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Full Length Research Paper

Endophenotype Growth Response Of *Allium Cepa* L. Under Salinity Stress

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This study investigated the Endophenotype growth kinetics of *Allium cepa* L., in response to variations in the concentration of aqueous sodium chloride (NaCl) solution; using cytogenetic approach. The aim was to establish an alternative parametric index (%proportion of mitotic cells per field), as well as to ascertain the possibility of cultivating *A. cepa* L in our saliferous coastal belt. Dried, shrivelled roots of *A. cepa* bulb were shorn off and the stem surface dipped in a beaker of distilled water, 0.00mMNaCl (control) and various concentrations, (10-500mMNaCl) sodium chloride solution. Fresh roots were harvested after 72 hours, fixed for 24 hours in Carnoy's fluid, macerated and squashed on slides. The number of dividing cells per field, per concentration was counted. The results, as depicted in the growth trajectory, showed that, the relative proportion of the dividing cells per field, increased with increase in sodium chloride concentrations; from control (12%) till 250mM NaCl (32%) and dropped pendulously to 350mM NaCl (13%). The concentrations of 400mM to 500mM did not support root emergence. The relevance of this approach to the discipline of plant Physiology, Cytogenetics, Ecology and Agriculture was implied.

Keywords: *A. cepa, endophenotype, cytogenetics, Growth kinetics, parametric index, trajectory, saliferous*

INTRODUCTION

Stress physiology in plant is a study aimed at evaluating phenotype productivity, relative to the environmental factors. Among these factors, is salinity – a stress condition, occasioned by the preponderance of sodium and chloride ion ($Na^+ Cl^-$) concentration in the natural substrate. High or low salt concentration within the plant environment influences its growth performance or the reproductive effort. (Jamil *et al.*, 2006).

Allium cepa L. (Onion) is a biennial herb, cultivated as annual bulb. The stem is a cone shaped base

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from which arises in succession, alternate rings of tubular leaf sheath. From the apex of the leaf sheath, emerge the cylindrical leaf blade. Inflorescence axis, push through the bulb to establish a terminal umbel of aggregate cyme, with between, 50- 2000 flowers. Each flower is pedicellate and trimerous . The fruit is a globular capsule with loculicidal dehiscence. The seeds are plump and smooth when fresh turning wrinkled and black when dry. (Nyananyo, 2006)

Phenological estimation of growth kinetics is exemplified in biomass accumulation, which is a plastic investment, manifesting as such growth parameters as; shoot height, number of branches, number of leaves, number of fruits, number of flower buds, nodule formation, seedling development, seed germination, fresh and dry weight of the vegetative and reproductive partitions (Mujeeb – ur – Rahman *et al*., 2008; Mahmoodzadeh, 2009; Demir and Mavi, 2008).

Hitherto, information on the range of tolerance to salinization, was based on the above growth parameters. These are found to be species dependent, fostered by the ontogeny of the plant. These data vary from age to age, and size to size of the plants investigated. This, invoke the need to generate data through another approach such as Cytogenetics.

Endophenotypes; in plant refers to the cell morph of the Anatomical features, they include the cells from the calyptrogen, pleromogens and the periblogens. The endogenous activities of these cell forms, especially, their meristematic kinetics manifest in their growth rates. Growth dynamics, therefore, can be determined by the number of dividing cells per variable factors of the environment such as the salinity stress. This study, therefore, aims at using the number of dividing cells per concentration of sodium chloride (NaCl) to evaluate plastic response of *Allium cepa* to salinity.

The objective is to subject the plant to varying concentrations of the stress factor, and measure performance, by enumerating the relative proportion of the population of dividing cell per endophenotype population, per field, per concentration of the stressing factor. The procedure involves, generating roots in different concentration of sodium chloride, and determine growth performance through the number of dividing cells per concentrations. We anticipate to get a growth curve that will reflect the metabolic cum ontogenic profile of the plant in the face of saline prone environment.

MATERIALS AND METHODS

Collection of materials

The bulbs of *Allium cepa,* were collected from Relief market, Owerri and taken to the laboratory, at the Department of Biotechnology, Federal University of Technology Owerri, where the study was carried out.

Owerri is the capital city of Imo state South Eastern Nigeria. It is located within latitude, 5^{o} $27^{/}$ N and longitude $7^{o}02^{/}$ E, in the Rain forest belt of Nigeria.

Salinization

Sodium chloride (common salt) and demineralised water were used to prepare different molar concentrations of the saline solution. Fifty-eight grams (58.5grms) of NaCl. Was weighed and dissolved 1,000ml of demineralised water to form a 1mole solution. Dilutions were made to produce milimoles of 10, 50, 100,150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000mM. Three beakers were filled with ¾ of each concentration and demineralised water is used as a control. The onion bulbs were properly shaved of their old dried roots, pierced with tooth pick to enable it hang on the beaker with the shaved surface touching the liquid. The sets were labeled and distributed randomly on the part of the laboratory devoid of direct sunlight, but had a temperature of 23 ± 1^{o}c. and allowed to grow for 72 hours.

Harvest and Fixation

The roots tip of the *Allium cepa* was used in this study. Healthy roots were excised, 2mm from the tip to capture the calyptrogen region, between, 6.30 to 7.30 am. The same were flooded with demineralized water and fixed in Carnoy's fluid (Absolute Alcohol and glacial acetic acid 3;1 v/v) and kept in a refrigerator at 4^{o}c for 24hours , following methods of Nwankiti (1983), Udengwu and Arukwe (2010). The roots tip were then transferred to 70% ethanol and kept in the laboratory, at 23^{o}c for further treatment.

Acid Hydrolysis

The roots tip maceration followed the method of Udengwu and Arukwe (2010). The excised roots tip were washed in three changes of de ionized water, then macerate in 20% v/v Hydrochloric acid (HCl) for 3minutes without further heating and squashed in FLP orecin stain. Slides with well spread out cells were selected. The number of cells at metaphase, Anaphase and telophase per field of view were counted. Cells at prophase were not counted to avoid confusion. . Photomicrograph using English version CE71694 Digital photomicroscope at X100 magnification, were taken. Computer printout of each considered field, were made from each concentration and five field of view, were considered per slide, totaling 50 field per concentration.

RESULTS

After 72 hours of incubation at room temperature, root emergence was observed at lower concentrations; from 0.00mM NaCl (controlled), to 350mM NaCl solutions, but no root emerge, from the concentrations of 400mM NaCl upwards. The emergent numbers and their relative length did not vary significantly (0.05) between the treatment (salinization) and the control, as well as between different concentrations.

Summary of the result of the effects of salinization on the endomorph growth effort of the *A cepa* is shown in Table 1. The effort index signified by the proportion of dividing cells per field per concentration, was highest at the concentration of 250mMNaCl solution (32%), minimum at 350mMNaCl (13%) and lowest in the control 0.00mMNaCl (12%), relative to the total number of cells in the respective microscopic field.

There was no significant change in the endophenotype, as the shapes and configuration of the dividing cells, did not differ from that of the control (figure 2 and 3).

Table 1: Showing growth proportion in response to varying degrees of sodium chloride concentrations

Conc (mM)	N of field	Total N of cells	N of div cells	prp.	%Prp
00	50	411	50	0.12	12
10	50	350	60	0.17	17
50	50	330	60	0.18	18
100	50	339	72	0.21	21
150	50	260	61	0.23	23
200	50	301	82	0.27	27
250	50	260	84	0.32	32
300	50	272	50	0.18	18
350	50	310	40	0.13	13
400	00	00	00	00	00
450	00	00	00	00	00
500	00	00	00	00	00
550	00	00	00	00	00
600	00	00	00	00	00
650	00	00	00	00	00
700	00	00	00	00	00
750	00	00	00	00	00
800	00	00	00	00	00
850	00	00	00	00	00
900	00	00	00	00	00
950	00	00	00	00	00
1000	00	00	00	00	00

Legend

mM: milimole

N: number

Divcells: dividing cells

Prp: proportion

Fig 1: Trajectile, showing the proportion of dividing cells relative to the sodium chloride concentrations

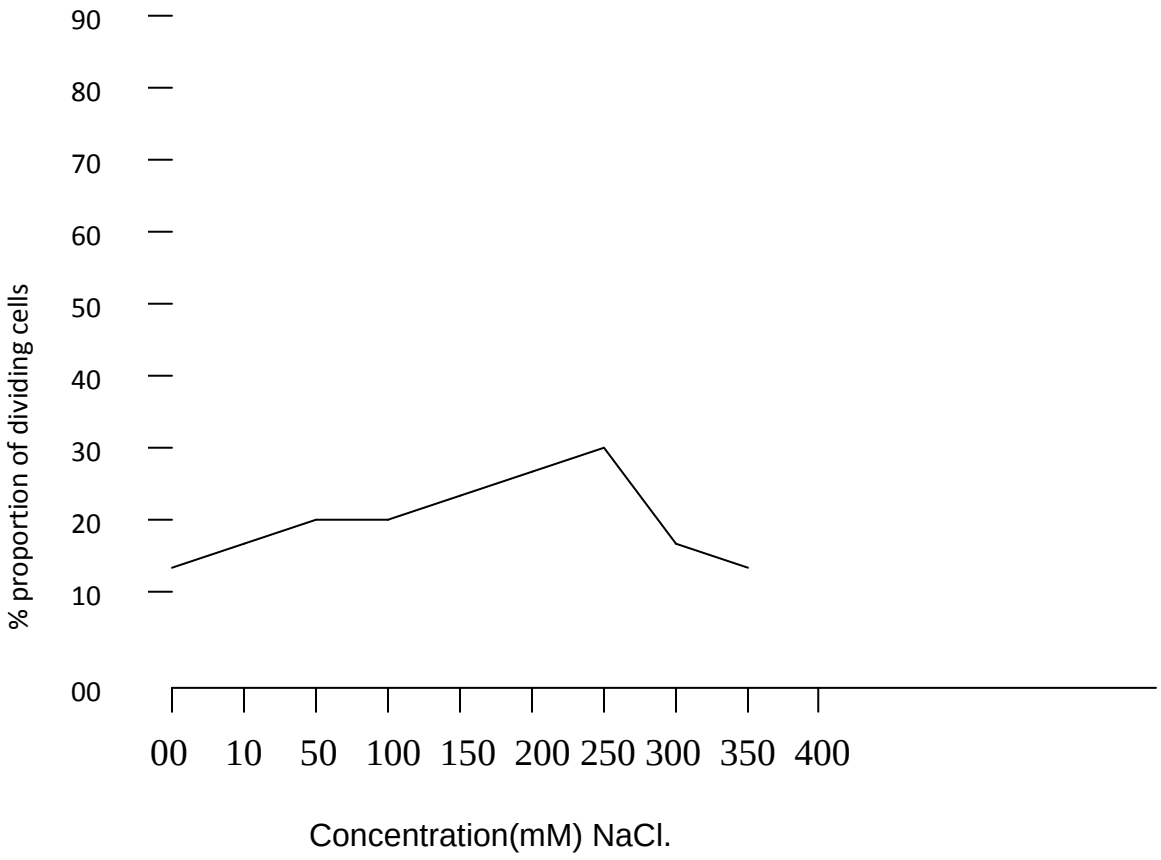

Fig. 2: showing, dividing cells of the control (0.00mM NaCl).

Note; the anaphase and telophase stages

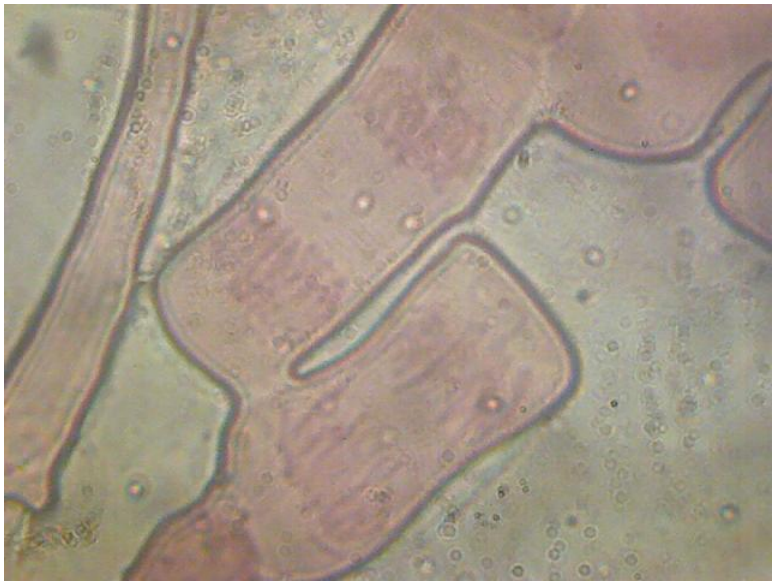

Fig. 3: showing dividing cells from (250mM NaCl)

Note; the anaphase and telophase stages

DISCUSSION

The choice of *A cepa* in this study is because the plant is a highly, cherished spice consumed, but not cultivated in our saline costal belt and Niger Delta region of Nigeria. The employment of cytogenetic approach is because information on endophenotype plasticity is, among other things, a veritable tool in micropropagation and physiological investigations. Again, much of the comparism will be on *Capsicum annum* because these two plants are grown wildly under the same ecological zone of the Sahel Savannah in Northern Nigeria; but *C annum* are cultivated in our coastal states while *A cepa* is not.

Higher concentrations, of 400mM and above did not support root emergence. This is in consonance with similar reports by Mgbeze *et al*., (2011), on *Capsicum annum*. It also concurs with the reports of Mujeeb-ur-Rahman *et al* (2008). The scenario may be due to endogenous osmotic tension, created by higher osmotic potential of the external brine medium. It means that the concentration is above the tolerance limit of the plant.

Table 1 and fig. 1, showed a tolerance range of up to 350mMNaCl of the sodium chloride concentration. This also agreed with parts, with the findings of Mgbeze *et al*. (2011), but at variance with the reports of other workers like Yildirim and Guvenc (2006), and Houimli *et al* (2008), who reported tolerant limits lower than that contained in this report. This condition may be due to species differences in tolerance range. From Table1 and figure 1, *A cepa* has growth stimulating range of between 10 – 250mM NaCl and mere survival range of between 250 – 350mMNaCl. Growth quotient (0.13) of the tolerant limit (350mMNaCl) is higher than that of the control (0.12 at 00mMNaCl). This shows that the plant is salinity stress tolerant to the extent of 350mMNaCl. It also indicates that some quantity of sodium is needed in the substrate (soil) to stimulate growth.

CONCLUSION

To the extent of this work, it is evident that *Allium cepa* (onion) can grow in a salty substrate which is above half the concentration of the waters of the ocean basin (approximately 600mM). The trajectile (fig. 1) is a good confirmation that the proportion of dividing cells per field of view of the endomorph, can be used to estimate growth.

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