WFL Publisher
Science and Technology
Food, Agriculture & Environment Vol.2 (2) : 265-272. 2004
www.world-food.net

Effect of increasing concentrations of sodium carbonate on pearl millet *Pennisetum americanum*

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Received 11 January 2004, accepted 22 April 2004.

Abstract

Significant reduction of seedling root length was observed, due to increased concentration of Na_2CO_3 and considerable variation was found between accessions for growth in salt. Analysis by non-linear least square inversion method using root length data revealed significant differences in pearl millet accessions on the basis of salinity threshold Ct, the Na_2CO_3 solution concentration at which root length begins to decrease. The concentration caused a 50% decrease in root length (C50) and the concentration causing zero root growth (C0). From the comparison of these three characters, C50 appears to be a useful character by means of assessing and quantifying salinity tolerance.

Key words: Pearl millet, *Pennisetum americanum*, sodium carbonate, salinity.

Introduction

Salinity problems are not caused only by NaCl, but by a range of salts present in soil, which inhibits plant growth and reduce plant growth to different degrees depending upon the plant species and electrolyte concentration[1]. The soluble salts that accumulate in soil, consist principally of various proportions of sodium, calcium, and magnesium cations and sulphate, chloride, potassium, bicarbonate, carbonate and nitrate anions. Salinity problems arise when the concentrations of sodium chloride, sodium carbonate, sodium sulphate, sodium bicarbonate or salts of magnesium are in excess, and the effect becomes increased.

The concentration of inorganic salts reduce the growth of most plants, but that depends upon the nature of the salts present, plant growth stage and the effectiveness of the tolerance or avoidance mechanisms of the plant. The mechanisms by which various salts reduce plant growth are not entirely clear, and may in fact be different for plants of different salt tolerance, and different plants vary greatly in their tolerance.

Sodium carbonate was shown to be more toxic than sodium sulphate and sodium chloride in pea plants [2]. Inhibition of plant growth, particularly in roots, was greater from Na_2CO_3 than NaCl. The greater effect of Na_2CO_3 on plant growth was attributed to higher pH around the root system, and lower plant availability of Ca^{2+}, Mg^{2+} and P in *Puccinellia tenuiflora* [3]. Wagner et al. [4] suggested that water with residual sodium carbonate should not be used unless it is treated with gypsum ($CaSO_4.2H_2O$) or contains enough gypsum to counteract the effect of sodium carbonate. The carbonate or bicarbonate saturation index showed that precipitation of Ca^{2+} or Mg^{2+} at 90% of the sites, causes dominance of Na^+, which may therefore, have an adverse effect on the soil in the medium or a long-term and a toxic effect on the bananas [4].

The presence of excess Na^+ with CO_3^{2-} as companion anion, results in high electric conductivity in soil solution and high pH of growing medium, which could possibly be responsible for growth reduction [5]. Little work to-date has been carried out concerning the effects of anions other than Cl^-. The CO_3^{2-} and HCO_3^- salts system may produce more damage than Cl^- or SO_4^{2-} salts. The objective of this study was to assess the effect of Na_2CO_3 on pearl millet growth. Considerable amounts of soils described as sodic are present in situations where crop growth is not possible. The effect of Na_2CO_3 on pearl millet has been examined involving seedling root growth response to Na_2CO_3 and the possibility of some degree of resistance to Na_2CO_3.

Evaluation of the tolerance and response of crop accessions to soil salinity can provide estimates of salinity treatments thresholds at which root and shoot growth separately begin to decline. The experiment design has been based upon the non-linear least square inversion model developed by Van Genuchten and Hoffman [6].

Materials and Methods

Plant material: Twenty-three pearl millet accessions were used in this experiment, all of which had been previously tested for NaCl tolerance. Seven were relatively tolerant, six susceptible and a further ten were taken at random from a total of 143 accessions.

Tolerance testing: Six different Na_2CO_3 concentrations 0 as control and 5, 10, 15, 20 and 25 mM were used. Seedlings were grown for 14-days in culture half strength nutrient solution using techniques described by Ashraf et al.[7] and using the Rorison's solution[8]. Growth room was maintained at 24±1°C, relative humidity 60-70% and day length of 16 h at an intensity of 27 Wm^2. Pots were covered by Perspex chambers having small holes to control the solution evaporation. Twenty seeds from each accession were sown on rafts of black alkathene beads, five layers deep floating on the nutrient solution containing the required Na_2CO_3 concentration in 200 cm^3 plastic beakers. Each experiment was replicated three times. Before planting, seeds were surface sterilised for 10 minute with 5% sodium hypochloride solution. Each experiment was set up as a completely randomised design. After 14 days, ten randomly chosen seedlings from each replicate were measured for longest root length from which relative tolerance was determined. The pattern of variability in the salinity responses of accessions was examined on the basis of data analysis methods.

Data analysis: Raw data were transformed to relative tolerance as:

$$\text{Relative tolerance} = \frac{\text{seedling root length in saline solution}}{\text{seedling root length in control solution}} \times 100$$

A non-linear least square method was used to show the observed salinity response data to the response models outlined below.

The models applied to the observed root length data were NOPT 5 / NOPT 2 and NOPT 12, [6] to study the salinity-yield response functions of the accessions at the seedling stage. The programme is structured such that it will convert its option automatically from NOPT 5 to NOPT 2, whenever all observed data points are to the right of the fitted threshold value.

1. NOPT 5/NOPT 2: A piece-wise response function similar to one proposed by Mass and Hoffman [9] is given by:

$$Y = \begin{cases} Ym & 0 \le C \le C_t \\ Ym - Ym^s (C - C_t) & Ct < C \le C_0 \\ 0 & C > C_0 \end{cases}$$

where Y = absolute yield;
Ym = absolute yield under non-saline conditions;
C = average root zone salinity during the growing season;
C_t = threshold concentration at which yield begins to decrease;
C_0 = concentration at which yield equals zero;
and "s" is defined as an absolute value of slope of the response function between Ct and C_0, and is obtained with the equation

$$s = \sum_{i=1}^{n} (Y_m - Y_i) \sum_{i=1}^{n} (C_i - C_t),$$

where (Ci, Yi) represents the i-th data point ($1 \le i \le n$), and "n" is the number of observed data points used in analysis.

2. NOPT 12: A sigmoid-form, which provides a much better fit to the data than the piece-wise NOPT 5/NOPT 2 linear response models, is the function given by:

$$Y = \frac{Y_m}{1 + (C/C_{50})^P}$$

where C_{50} determine that salinity at which yield decreases by 50%, where P is an empirical constant that specifies the steepness of the curve. The computer programme, "SALT", [10] was used to carry out these computations. This programme, provides estimates for C_t, C_0 and s (NOPT 5/NOPT 2), and C50 and P (NOPT 12), as well as fitted response curve.

Root length means of five plants per replicate of each accession in each salt concentrations were also subjected to analysis of variance using PROC ANOVA of SAS [11]. Genotypic and phenotypic components of variance were estimated to give broad sense heritability [12], where broad sense heritability (h^2_B) = V_G/V_P; VG and V_P are estimates of the genotypic and phenotypic variances, respectively. The large set of increasing concentrations used in this experiment was designed to provide a wide range of response to salt, thus providing a comprehensive data set for analysis followed by Van Genuchten and Hoffman[6].

Results

Relative root length: The results of analysis of variance for the absolute root length data for 23 pearl millet accessions are presented in Table 1. There are highly significant differences ($P \le 0.001$) between accessions. The interaction (accessions x Na_2CO_3 concentrations) was also highly significant ($P \le 0.001$) showing that accessions root length responded differently to Na_2CO_3 treatments.

Relative root lengths of 23 accessions in control to 25 mM Na_2CO_3 concentrations are given in Table 2. Root lengths as would be expected, decreased significantly with increase of Na_2CO_3. Of the 23 accessions four 18406 (101 %), PARC-MS-1 (108 %), DB-V (103 %) and ICMV-93753 (103 %) were unaffected at 5 mM Na_2CO_3. There was a marked reduction in relative root length however, between 5 to 10 mM Na_2CO_3 and at all further concentrations there was a continuing reduction of root length. On the basis of relative root length, which ranged from 2.12 to 5.45 cm at 25 mM, eight accessions 10878, 18570, 20665, 20728, PARC-MS1, DB-V, ICMV-93753, and ICMV-94474 had root length more than 4 cm, suggesting some degree of tolerance. Eleven accessions grown in 15 mM Na_2CO_3 had relative root values at in excess of 20% and two accessions (ICMV-93753 and PARC-MS-2) had the highest relative values of 30.64% and 25.06%, respectively. Whereas, at 25 mM concentrations accessions 18570 (4.87%) and DB-V (4.63%) showed their tolerance, and 20702 and PARC-MS-2 had considerably reduced tolerance, moderate equivalent to the tolerant group. Thus accessions behave differently in different salts and with different concentrations.

NOPT analysis Ct, C50 and C0: The absolute root length data of the nine accessions representing three tolerant accessions, three moderately tolerant accessions, and three sensitive accessions are presented in Figs 1-3. Results obtained from NOPT analyses, NOPT 5, NOPT 2, and NOPT 12 are presented in Table 3. They provide estimates of threshold (Ct), fifty-percent reduction in root length (C50), and the concentration at which root growth ceases (C0) for each accession. These data show considerable variation in response to sodium carbonate.

The responses for absolute root length data of three tolerant accessions DB-V, ICMV-93753 and ICMV-94474 (Fig. 1), three moderately tolerant accessions 10876, 20728 and 10525 (Fig. 2) and three sensitive accessions 25233, 5960, and 5995 are presented in Fig. 3. Clear differences are evident in these three groups of accessions to Na_2CO_3 for the parameters Ct, C50, and C0. Tolerant accessions in general had higher Ct and C50 values, and also had higher C0 values suggesting that tolerant accessions had a general trend for tolerance to Na_2CO_3 in terms of Ct, C50, and C0. However, moderately tolerant lines showed variable tolerance levels for these parameters. The values of the 23 accessions for three characters Ct, C50, and C0 with their tolerance ranking are presented in Table 3. To facilitate interpretations, all accessions examined in Na_2CO_3 have been ranked on an arbitrary scale of I to III for tolerance in

Table 1. Analysis of variance for absolute root lengths of 23 accessions of pearl millet, grown in 6 sodium carbonate concentrations in solution culture for 14 days.

Source of variation	Degrees of freedom	Mean square	P	
Replications	2	2.00	0.216	NS
Accessions (Acc)	22	4.64	<0.00	***
Na_2CO_3 concentrations (Conc)	5	4367.31	<0.00	***
Acc x Conc	110	5.16	0.00	***

Table 2. Relative tolerance of root length (%) of 14-day old seedlings from 23 pearl millet accessions in response to increasing Na_2CO_3 concentration.

Accession	Control root length (cm)	5 mM	10 mM	15 mM	20 mM	25 mM
25233	23.50	80.32	34.31	7.89	4.66	2.81
5960	21.56	62.04	29.41	9.03	4.17	2.12
5995	22.34	75.37	47.46	16.22	7.18	3.31
10501	19.21	89.60	35.94	17.77	5.66	3.87
10525	20.78	91.76	38.59	18.46	6.38	3.87
10528	21.74	84.17	34.30	15.43	5.28	2.51
10876	17.17	83.15	50.76	18.16	5.86	3.61
10878	21.87	78.74	42.72	19.31	7.47	4.16
17407	21.56	74.22	47.97	15.96	5.38	3.27
17410	18.57	85.91	63.38	20.80	9.31	3.82
17418	17.80	94.27	61.20	22.51	8.22	3.79
18278	18.70	93.00	45.79	17.45	7.77	3.35
18406	16.96	100.79	53.83	23.27	11.38	5.05
18570	17.93	96.36	48.87	21.85	11.86	4.87
20665	18.53	93.47	59.07	22.11	13.08	4.00
20702	16.63	89.81	54.76	23.85	12.07	3.33
20728	19.56	80.54	50.71	17.50	6.10	4.12
20735	19.03	90.85	62.25	21.29	6.67	2.65
PARC-MS-1	17.27	108.03	69.11	21.92	7.07	4.79
PARC-MS-2	18.03	98.30	55.33	25.06	9.81	3.88
DB-V	15.75	102.94	80.32	19.42	11.53	4.63
ICMV-93753	17.21	102.59	62.78	30.64	13.39	5.45
ICMV-94474	17.59	95.18	58.15	23.95	9.98	4.21
Mean	19.10	89.19	49.87	19.56	8.27	3.80

terms of root length, I representing high tolerance and III most sensitive response. The accessions PARC-MS-1, DB-V, ICMV-93753, 18406, 18278 and PARC-MS-2 had relatively higher Ct values, root length began decreasing at 5 mM Na_2CO_3, and were ranked I. The accessions PARC-MS-1, DB-V, ICMV-93753, 17410, and 20735 showed relatively higher values for C50 indicating some degree of tolerance. Accessions ICMV-93753, 20702, 20665, 17410, 18570, PARC-MS-2, DB-V and 18406 showed relatively higher values for C0. Two accessions, DB-V and ICMV-93753 are the most tolerant to Na_2CO_3, having the highest values for all three characters (C50, Ct, and C0). By contrast only one accession 5960, was ranked III.

Heritability: Broad sense heritability estimates obtained from the root lengths of the 23 accessions grown at varying levels of Na_2CO_3 are presented in Table 4. Maximum heritability was exhibited for plants grown in control, and it was progressively reduced as the Na_2CO_3 concentration in the rooting medium increased. Broad sense heritability varied from 0.22 to 0.66 under different concentrations but under stress conditions heritability was maximum (0.50 at 20 mM Na_2CO_3). High estimates of heritability indicate that selection in this material may produce material with increased tolerance to Na_2CO_3.

Discussion

The mechanism by which various salts reduce plant growth are not entirely clear and may in fact, be different for plants of different salt tolerance. Plants vary greatly in their tolerance to salt[13]. Salt tolerance is considered to be a developmentally regulated phenomenon, the early seedling stage being the most sensitive[14] and tolerance at one stage of plant development does not necessarily correlate with tolerance at other development stages[15]. Root growth as an indicator for the whole complex of characteristics determining salt resistance was found to be useful in the first steps of screening programmes for salts resistance[16], the consequence of plant growth inhibition, which has been considered to be the most relevant effect of salinity [17], and the first effect of salinity on plant growth appears to be in roots. This has been used to assess salinity tolerance in several crop species, e.g. at the seedling stage of sorghum[18], barley[19], wheat[20], maize[21], millet[22], alfalfa[23] and cotton[24]. Differences in tolerance to Na_2CO_3, Na_2SO_4 and NaCl were examined[2] and Na_2CO_3 was found to be more toxic than either Na_2SO_4 or NaCl. From the work reported in this article it is clear that Na_2CO_3 has higher toxicity than NaCl as has been shown in pea plants, on germination and seedlings of pea plant growth [2, 25].

Among 23 accessions (Table 3) tolerance assessed for Ct, C50, and C0 values, only two accessions, DB-V and ICMV-93753 had high Ct, C50, and C0, and only one accession (5960) had lower values for all three characters for Na_2CO_3. This was not found in other tolerant, moderate tolerant and sensitive accessions, and large differences existed in Ct, C50 and C0 estimates. For example, accession 17410 with the lower Ct value had poor C0 and accession 18278 with higher Ct value had lower C50 and C0.

In similar studies for millet[21] and maize[20] for NaCl tolerance, there was no relationship between Ct and C50. Of the three characters

Table 3. Calculated values of C_t and C_0 (NOPT 2/NOPT 5) and C_{50} (NOPT 12) for 23 accessions of pearl millet with tolerance ranking for sodium carbonate.

Accessions	C_t	Rank	C_{50}	Rank	C_0	Rank
25233	0.00	III	7.93	II	18.29	II
5960	0.00	III	6.33	III	15.00	III
5995	0.00	III	8.86	II	19.64	II
10501	2.73	II	8.68	II	16.64	III
10525	3.14	II	8.97	II	16.75	III
10528	1.98	III	8.27	II	16.44	III
10876	2.40	II	9.85	II	17.81	II
10878	1.10	III	8.71	II	17.87	II
17407	0.00	III	8.82	II	19.47	II
17410	2.38	III	11.47	I	20.69	I
17418	3.42	II	11.15	I	20.30	I
18278	3.59	I	9.55	II	16.86	III
18406	3.66	I	10.50	II	20.40	I
18570	2.60	II	9.99	II	20.46	I
20665	2.96	II	10.97	I	20.92	I
20702	2.11	II	10.58	II	21.01	I
20728	2.01	II	9.68	II	17.86	II
20735	3.13	II	11.30	I	20.16	I
PARC-MS-1	6.34	I	11.48	I	17.25	II
PARC-MS-2	3.56	I	10.67	II	20.41	I
DB-V	5.60	I	12.20	I	20.37	I
ICMV-93753	4.51	I	11.58	I	21.21	I
ICMV-94474	3.38	II	10.95	I	20.53	I

C_t Above 3.5 I, 2-3.4 II, Below 2 III
C_{50} Above 11 I, 7-10 II, Below 7 III
C_0 Above 20 I, 17-19 II, Below 17 III

Table 4. Component of variance and broad sense heritability h^2b of Na_2CO_3 tolerance in pearl millet seedling at each concentration (Vg = genetic variance; Vp = phenotypic varience).

Component	Control	5 mM	10 mM	15 mM	20 mM	25 mM
Vg	3.95	1.38	1.79	0.25	0.14	0.1
Vp	5.98	3.14	4.84	1.13	0.28	0.03
h^2_b	0.66	0.44	0.37	0.22	0.50	0.33

(Table 3), C50 appears to be the best parameter for assessing salinity tolerance for selection in Na_2CO_3, particularly because it showed good positive relationship with Ct and C0. This opinion is supported by the previous study [26] and comes to the conclusion that in wheat, the C50 is a much better parameter than Ct and C0 for assessing tolerance, further more tolerant (Fig. 1), moderate (Fig. 2) and sensitive (Fig. 3) accessions showed clear response to these characteristics, and suggested that selection can be possible for tolerance.

The data showed that those plants having smaller root lengths were generally more Na_2CO_3 tolerant as were found in accessions ICMV-93753 and DB-V. Both had smaller root length in control but also had higher relative root lengths and higher threshold (Ct) values at higher concentrations. From an examination of response of a group of 24 barley cultivars to germination, Martinez–Cob et al.[27] concluded that the Ct threshold salinity was the appropriate parameter used for quantifying salinity tolerance. In contrast to that data (Table 2) from accession 18570 with higher relative tolerance, had lower Ct, and accession PARC-MS-2 with a lower relative tolerance, had higher Ct, and from these data, Ct does not appear to be a selection criteria for Na_2CO_3. Thus it has been concluded again that C50 is the best parameter for selection in Na_2CO_3.

Variability for any particular character used to estimate tolerance to an environmental stress, depends upon the extent to which that character is heritable. From the plant breeding point of view, the data obtained (Table 4) are promising enough that variation exists to make selection for Na_2CO_3 tolerance possible. Other crops have shown considerable differences in broad sense heritability estimates for salinity tolerance in seven grasses and forage species in maize[28] and tomato[29, 30]. Similarly, the present results, broad sense pearl millet heritability estimates at six Na_2CO_3 concentrations (h^2b = 0.22 to 0.66) suggests that the prospects of improving Na_2CO_3 resistance through selection and breeding are considerable, provided the genetic system controlling the variation is mainly predominantly quantitative with additive effects.

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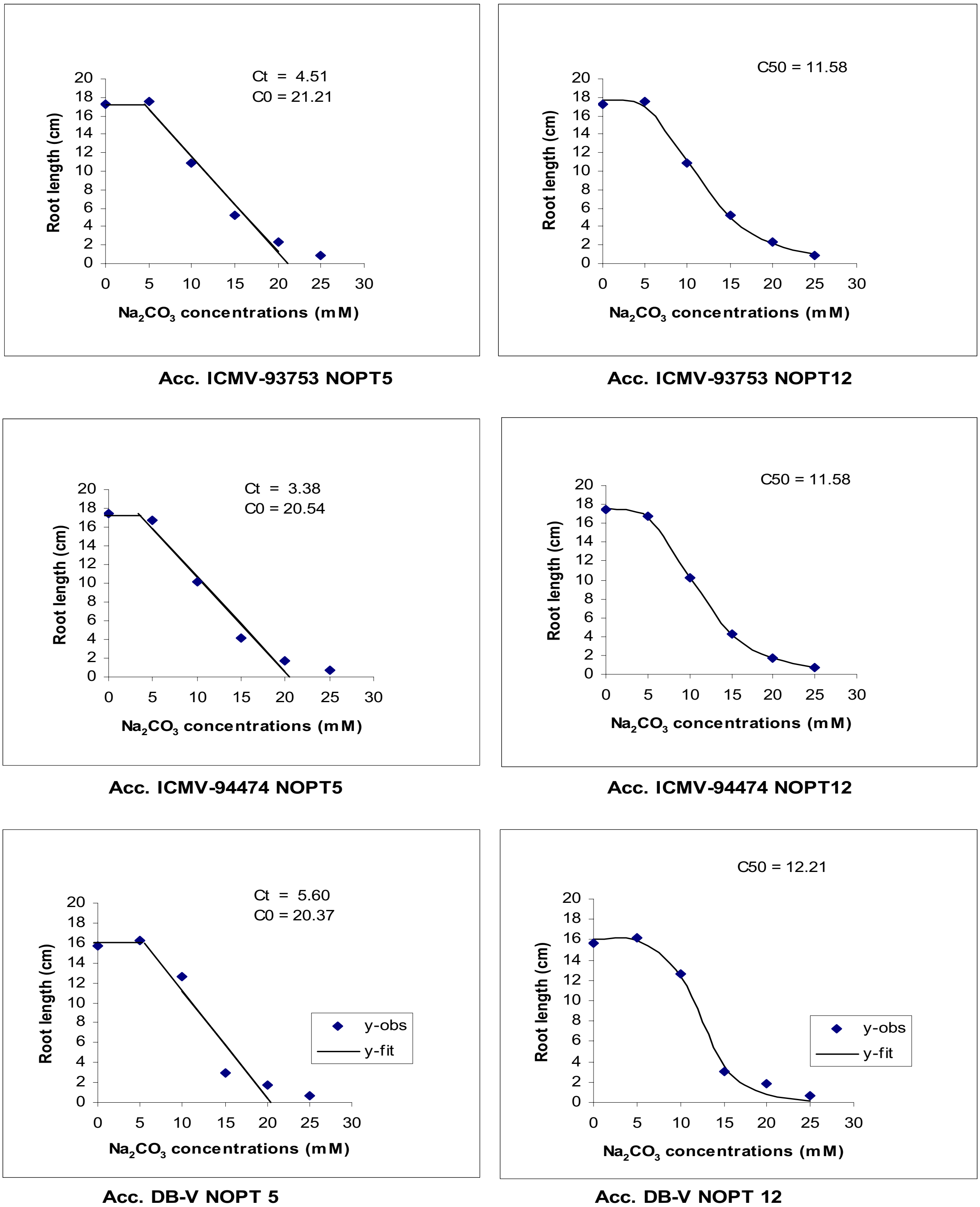
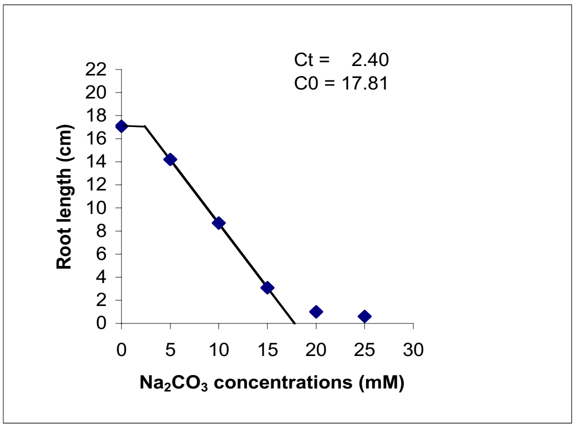


Figure 1. Response functions between Na_2CO_3 concentrations and root length (cm) of pearl millet seedlings from three tolerant accessions.



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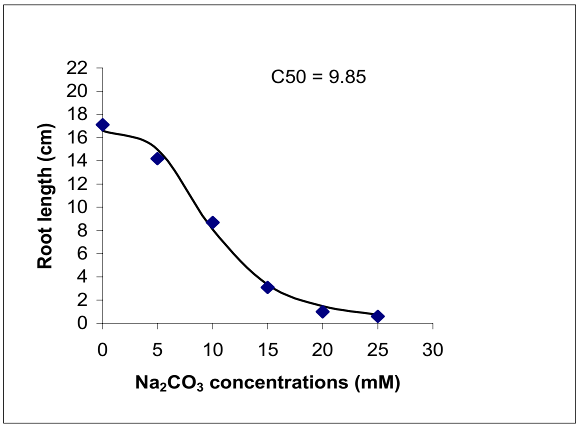

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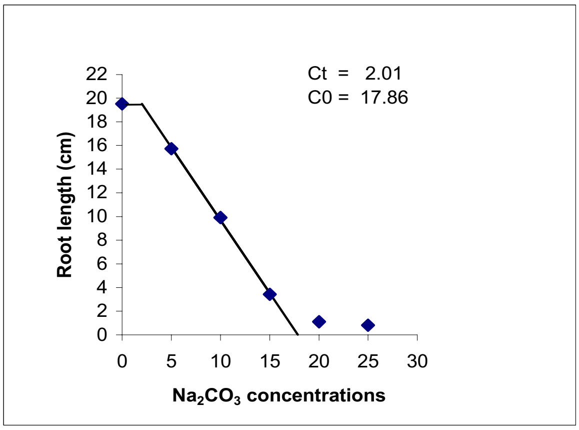

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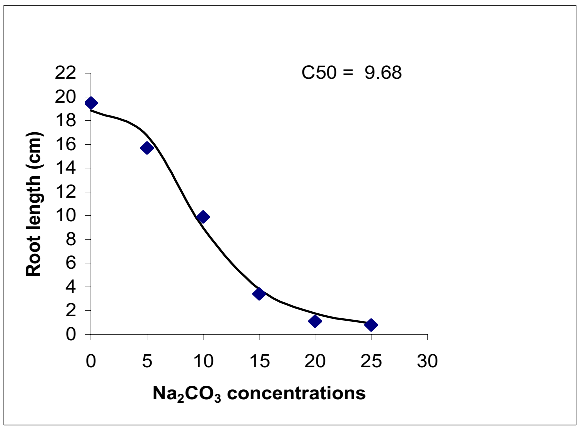
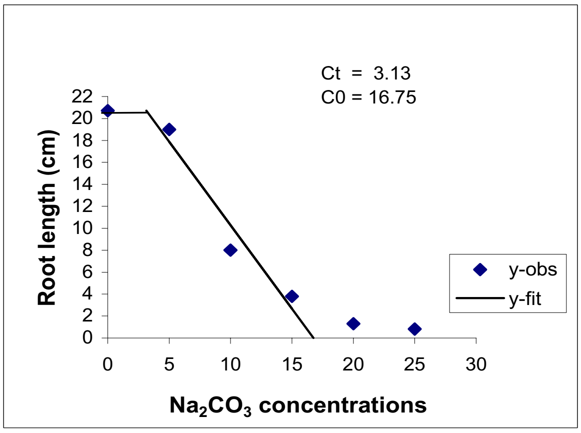


Acc. 20728 NOPT 12



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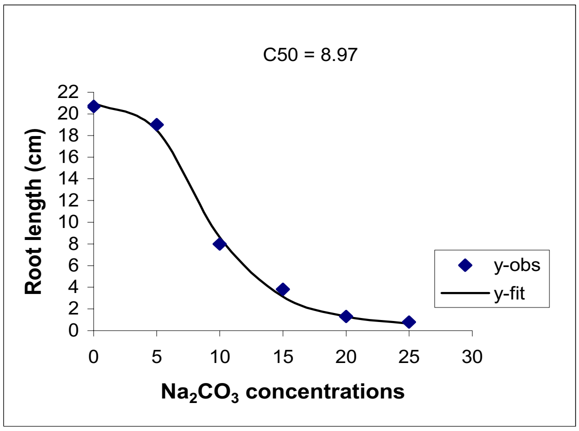

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Figure 2. Response functions between Na_2CO_3 concentrations and root length (cm) of pearl millet seedlings from three moderate accessions.

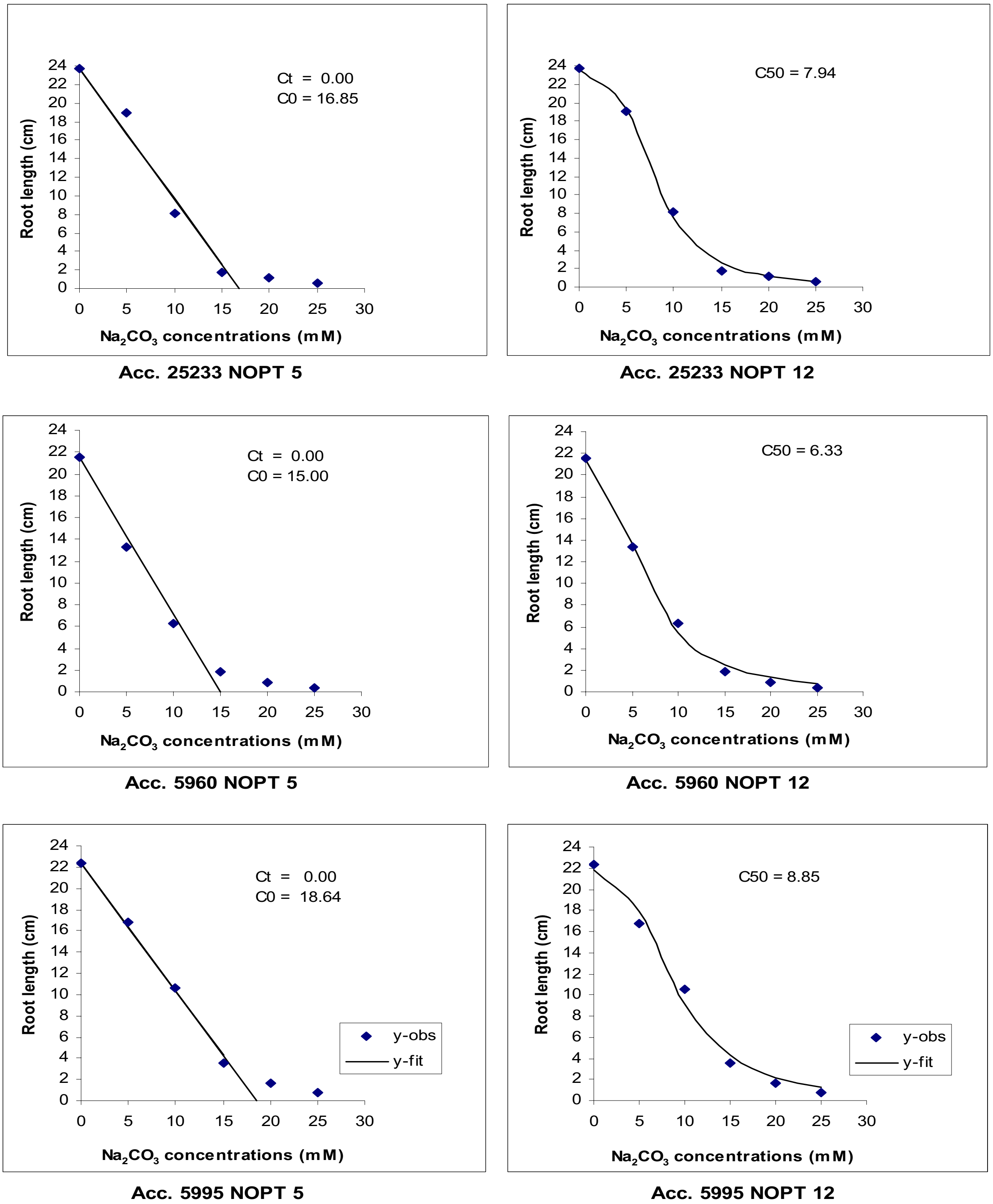

Figure 3. Response functions between Na_2CO_3 concentrations and root length (cm) of pearl millet seedlings from three susceptible accessions.