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IN VITRO SCREENING OF GROUNDNUT (*ARACHIS HYPOGAEA* L.) VARIETIES FOR DROUGHT TOLERANCE USING POLYETHYLENE GLYCOL (PEG 6000)

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ABSTRACT
Drought has been a major environmental factor contributing to reduced crop productivity. Identifying groundnut varieties that are drought tolerant will be of immense importance to the improvement of the crop. The study aimed at screening groundnut varieties for drought tolerance under *in vitro* condition using polyethylene glycol (PEG 6000). Treatments comprise of six varieties of groundnut (SAMNUT 14, SAMNUT 22, SAMNUT 23, SAMNUT 24, SAMNUT 25 and SAMNUT 26) and different concentration of PEG 6000 (0, 20, 40, 60g/L). The treatments were laid out in a Completely Randomized Design with three replications. Embryonic axes explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of PEG for shoot formation. Data were collected on shoot lengths, number of leaves per plantlet, number of roots and root length. A significant ($P<0.05$) reduction was observed in all the treatments as the concentration of PEG increases. Significant ($P<0.05$) variation was also observed among the varieties in response to the PEG treatment. The highest number of roots was observed in SAMNUT 24 (8.9), SAMNUT 25 (8.8), and SAMNUT 26 (7.5). These varieties also recorded the root lengths of 3.1cm, 3.7cm and 2.5cm respectively, which were the highest, suggesting these varieties to be drought tolerant. The results indicated that PEG (6000) can be used for simulating water stress under *in vitro* condition. This study will serve as a baseline for future *in vitro* screening for drought tolerance in groundnut.

Keywords: Drought tolerance, groundnut, *in vitro* screening, PEG (6000)

INTRODUCTION

Drought is the most devastating abiotic stress affecting crop productivity, which is caused by insufficient rainfall and or altered precipitation patterns (Toker et al., 2007). Groundnut (Arachis hypogaea L.) is one of the world's most important legumes. It is grown primarily for its high quality edible oil and protein. The crop is grown on 25.5 million hectares across 100 countries in the world (FAOSTAT, 2015). More than half of the production area, which accounts for 70% of the groundnut growing area fall under arid and semi-arid regions; where groundnuts are frequently subjected to drought stresses for different duration and intensities (Reddy et al., 2003). Identifying groundnut varieties that are tolerant to drought stress will be of immense importance to improve crop yield . Screening of large number of genotypes for drought tolerance is tedious under field condition. Tissue culture technique offers an opportunity for rapid screening of large set of germplasm for drought tolerance. Polyethylene glycol (PEG) is a chemical commonly used in physiological experiments to induce controlled drought stress in nutrient solution cultures and in vitro cultures. One of the benefits of PEG-simulated draught is that most often there is a positive correlation between drought tolerance of genotypes in the laboratory experiments and in the field (Kosturkova et al., 2014; Turhan and Baser, 2004). Successful in vitro screening technique for drought has been developed in other crops (Aazami et al., 2010; El Siddig et al., 2013; Turhan and Baser, 2004). However, there is paucity of knowledge on in vitro screening for drought tolerance in groundnut. Hence the present study was undertaken to develop a protocol for screening groundnut varieties for drought tolerance under in vitro condition.

MATERIALS AND METHODS

The study was conducted at the Biotechnology laboratory, Department of Plant Science Institute for Agricultural Research (IAR), Ahmadu Bello University Zaria. Six IAR improved varieties of groundnut were used in the study, which were obtained from the groundnut breeding unit of IAR. Origin and description of the varieties is given in Table 1. The seeds were surface sterilized using double sterilization sequence under the laminar flow hood. The first sterilization sequence involves treatment in 70% alcohol for 5min, followed by 10% Chlorox (commercial bleach containing 3.5% NaOCl)) plus 2-3 drops of Tween 20 for 20min with occasional stirring. The seeds were then rinsed thrice with sterile distilled water. This was followed by the second sterilization sequence when the seeds were immersed in 5% Chlorox plus 2-3 drops of Tween 20 for 10min with occasional stirring and then rinsing thrice with sterile distilled water. Embryonic axes were dissected from the sterile seeds and cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with different concentrations of polyethylene glycol (PEG 6000) (0, 20, 40, 60g/L) and solidified with 8g/L agar. Media were adjusted to pH 5.8 before autoclaving at 121^0C and 15psi air pressure for 20 min. The cultures were maintained at 26±2ºC under 16/8hr light/dark photoperiod provided by white fluorescence lamps. The experiment was arranged in a 4 x 6 factorial experiment in a completely randomized design of three replicates. Data were recorded at four weeks on shoot height (cm), number of leaves/plantlet, root length (cm) and number of roots/plantlet. The data recorded were subjected to analysis of variance using the general linear model (procedure of the statistical analysis system (SAS Institute Inc., 1990). Means were compared using least significant difference (LSD) at 0.05 probability level.

Table 1: Origin and description of the Groundnut varieties.

Varieties	Characteristic
SAMNUT-14	Developed at IAR; Potential pod yield 2000kg/ha; Late maturing (135-150 days); Rosette resistant; Seed colour-variegated (tan and white); High oil content 55-52% (Dry matter basis); Adaptation-Guinea savannah and forest
SAMNUT-22	Developed at IAR & ILRI-ICRISAT; Potential pod yield 2400kg/ha; Potential haulm yield 4000kg/ha; Medium maturing (110-120 days); Seed mass- 45-50g; Seed colour- tan; Oil content: 45%; Adaptation- Sudan and guinea savannah.
SAMNUT-23	Developed at ICRISAT& IAR; Potential pod yield 2000kg/ha; Early maturing (90-100 days); Rosette resistant; Seed mass- 35-38%; Seed colour-red; Oil content- 53%; Adaptation-Sudan and Sahel savannah.
SAMNUT-24	Developed at ICRISAT& IAR; Extra-early maturing; High oil content; Seed colour-red; Adaptation-Sudan and Sahel savannah
SAMNUT-25	Developed at ICRISAT& IAR; Extra-early maturing; Rosette resistant; Seed colour-red; Adaptation-Sudan and Sahel savannah
SAMNUT-26	Developed at ICRISAT& IAR, Extra-early maturing Rosette resistant, High oil content, Seed colour-red, Adaptation-Sudan and Sahel savannah

RESULTS AND DISCUSSION

PEG is the most commonly used osmotic agent for simulating drought under in vitro condition. In this study water potential of the culture media decreases with increasing PEG concentration which shows a significant ($P<0.05$) reduction in the growth of the cultures (Table 2; Figure 1). Maximum shoot height (5.4cm) was observed in the control treatment (medium devoid of PEG). Among the PEG treatments, medium supplemented with 60g/L PEG recorded the least shoot height (1.6cm). As regards to number of leaves/ plantlet, the highest number of leaves (3) was observed in the control while the PEG treatments recorded comparable lower number of leaves (2). Root length and number of roots/plantlet were also significantly ($P<0.05$) affected by the presence and concentrations of the PEG. The highest root lengths (4.5cm) and number of roots (5) were observed in the control, while the least root length (0.9cm) and number of roots/plantlet (2) were observed in MS medium supplemented with 60g/L PEG. PEG exposure causes osmotic stress, reduction of turgor pressure, limitation of nutrient uptake and inhibition of photosynthetic CO_2 uptake (Pugnarie et al., 1994). This could probably explain the observed reduction in growth of the groundnut cultures with increasing concentration of PEG. Turhan and Baser (2004) also observed reduction in shoot length, number of leaves and number of roots with increasing concentration of PEG in sunflower cultures. Similarly, Mengesha et al. (2016) observed reduction in shoot height, root length and number of roots with increasing concentration of PEG in cactus. There was also an observed significant ($P<0.05$) variation among the varieties in response to the PEG treatment for shoot height, with SAMNUT 24 (3.5cm), SAMNUT 26 (3.2cm), SAMNUT 25 (3.1) and SAMNUT 23 (3) having the longest shoot height. Maximum number of roots was observed in SAMNUT 24 (8.9), SAMNUT 25 (8.8), and SAMNUT 26 (7.5). These varieties also recorded the longest roots of 3.1cm, 3.7cm and 2.5cm, respectively (Table 3). Suggesting these varieties to be more drought tolerant as they had better rooting, which could have enhanced their capability to absorb water even under PEG induced water stress. Water deficit influenced mostly the number of lateral roots and the variety with a greater increase of its lateral root numbers could be considered a drought tolerant variety (Badiane et al., 2004). Genotypic variation under PEG-simulated drought has also been reported in tomato, sunflower and cactus cultures (Aazami et al., 2010; Turhan and Baser, 2004; Mengesha et al., 2016). This suffices the deployment of this current procedure for in vitro screening for drought particularly with the groundnut genotypes used in this study.

Table 2: Effect of different concentrations of polyethylene glycol on growth of in vitro plantlets of groundnut.

Treatments	Shoot height (cm)	No. leaves/plantlet	Root length (cm)	No. Roots/plantlet
PEG (g/L)				
Control	5.36a	2.50a	4.47a	4.58a
20	2.77b	2.00b	1.98b	4.42b
40	2.14c	2.00b	1.69b	3.50bc
60	1.55d	2.00b	0.89c	1.58c
CV%	14.48	9.61	36.04	45.67

Means followed by the same letter(s) within a column are not significantly different at ($P<0.05$) level of significance using LSD

Fig. 1: Effect of PEG on growth of *in vitro* plantlets of groundnut. a) plantlet on MS medium without PEG b) plantlet on MS medium + 20g/L PEG c) plantlet on MS medium + 40g/L PEG d) plantlet on MS medium + 60g/L PEG

Table 3: Response of four groundnut varieties to polyethylene glycol-simulated water stress.

Treatments	Shoot height (cm)	No. leaves/plantlet	Root length (cm)	No. Roots/plantlet
Variety				
SAMNUT 14	1.98c	2.00b	1.03d	3.00b
SAMNUT 22	3.30a	2.00b	1.00d	3.50b
SAMNUT 23	2.64b	2.25a	2.21c	4.50b
SAMNUT 24	3.48a	2.25a	3.09ab	8.88a
SAMNUT 25	3.14a	2.13ab	3.66a	8.75a
SAMNUT 26	3.20a	2.13ab	2.56bc	7.50a
CV%	14.48	9.61	36.04	45.67

Means followed by the same letter(s) within a column are not significantly different at ($P<0.05$) level of significance using LSD

CONCLUSION

Results from this study indicated that PEG (6000) at a concentration of 60g/L can be used for simulating water stress in groundnut under in vitro condition. Among the varieties evaluated SAMNUT24, SAMNUT25 and SAMNUT26 proved more tolerant to the PEG-simulated drought, suggesting these varieties to be drought tolerant. This study will serve as a baseline for future in vitro screening for drought tolerance in groundnut germplasm.

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