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In Vitro Screening of *Zea mays* L. for drought tolerance

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Abstract

The drought tolerance of two local varieties of *Zea mays* L. (var. *indentata* and var. *everta*) were studied using different concentrations (0 g/l, 3 g/l and 5 g/l) of polyethylene glycol (PEG) in Murashige and Skoog (MS) medium. The maize seeds were surface sterilized with 10% (v/v) sodium hypochlorite for 15 minutes and subsequently in 70% (v/v) ethanol for 1 minute and thereafter rinsed thrice with sterile water. The sterile seeds were inoculated in the growth media and observed for growth. The experiment was done in a completely randomized design (CRD) with ten replicates for a period of 15 days and growth parameters were evaluated. The result showed that for var. *indentata*, the shoot length (19.15 ± 1.34cm), leaf length (16.62 ± 1.27cm), leaf width (1.19 ± 0.07cm) and leaf number (3.00 ± 0.15cm) of the plants grown in the control medium were significantly higher (P < 0.05) than those grown in media containing 3g/l and 5g/l PEG, but the root number (8.80 ± 1.03cm) was lower in the control compared to the media containing PEG. The same pattern of increase in control compared to media containing PEG were also observed for var. *everta*. With the growth parameters of the control being the highest for both varieties, it therefore shows that both var. *indentata* and var. *everta* are susceptible to drought.

Key words: *In Vitro, Zea mays,* Drought tolerance**,** Polyethylene glycol.
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Introduction

Maize (*Zea mays*) of the family Poaceae is a monoecious, fast growing, vertically erect, short-lived, annual, allogamous plant. It is one of the oldest, high producing and the world's most cultivated cereals with the global average yield of about four tonnes per hectare (Farnham et al., 2003; Shah et al, 2016). The rate of self-pollination in maize is about 5%, showing that natural maize populations have individuals that are heterogeneous at different loci (Sleper and Poehlman, 2006). Africa produces 7% of the world's maize population, with Nigeria as the largest Sub-Saharan producer. As an essential food nutrient source, it is rich in carbohydrate, minerals, iron, vitamin B and protein (International Institute of Tropical Agriculture (IITA), 2009; Olaniyan, 2015). However, its growth is supported in a wide range of environmental conditions. There are various varieties of *Z. mays* all of which can be known by their endosperm and kernel composition and they include: dent maize (var. *indentata*), popcorns (var. *everta*), flint maize, floury maize, sweet maize and waxy maize (Purseglove, 1972; Qayyum et al, 2012). Being the most important cereal crop in the economies of African countries, it is usually processed for a variety of uses such as food ingredients e.g. corn syrup; and industrial uses e.g. utilization in paper industry. It is also a major source of starch in the world which can be fermented into ethanol for fuel or drinks production among other economic relevance

(Boyer and Hannah, 1994; Paliwal, 2000; Shah et al, 2016).

Like in most crop farming, maize production is affected by the unpredictable weather and climatic factors especially that of drought (Wilhite and Glantz, 1985; Cudjoe et al, 2021). A plant is said to be undergoing drought stress if the available water in the soil required for optimum growth and biomass production of the plant has dropped below the plant rhizosphere (Osmolovskaya et al, 2018). Drought is often caused by changing weather patterns exacerbated by human activities leaving many lives with devastating effects such as mass starvation, famine and cessation of economic activity especially in areas where agriculture is the major booster of the economy. Drought is one of the natural disasters in the world that has a great damaging effect, it affects more people than any other natural disaster (Chibueze, 2016). In Nigeria, drought is more prevalent in the northern regions - Sudano-Sahelian region which is the base for agricultural activities and has been increasingly unstable in the past recent years. The recent constant bouts of drought create concern and a fear for the future of agriculture in the country as the recent trends in the climate has the capacity to cripple the economy and devastate food resources. In fair situations, plants in such adverse environment might have water stress tolerance ability through certain physiological, biochemical or morphological adaptations and avoidance of cell injury (Xonocostle-Cazares et al, 2011; Lamaoui et al, 2018), but in severe cases, growth and production processes are impeded.

The mechanism of drought tolerance involves the decrease of cell size via protoplasmic resistance, increase in cell elasticity and turgor pressure maintenance through osmotic adjustments (Azhar and Rehman, 2018). Crop plants that display drought tolerance are usually bred for this characteristic or genetically engineered to express this quality. These plants do better in arid conditions and provide more yield with less input (Muhammad and Abdul, 2018). Hence, the cultivation of such will require less water.

In a bid to stimulate drought, PEG (polyethylene glycol); a high molecular weight solute (polyether compound) that causes cytorrhysis but cannot be taken up by the roots is used. It has little toxic effect on the plant and when introduced to a growth medium can stimulate drought conditions. Overtime, it has proven to be the best solute for imposing low water potential on plants (Verslues and Bray, 2004; Saglam et al, 2014). It is a non-ionic water-soluble polymer that has been successfully used on dicots, monocots, fungi, yeasts and gymnosperms (Kulkarni and Despande, 2007). This method consisting of the application of PEG is desirable because the experiments can be controlled precisely and large numbers of treatment can be carried out in a short period of time (Verslues et al., 2006; Saglam et al, 2014).This study therefore investigated the various changes in the morphological parameters of two varieties of *Z. mays* placed under drought stress condition.

Materials and Method

Sample collection and experimental location.

The *Zea mays* varieties used in this study- var*. indentata* (pop maize) and *everta* (dent maize) were obtained from Ogige Main Market in Nsukka, Enugu State, Nigeria This experiment was carried out at the Plant Tissue Culture Laboratory of the Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Nigeria, latitude 6°51′28.19″ N and longitude 7°23′44.77″ E.

Sample preparation, media preparation and inoculation

The plant varieties used in this study were tested for viability by soaking in water and the floating seeds discarded. Viable seeds were washed with distilled water, immersed in 10% (v/v) sodium hypochlorite for 15 minutes and subsequently in 70% (v/v) ethanol for one minute and rinsed thrice with sterile distilled water. Murashige and Skoog culture media was prepared using standard protocol under aseptic conditions according to Murashige and Skoog (1962); the media contained Murashige and Skoog, Mio-Inositol 100 mg/l, commercial sugar 30 g/l, and pH adjusted to 5.8 before adding Agar 7g/l and sterilised by autoclave at 121^{0}C. Different media containing varying concentrations (3 g/l and 5 g/l) of PEG-6000 served as the treatment and those prepared without PEG-6000 served as control.

The culturing was done under sterile conditions. Each treatment and control were replicated 10 times for each of the varieties studied. A total number of 60 test tubes containing *Z. mays* were used. The set up were transferred and maintained in the growth room in a completely randomized design (CRD) at a temperature of 25±20℃ under 16 hours light and 8 hours dark cycles.

Data collection

The parameters evaluated include – number of sprouted seeds, number of leaves, leaf width, length of shoot, number of roots and length of roots. The readings were taken for a period of eighteen days and photographs were taken at intervals.

Statistical analysis

The data obtained were subjected to Analysis of Variance (ANOVA) at P≤ 0.05. Treatment means were separated using Duncan's New Multiple Range Test (DNMRT).

Results and Discussion

The results from this study (table 1) revealed that the control had a significant (p<0.05) effect on the growth parameters of *var. indentata* studied while there was no significant difference between 3g/l and 5g/l of the treatment on the growth parameters, there was a significant difference between the control and treated plants on all the growth characters evaluated. Table 2 revealed effect of treatment on *Z. mays* var. *everta.* It showed that though there were differential response of each treatments on the variety, however there was a significant difference between control and the treatment on shoot length, leaf length and number of roots. The control had the highest value for all the parameters measured except for the number of roots where it was least and these could be treatment effect causing more roots in response to the drought stress. This supported the findings of Qayyum et al. (2012) and Olaniyan (2015), they revealed that *Z. mays* is susceptible to drought conditions as it could significantly reduce the growth of the plants. The shoot length, leaf length and leaf width for var. *everta* decreased with increase in concentration of PEG medium, while there was a slight increase with increased concentration of PEG medium for var. *indentata* using the same parameters. This is in line with the findings of Khodarampour (2012) and Quieroz et al. (2019). Their work reported growth reduction as a result of fall in cellular expansion as the first effect measurable due to water deficit. They also reported that the susceptibility of the cell elongation process and synthesis of the carbohydrate wall to water deficit and the growth deficit was as a result of the decrease in the turgidity of the cells.

The control and treated seeds of the *Z. mays* varieties studied - var. *indentata* and *everta* sprouted on the third day after inoculation and exhibited healthy growth throughout the study period.

Table 1: Main effects of different concentrations of PEG on the growth parameters of Z. *mays* var. *indentata*

YELLOW DENT CORN (var. *indentata*)						
Treatment	**SL (cm)**	**NL**	**LL (cm)**	**LW (cm)**	**NR**	**LR (cm)**
0g/l	19.15± 1.34[a]	3.00± 0.15[a]	16.62± 1.27[a]	1.19± 0.07[a]	8.80± 1.03[b]	18.59± 1.27[a]
3g/l	6.25± 0.85[b]	1.10± 0.31[b]	1.84± .66[b]	0.67± 0.18[b]	13.50± 1.24[a]	5.28± 1.34[b]
5g/l	7.64± 0.86[b]	1.10± 0.31[b]	2.57± .81[b]	0.64± 0.18[b]	13.50± 1.54[a]	6.58± 0.94[b]

SL= shoot length, NL= leaf number, LL = leaf length, LW= leaf width, NR = number of roots, LR = root length.

Mean values within a column having different letters are significantly different from each other at P≤0.05

Table 2: Main effects of different concentrations of pegon the gwroth parameters of *z. mays* var. *everta*

POP MAIZE (var. *everta*)

Treatment	SL (cm)	NL	LL (cm)	LW (cm)	NR	LR (cm)
0g/l	13.40± 1.77[a]	1.90± .28[a]	7.83± 1.49[a]	0.54± 0.09[a]	7.80± 1.29[a]	12.97± 1.64[a]
3g/l	6.48± .88[b]	1.50± 0.37[a]	2.11± 0.53[b]	0.54± 0.15[a]	9.50± .76[b]	3.53± 0.30[a]
5g/l	5.09± .27[b]	0.00± 0.00[b]	0.00± 0.00[b]	0.00± 0.00[b]	9.00± 1.15[b]	3.46± 0.35[b]

SL= shoot length, NL= leaf number, LL = leaf length, LW= leaf width, NR = number of roots, LR = root length.

Mean values within a column having different letters are significantly different from each other at P≤0.05

Figure 1 revealed that var. *indentata* (control) had the highest percentage sprouting per day after third day of inoculation while var. *everta* treated with 5 g/l PEG had the lowest percentage sprouting. All treatments of var. *indentata* and control had higher percentage sprouting than those of var. *everta*. this supported the work of Olaniyan (2015); Also Khodarampour (2011) reported that an increase in the concentration of PEG led to a reduction in the sprout rate.

Generally, *Zea mays* var. *indentata* was found to have higher growth rate than *Zea mays* var. *everta*. Its shoots, leaf and root growth had greater measured values than those of var. *everta*. This can be linked to the genetics of the variety as reported by the studies of Malosetti and Abadie (2001) who noted that var. *indentata* produces longer shoots, leaves and roots per time than var. *everta* although it takes a longer period to complete its life cycle.

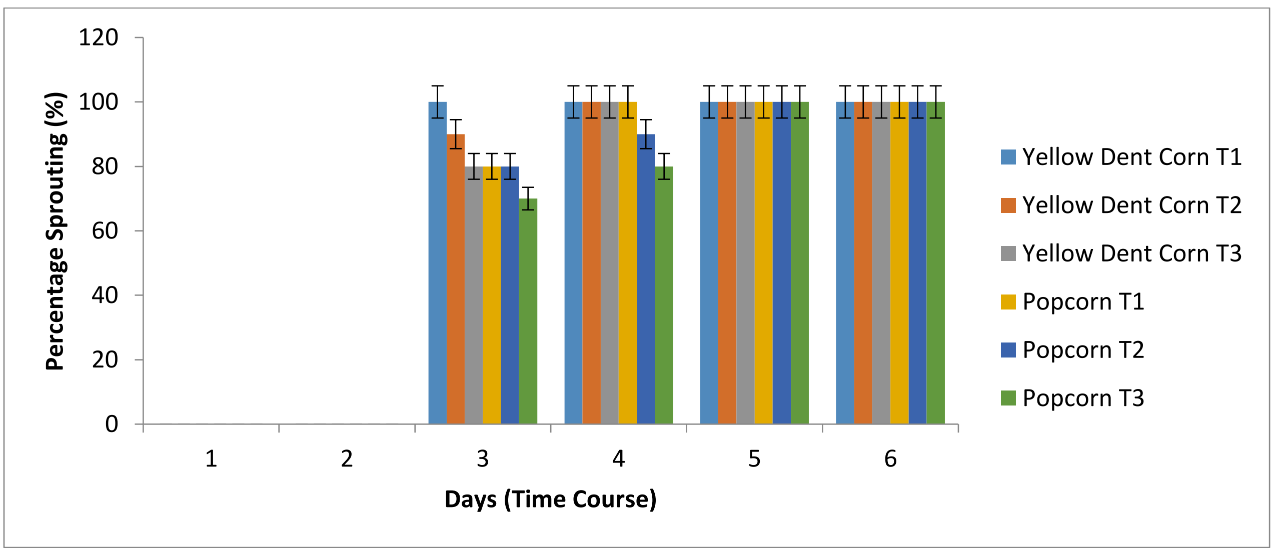
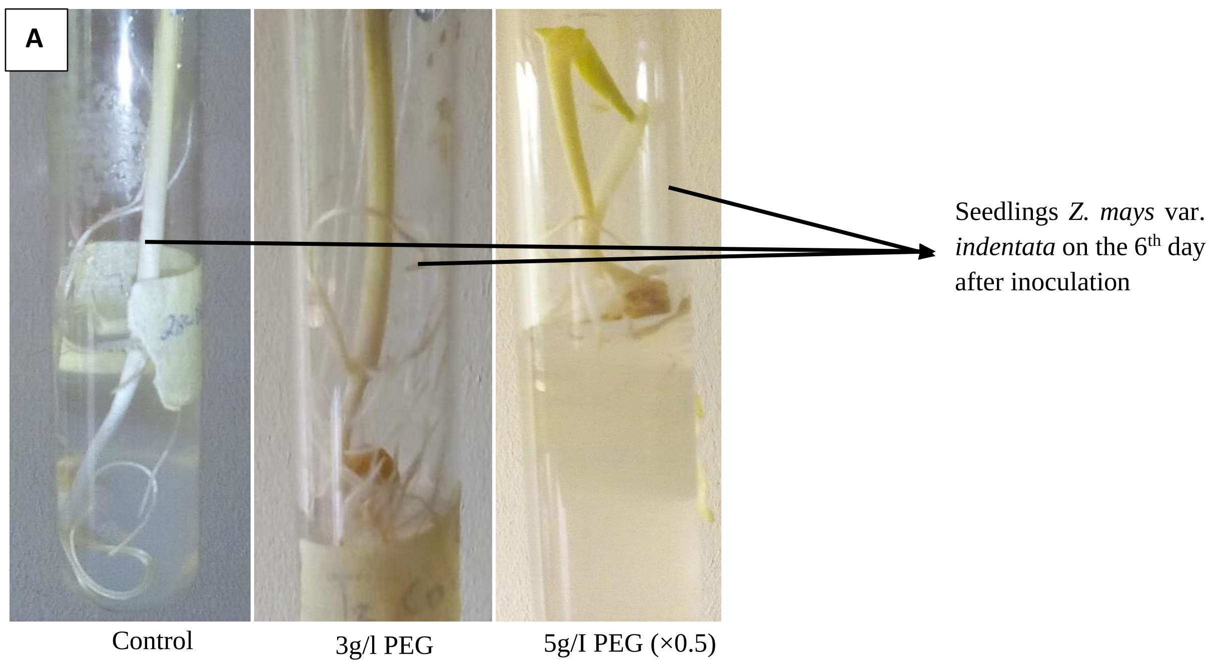
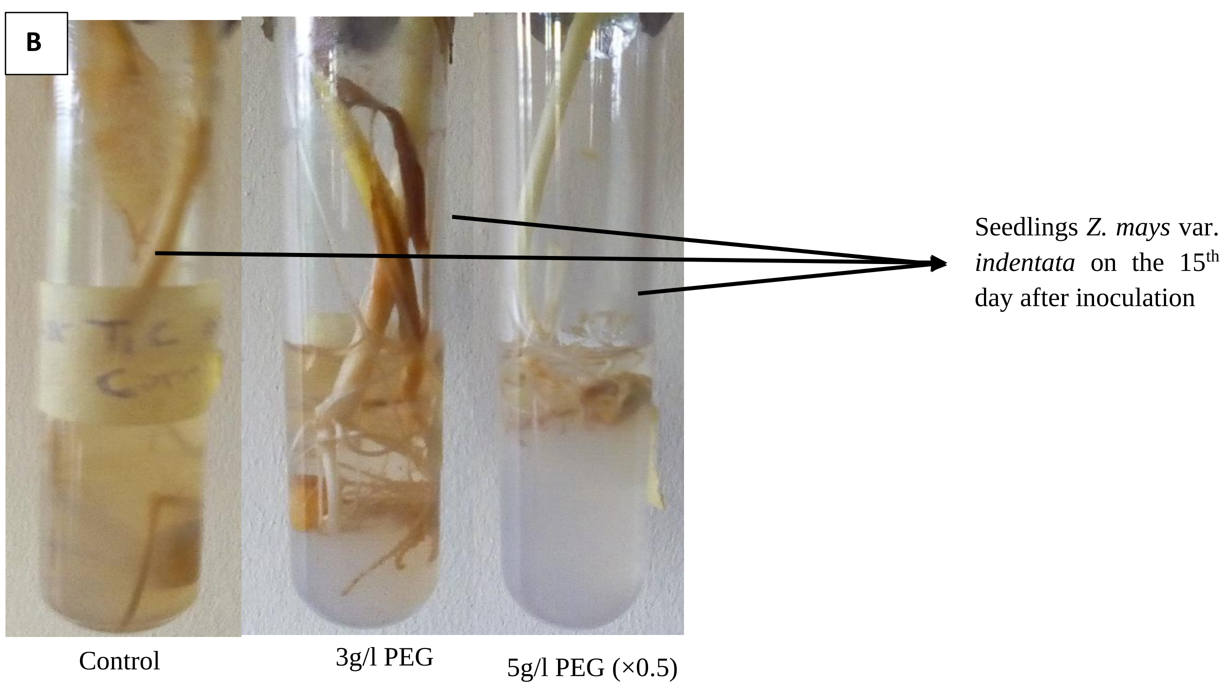
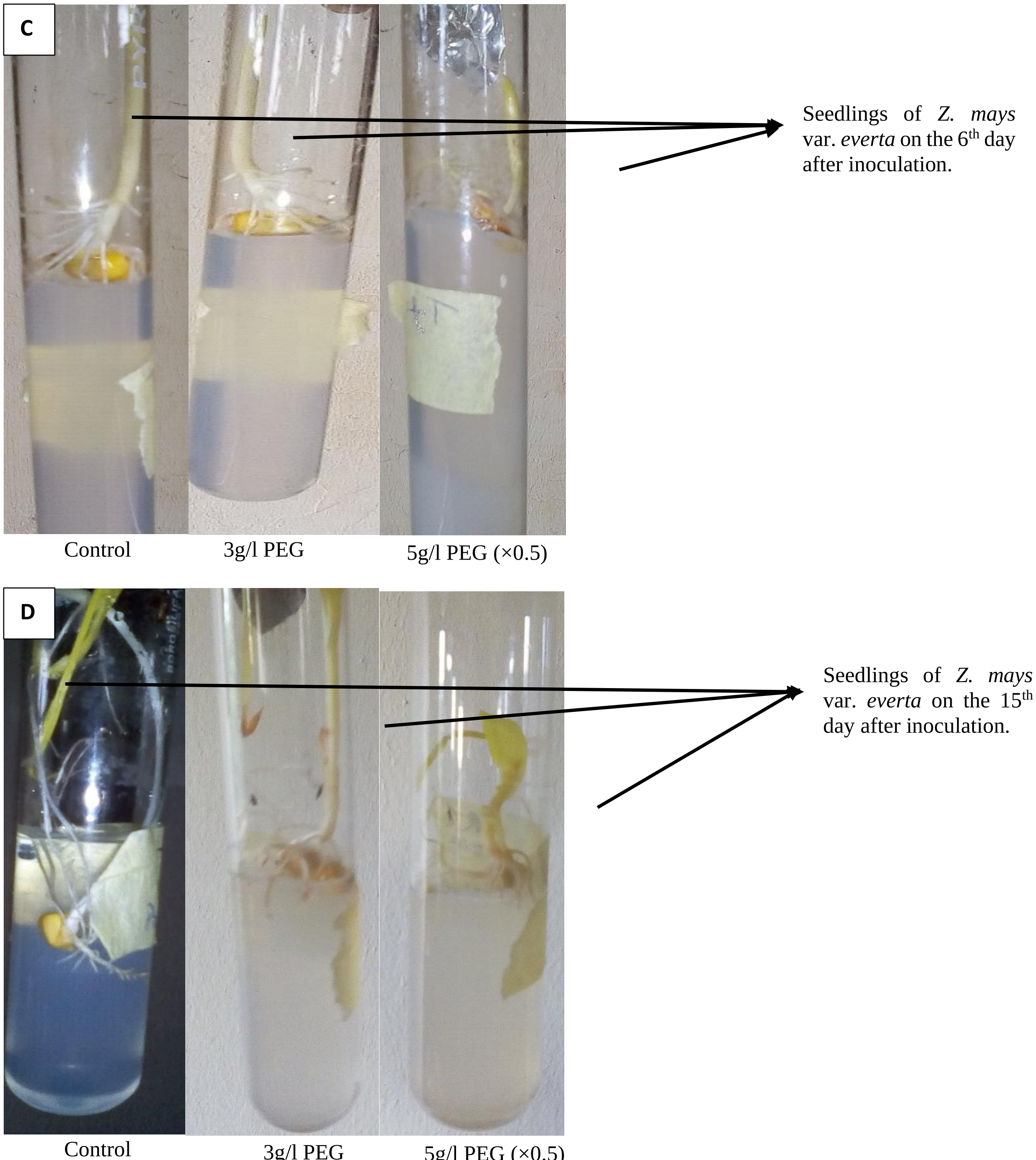


Fig 1: Time course in percentage sprouting of mature zygotic embryo of *Z. mays* var *indentata*(yellow Dent Corn) and *everta* (popcorn) treated with control(T1), 3 g/l PEG(T2) and 5 g/l(T3) PEG.


A
Seedlings *Z. mays* var. *indentata* on the 6th day after inoculation
Control
3g/l PEG
5g/I PEG (×0.5)


B
Seedlings *Z. mays* var. *indentata* on the 15th day after inoculation
Control
3g/l PEG
5g/l PEG (×0.5)



Plates A-D: showing the seedling growth of *Z. mays indentata* and *everta* on the 6th day and 15th day respectively.

Conclusion

This study revealed that the two *Z. mays* varieties screened for drought tolerance *In Vitro* showed susceptibility to the induced drought stress. However, *Zea mays* var. *indentata* exhibited a more tolerance response to the drought stress than var. *everta* and can be used as a better variety for further breeding work. It is therefore recommended that cultivation of maize should be planted on well irrigated soil for maximum yield. From the results gathered in this work, studies should be carried out so as to facilitate the development of more drought resistant maize varieties in other to keep up with the production and supply of maize in the world as the effects of global warming are upon us.

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