However, the differences were only significant at eight weeks after planting.

COMPENDIO

Se realizó un estudio para investigar los efectos de niveles de nitrógeno y déficit hídrico sobre el estado de hidratación de la hoja así como la actividad de reductasa del nitrato (ARN) en caña de azúcar (*Saccharum officinarum*). Se estableció un experimento en potes con dos variedades de caña, Co 957 y Co 1001, a las cuales se les aplicó tres niveles de fertilizante nitrogenado equivalente a 0, 100, y 200 kg N/ha, además de estrés hídrico por un período de siete días a dos diferentes edades del cultivo. También se determinó las tasas de recuperación de las plantas midiendo la actividad de NR y el estado hídrico de las plantas después de rehidratar las mismas. La actividad de la NR aumentó con los niveles de N si la humedad era adecuada. Sin embargo durante el período de estrés la actividad de la RN desapareció a una tasa más rápida en los niveles altos de N conforme el estrés avanzaba. De igual manera la actividad de la RN bajó con la edad de la planta, más aun las variaciones en la actividad de la NR causadas por el déficit hídrico aumentaron con la edad. En todos los niveles de N el cultivar Co 957 mostró mayor actividad enzimática que el cv. 1001 sólo después de ocho semanas de sembradas.

INTRODUCTION

The productivity of agricultural plants is limited largely by nutrient deficiency and drought. Nutrient deficiency of field crops is usually diagnosed by appraisal of visual symptoms and leaf or soil analysis. However, these methods may be inaccurate, labourious, or symptoms may appear too late for the cause to be remedied without significant loss of productivity (10, 19). Moreover, leaf analysis usually fails to distinguish between the biochemically active ions of an element and the relatively inactive or the total ion amount. Bar-Akiva and Sternbaum (3) were of the opinion that the examination of element activity in the plant cell may increase the effectiveness of plant analysis as a tool for appraisal of nutrient status of field and horticultural crops. Thus, there has been increased interest in the use of enzymes as biochemical markers of nutrient deficiencies (2, 3, 5, 10).

The suitability of nitrate reductase assay as an indicator for the determination of nitrogen status in plants, following the demonstration of the activity of the enzyme systems in the leaves, has been reported (3). The level of nitrate reductase activity could also be used as a physiological nitrogen status of the plant (3), while the magnitude of its inducibility could be used as a measure of the capacity of the plant to utilize nitrate from the soil (11). The non-constitutive nature of NR and the dependence of the enzyme on the presence of nitrate have been widely noted in higher plants (13, 15, 25, 27, 28, 29).

However, other reports have indicated that water deficit may cause loss of activity of the NR enzyme in leaves of higher plants. Mattas and Pauli (21) have reported a reduction of about 84 percent in NR in corn after wilting. Similarly, a decrease of about 50 percent was obtained in barley (*Hordeum vulgare*) after three days of drought (16). Bardzik *et al.* (4) observed that NR decreased markedly with a water deficit of 10-20 percent, while Viqueira *et al.* (32) reported a significant negative correlation between water deficit and NR in sugarcane leaf.

1 Received for publication 20 March 1987

* Sugar Research Institute, University of Ilorin, Nigeria.

** Biological Sciences Department, University of Ilorin, Nigeria

The sugarcane plant has been found to respond in a linear fashion to nitrogen fertilization. However, NRA has been implicated in the assimilation of nitrate. The objective of the present study was to determine the effect of variations in external nitrogen supply and water deficit on nitrate reductase activity in sugarcane leaf

MATERIALS AND METHODS

Two cultivars of sugarcane – Co 957 and Co 1001 (both of which constitute about 80% of commercial sugarcane cultivars in Nigeria) were grown (using one-budded sets) in pots filled with loamy sand soil (Table 1). Three levels of nitrogen fertilizer in the form of calcium ammonium nitrate (CAN) at rates equivalent to 0, 100 and 200 kg N/ha were applied to the cane plants in a factorial experiment replicated four times. The plants were watered twice weekly from planting until eight weeks after planting, when the first moisture stress was initiated by withholding water for seven days, after which water was re-introduced in a recovery trial. These processes were repeated at 16 weeks after planting. During the stress/recovery periods, plant leaves were sampled for water status and nitrate reductase activity determination at zero, three and seven days after irrigation was suspended; and at one, three and seven days after water was re-introduced. The study was carried out between November 1985 and February 1986 (Atmospheric conditions showed a relative humidity of 49.7-64.7% and a temperature of 34°C).

Leaf Water Status Measurement. Leaf status was estimated by the method of Weatherly and Slayter (30), as described by Naidu *et al.* (23). Duplicate 10 cm sections were taken from the mid-point of the No. 2 leaf of a plant. The original weight was determined immediately after harvest, and the tissue was floated in distilled water for 24 hours in a closed chamber. At this time, the saturated weight was taken, and the tissue was dried at 95°C for 24 hours for dry weight determination. From the data obtained, the percent relative water content (RWC) and water saturation deficit (WSD) were estimated as follows:

$$\%\ RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times \frac{100}{1}$$

$$WSD = 100 - \%\ RWC$$

Nitrate reductase assay. The *in vivo* assay technique of Mulder *et al.* (22), as described by William and Daniel (31), was used. A 10 cm section of No. 2 blade [the spindle leaf half or more open was designated No. 1, the next most mature leaf was No. 2 (20)] was rinsed with distilled water to remove all traces of soil. Then the leaf was chopped into 1-2 mm segments, out of which 200 mg was immediately put into a test tube containing the incubation medium. The medium is a mixture of 5 ml 0.1M KH_2PO_4 (p^H 7.5), 3 ml of 1% (vlv) 1 – propanol and 2 ml of 0.1M KNO_3. The contents were infiltrated three times for about two minutes each time. The tubes were subsequently incubated for one hr in the dark at 37°C. The nitrate formed was assayed by adding 1 ml of 1% (wlv) sulphanilic acid in 3N Hcl, and 1 ml 0.02% (wlv) of 1 – napthylamine, 7 – sulphoric acid to the incubated medium. Colour was allowed to develop for 30 minutes and its absorbance was measured in a Bausch & Lomb Spectronic 20 at 540 nm. A standard curve was prepared with sodium nitrite, and the amount of NO_2^{-1} in the sample was estimated from the curve. The enzyme activity was expressed as μ moles NO_2^{-1} produced per hour incubation period per gramme fresh leaf weight.

Table 1. Some physical and chemical properties of soil used in the study.

Sand (%)		62.4
Silt (%)		28.0
Clay (%)		9.6
Textural class		Sandy loam
p^H (in 0.1M $Cacl_2$)		4.40
Na	(ppm)	42.92
K	(ppm)	76.67
Total N	(%)	0.16
Organic matter	(%)	2.20
C.E.C. meg/100 g soil		16.17
Available P	(ppm)	302.42

RESULT AND DISCUSSIONS

Cv. Co 957 recorded consistently higher nitrate reductase activity (NRA) at all nitrogen levels than Cv. Co 1001 during the two periods of sampling when soil moisture is non-limiting. However, the differences were only significant at the early age (8 WAP) (Fig. Similar differences in NR activity among cultivars of sugarcane have been reported (32). The level of NR activity could be used as a physiological nitrogen status of a plant (3), while the level of its inducibility had been used as a measure of the capacity of the plant to utilize nitrogen from the soil (11). Cv. Co 957 could then be said to have higher physiological nitrogen status than Cv. Co 1001, and thus possess the capacity to take up and utilize more nitrogen from the soil, as high NR activity in the plant suggests a higher rate of nitrate reduction and utilization of reduction product (24) The importance of nitrogen in cane yield had been widely noted (7, 8, 26). The ability of Cv Co 957 to utilize nitrogen from the soil better could then explain its usual significantly better yield as compared to Cv Co 1001 Similarly, heavy nitrogen fertilizer application had been observed as an important factor in flowering control in sugarcane (6, 12) This presumably was to make the plant take up excess nitrogen, thereby prolonging its vegetative growth Thus, Cv Co 957, with its ability to take up higher concentrations of nitrogen from the soil, has not been observed to flower under Nigerian conditions, while Cv Co 1001 is known to flower profusely. Both cultivars had higher relative water content and hence low water saturation deficits (Fig 1)

NR activity showed a positive linear relationship with increasing nitrogen level when soil moisture is non-limiting (Fig 1) This linearity was significant (at P = 0 05) for the differences between the two nitrogen levels and the control only at eight weeks after planting. Like enzyme activity, the relative water content (RWC) showed signs of a positive linear relationship with nitrogen levels. However, water saturation deficit (WSD), negatively correlated with RWC, decreased with increasing nitrogen levels

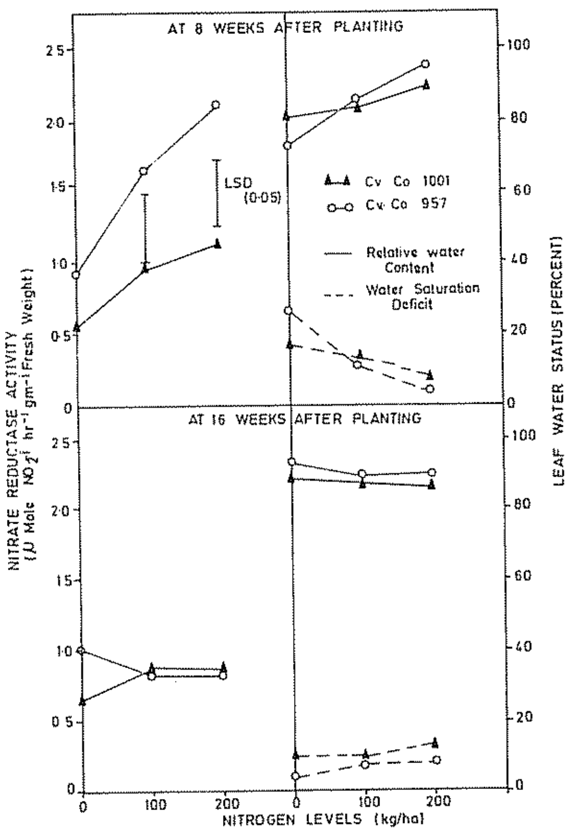

Fig 1 Effect of nitrogen levels and plant age on nitrate reductase activity (NRA) and water status in leaves of two sugar cane cultivars

The increased enzyme activity with increasing nitrogen levels observed in this study supports the view that nitrate reductase is a non-constitutive, inducible enzyme (1, 14) The dependence of the enzyme on the presence of nitrate has been widely noted in other higher plants (25, 28, 29) A constantly low level of the enzyme activity in roots of maize in the absence of nitrate has been reported (27) Thus, in the present study very low activity of NR was recorded from plants given zero N (at 8 WAP) and in all plants at 16 WAP. The low level of enzyme activity exhibited by all plants (including those given higher N levels at planting) at 16 weeks after planting was an indication that the fast-growing plants had exhausted the applied nitrogen

The increase in leaf RWC with increased nitrogen levels was in line with the observation of Clements and Kubota (9), who reported a close relationship between nitrogen concentration and leaf moisture content in the sugarcane plant. The report of these authors implied that plants growing under conditions of inadequate nitrogen supply are likely to have low moisture content no matter what the moisture conditions in the soil are. A significant positive correlation similar to the one observed in this study (r = 0 63) has been reported between NR activity and RWC content of sugarcane leaf (32). This explains the dependence of the response of cane plants to additional nitrogen applications in the soil moisture regime.

Tables 2 and 3 show the effect of water stress on NR activity and WSD of the leaves of the two sugarcane cultivars at eight and 16 weeks after planting respectively. NR activity reduced with increased water stress period, while WSD increased simultaneously during the same period. The results further showed that a zero N level showed less variation in enzyme activity during the stress period, while the higher nitrogen levels showed greater variations at 8 WAP. Similarly, Cv Co 957 showed a higher variation in NR activity than Cv Co 1001 at both ages during the drought periods (Table 4). Seven days after irrigation was suspended, decreases in enzyme activity up to 64 38 (Cv Co 957), 4742 (Cv Co 1001), 92 41 (Cv Co 957), and 91 12 (Cv Co 1001) percent were observed at eight and 16 weeks after planting, respectively. In both cultivars, enzyme activity decreased with age, while variations in NR activity occasioned by water stress increased with age.

Table 2. Effect of moisture stress on nitrate reductase activity (μ moles NO_2^{-1} hr^{-1} g^{-1} fresh weight) and water saturation deficit (%) in leaves of two sugarcane cultivars given different levels of nitrogen fertilizer at 8 WAP.

Nitrogen level (kg/ha)	Cv. Co 957						Cv. Co 1001					
	NR activity			Water saturation deficit (%)			NR activity			Water saturation deficit (%)		
Stress period (days)	0	3	7	0	3	7	0	3	7	0	3	7
0	0.912	0.781	0.775	5.69	17.28	26.90	0.562	0.385	0.312	6.31	15.39	17.47
100	1.687	0.369	0.206	4.23	14.63	34.30	0.919	0.656	0.500	8.35	12.29	21.26
200	2.125	0.337	0.206	3.64	11.15	24.59	1.112	0.587	0.531	13.47	15.00	23.56
LSD (0.05)	0.487	0.380	0.229	10.73	4.19	4.79	0.487	0.380	0.229	10.73	4.19	4.79

These results supported the earlier report of Viqueira *et al.* (32), who observed decreases in NR activity of two sugarcane cultivars with increasing plant age and drought advancement. Similar decreases in nitrate reductase activity at different times after irrigation was suspended have been earlier observed for other crops (4, 16). At higher N levels, NR activity disappeared faster with increased drought period as plants with higher nitrogen levels lost water more rapidly than the control. This could be due to higher leaf surface areas and hence higher transpiration rate. Moreover, higher physiological activity as epitomised by higher NR activity will result in a higher rate of water loss in plants. This observation supports the view of Humbert (17), who reported that higher nitrogen encouraged larger leaf area, perhaps a disadvantage during drought.

The rate of disappearance of enzyme activity and leaf water status are indications of drought tolerance/susceptibility (23, 32). NR activity was observed to decrease, and WSD to increase faster and more in Cv. Co 957 than in Cv. Co 1001 with moisture stress severity (Tables 2 and 3). This tends to suggest that the latter is more tolerant to drought than the former. However, field observations showed Cv. Co 957 to tolerate drought conditions better than Cv. Co 1001. Moreover, Cv. Co 957 had more dense and deeper rooting habits, as well as thinner stalks, than Co 1001. Lal and Mehrotra (18) reported that a drought-tolerant variety generally possesses higher green leaf numbers, larger shoot numbers per plant and thinner stalks. This supports the observation that Cv. Co 957 (usually with more green leaves and shoots per plant, as well as thinner stalks) can tolerate drought better. However, the rationale for the rapid loss of enzyme activity could then be as suggested by Viqueira *et al.* (32): a greater decrease of enzyme activity in a drought-tolerant variety could be considered as an adaptation mechanism for reducing energy requirements during moisture stress.

Tables 5 and 6 show that NR activity and leaf water status recovered rapidly when plants were re-watered during the two periods. By the third day after the plants had been re-watered, NR activity in

Table 3. Effect of moisture stress on nitrate reductase activity (μ moles NO_2^{-1} hr^{-1} g^{-1} fresh weight) and water saturation deficit (%) in leaves of two sugarcane cultivars given different levels of nitrogen fertilizer at 16 WAP.

Nitrogen level (kg/ha)	Cv. Co 957						Cv. Co 1001					
	NR activity			Water saturation deficit (%)			NR activity			Water saturation deficit		
Stress period (days)	0	3	7	0	3	7	0	3	7	0	3	7
0	1.00	0.400	0.055	5.43	10.02	14.43	0.662	0.500	0.062	9.63	11.85	14.88
100	0.825	0.337	0.062	8.45	9.80	13.61	0.862	0.450	0.082	10.94	13.16	13.46
200	0.825	0.212	0.080	7.96	19.44	21.10	0.850	0.525	0.065	12.10	13.38	13.85
LSD 0.05	0.299	0.175	0.021	2.21	5.08	1.94	0.299	0.175	0.021	2.21	5.08	1.94

Table 4. Variations in nitrate reductase activity (NRA) in sugarcane leaf seven days after irrigation was suspended at eight and 16 weeks after planting.

Level of nitrogen (kg/ha)	Enzyme activity variations (%)			
	Eight weeks after planting		16 weeks after planting	
	Cv. Co 957	Cv. Co 1001	Cv. Co 957	Cv. Co 1001
0	15 02	43.95	94.50	90.63
100	87 79	45.59	92.48	90.49
200	90.30	52.25	90.30	92.35
Mean	64.37	47 26	92 43	91.16

Table 5. Effect of plant recovery (after re-watering) on NRA and WSD in leaves of two sugarcane cultivars given different level of nitrogen fertilizer. Data taken at 8 WAP.

Nitrogen level (kg/ha)	Cv. Co 957						Cv. Co 1001					
	NR activity			Water saturation deficit (%)			NR activity			Water saturation deficit (%)		
Recovery Period (days)	1	3	7	1	3	7	1	3	7	1	3	7
0	0.887	1.062	0 919	0.96	5.35	4.48	0.412	0.706	0.487	5.45	11.80	10 55
100	1.206	2.306	2.425	7.34	8.80	9 61	0.912	1 075	1.400	10.26	14.40	9 12
200	1.916	3.15	2.537	12 36	15 57	7.06	1.381	1.687	1 400	7.37	14.86	9.63
LSD (0.05)	0.440	0.704	0.638	2.91	2.89	4 11	0.440	0 704	0.638	2.91	2.89	4 11

Table 6. Effect of plant recovery (after re-watering) on NRA and WSD in leaves of two sugarcane cultivars given different levels of nitrogen fertilizer. Data taken 16 WAP.

Nitrogen level (kg/ha)	Cv. Co 957						Cv. Co 1001					
	NR activity			Water saturation deficit (%)			NR activity			Water saturation deficit (%)		
Recovery Period (days)	1	3	7	1	3	7	1	3	7	1	3	7
0	0.135	1.125	0.837	9.22	11.45	12.37	0.175	0.812	0 475	12.11	11.33	14.52
100	0 200	0.937	0 650	7.25	13 65	11.31	0.175	0.975	0.675	9.21	12.86	11 33
200	0.150	0.925	0.520	12.59	15.46	10.42	0.150	0.737	0.537	9 91	11.70	11 99
LSD (0.05)	0.058	0 429	0.237	4.23	4.79	3.24	0.058	0.429	0 237	4.23	4 79	3.24

leaves of the two cultivars of sugarcane had increased appreciably, with values greater than the pre-stress values being obtained. However, by the seventh day after re-watering, the NR activity had stabilized to near the pre-stress levels. The increase in NR activity by the third day of recovery could be due to influx of N through rapid water intake by plants. However, as the physiological activities of plants increased, the concentration of nitrogen dropped, hence the drop in NR activity. The high rate of recovery is an indication of the fact that there was no permanent loss of activity during the stress period.

From the foregoing, it could be suggested that the higher yield of sugarcane usually associated with increased nitrogen application is due to increased nitrate reduction (as indicated by increased NR activity with increased nitrogen levels) and the utilization of products of reduction in protein synthesis. Moreover, nitrate reductase is an inducible enzyme which functions well in adequate moisture.

LITERATURE CITED

1. AFRIDI, M.M.R.K.; HEWITT, E.J. 1964. The inducible formation and stability of nitrate reductase in higher plants. J. Exptl. Bot. 15(44):251-271.

2. BAR-AKIVA, A. 1971. Functional aspects of mineral nutrients in use for the evaluation of plant nutrient requirement. In Recent advances in plant nutrition. Ed. by R.M. Sanish. New York, Gorden and Breach Sc. Pub. v.1, p. 115-142.

3. BAR-AKIVA, A.; STERNBAUM, J. 1975. Possible use of the nitrate reductase activity of leaves as a measure of the nitrogen requirements of a citrus tree. Plant and Cell Physiology 6:575-577.

4. BARDZIK, J.W.; MARSH JUNIOR, H.V.; HAVIS, J.R. 1971. Effects of water stress on the activities of three enzymes in maize seedlings. Plant Physiology 47:828-831.

5. BARRETT-LENNARD, E.G.; ROBSON, A.D.; GREENWAY, H. 1982. Effect of phosphorous deficiency and water deficit in phosphate activities from wheat leaves. J. Exptl. Bot. 33:682-693.

6. BURR, G.O.; HARTT, C.E.; BRODIE, H.W.; TANIMOTO, T.; KORTSCHAK, H.P.; TAKAHASHI, D.; ASTON, F.M.; COLEMAN, R.E. 1957. The sugarcane plant. Annual Review of Plant Physiology 8:275-308.

7. CASTELLANOS, A.; GERRET, J.L.; LOPEZ, E. 1978. Study of different N.P.K. levels in sugarcane (*Saccharum officinarum*) Var. CP 5243. Agrotecnia de Cuba 10:35-49.

8. CHAUDHRY, B.A. 1983. Low sugarcane productivity in Negros – why? Sugar Land 20(6):4-7

9. CLEMENTS, H.F.; KUBOTA, T. 1942. Internal moisture relations of sugarcane. The selection of a moisture index. Hawaiian Planters' Record 46:17.

10. COX, W.J.; ROBSON, A.D. 1980. Optimization of plant nutrition in improving the efficiency of fertilizer use. In Proceedings of the Australian Agronomy Conference. p. 157-176.

11. CROY, L.I.; HAGEMAN, R.H. 1970. Relationship of nitrate reductase activity (NRA) to grain protein production in wheat. Crop Science 10(3):280-285.

12. EL-MANHALY, M.A.; FADAYOMI, O.; ABAYOMI Y.A.; OLOFINBOBA, M.O. 1984. Control of flowering in two commercial sugarcane varieties. Journal of Agricultural Science 103:333-338

13. HAGMAN, R.H.; FLESHER, D. 1960. Nitrate reductase activity in corn seedlings as affected by light and nitrate content of nutrient media plant physiology 35:700-708

14. HEWITT, E.J.; AFRIDI, M.M.R.K. 1959. Adaptive snynthesis of nitrate reductase in higher plants. Nature 183:57-58.

15. HEWITT, E.J.; FISHER, E.G.; CANDELLA, M.C. 1955. Factors affecting the activity of nitrate reductase in cauliflower plants. Ann. Rep. Long Ashton Rep. Station. p. 202-210.

16. HUFFAKER, R.C.; RADIN, T.; KLEINKOFF, G.E.; COX, E.L. 1970. Effects of mild water stress on enzymes of nitrate assimilation and carbosylative phase of photosynthesis in barley. Crop Science 10:471-473

17. HUMBERT, R.P. 1968. The growing of sugarcane. Amsterdam, Elsevier Publishing Company. p. 780.

18. LAL, K.N.; MEHROTRA, O.N. 1951. Physiological basis of drought resistence in sugarcane. Bienn. Conf. Sugarcane Res. Wkrs. Combatoure 1 Proceedings. Pt. 202, p. 33-40.

19. LEECE, D.R. 1976. Diagnosis of nutritional disorders of fruit trees by leaf and soil analyses and biochemical indices. J. Aust. Hort. Agric. Sci. 42:3-19.

20. MARETZKI, A.; DE LA CRUZ, A. 1967. Nitrate reductase in sugarcane tissues. Plant and Cell Physiology 8:605-611.

21. MATTAS, R.E.; PAULI, A.W. 1965. Trends in nitrate reductase and nitrogen fractions in young corn (*Zea mays* L.) plant during heat and moisture stress. Crop Science 5:181-184.

22. MULDER, E.; BOXMA, R.; VAN VEEN, W.L. 1959. Nitrate reduction in plant tissues. Plant and Soil 10:335-355.

23. NAIDU, K.M.; VENKATARAMANA, S.; GURURAJA RAO, P.N. 1983. Varietal variation in stomata inductance and diffusion resistance during moisture stress and recovery in sugarcane. ISSCT. Proceedings 18:567-584.

24. REED, A.J.; HAGEMAN, R.H. 1980. Relationship between nitrate uptake, flux, and reduction and the accumulation of reduced nitrogen in maize (*Zea mays* L.) II: Effect of nutrient nitrate concentration. Plant Physiology 66:1184-1189.

25. SANDERSON, G.W.; COCKING, E.C. 1964. The enzymic assimilation of nitrate in the tomato plant 1: Nitrate reductase. Plant Physiology 39:416-422.

26. SINGH, P.P. 1974. Ratooning potential of sugarcane varieties under different levels of nitrogen. Proc. of the annual Conven. of the Sugarcane Tech. Assoc. of India 40:A1-A5.

27. STEVENS, D.L.; OAKS, A. 1973. The influence of nitrate on the induction of nitrate reductase in Soybean leaves. Plant Physiology 49:448-450.

28. TANG, P.; WU, H.Y. 1957. Adaptive farmation of Nitrate reductase in rice seedlings. Nature 179:1355-1356.

29. WALLACE, W.; PATE, J.S. 1965. Nitrate reductase in the field pea (*Pisum arrense* L.) Annals of Botany 29:655-667.

30. WEATHERLEY, P.E.; SLAYTER, R.O. 1957. Relationship between relative turgidity and diffusion pressure deficit in leaves: Nature 179:1985-1086.

31. WILLIAM, L.B.; DANIEL, T.W. 1978. Effects of nitrate, ammonium and pH on the growth of conifer seedlings and their production of nitrate of reductase. Plant and Soil 50:371-385.

32. VIQUEIRA, L.; GONIEZ, L.; RODRIGUEZ, C. 1983. Effect of water deficiency on two sugarcane varieties. 18th Congr. ISSCT. Proceedings:539-565.