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Optimum Polyethylene Glycol Level Suitable for Inducing Water Stress in Potato (*Solanum tuberosum L.*) Genotypes in Kenya

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ABSTRACT
Water stress is a critical abiotic constraint that reduces crop productivity by preventing the expression of the genetic potential. Breeding programs prioritise developing climate smart resilient potato cultivars, that are pathogen-resistant, high-yielding and commercially viable. Modern biotechnological approaches, including *in vitro* drought stress screening using polyethylene glycol (PEG) 6000, have enhanced efficiency in identifying tolerant genotypes. PEG uniformly reduces water potential, mimicking soil moisture deficits. This study determined the optimal PEG level for inducing water stress in selected potato genotypes including; CIP390478.9, CIP397077.16, CIP392797.22, CIP398190.735, and local varieties *Unica* as tolerant and *Shangi* as sensitive checks. A randomized complete block design (RCBD) with five PEG levels (0.0–0.04 gL^{-1}) was replicated thrice, conducted at ADC Molo Tissue Culture Laboratory. Significant differences (P<0.05) in performance were observed among genotypes and PEG levels. Morphological parameters were highest at 0.02 gL^{-1} PEG, with CIP392797.22 exhibiting superior tolerance, followed by CIP398190.735. Shoot and root development declined at higher PEG levels (0.03 - 0.04 gL^{-1}). These finding underscored the use of PEG in screening drought-tolerant genotypes for other areas in Kenya.

Keywords: potato, *in-vitro*, PEG, genotype, moisture stress

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the predominant non-cereal food crop in the world ranking fourth after maize (*Zea Mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) (FAO, 2020). In Kenya, it is the second most consumed food crop after maize (*Zea mays*) and has recently emerged as one of the promising crops as a source of valuable nutrients and vitamins playing a great role towards agricultural food policy on self-sufficiency (Maina et al., 2016). The crop has been recognized as the government agenda crops providing food security and nutrition diversity to the rising population, under the agricultural transformation pillar (NPS, 2025).

Despite its importance, potato is highly sensitive to drought with water scarcity drastically reducing tuber productivity and quality (Mir et al., 2012). The crop's susceptibility is mainly attributed to its shallow root system and limited root length per unit area, which impair its capacity to access water effectively (Puertolas et al., 2014; Obidiegwu et al., 2015). Water scarcity frequently affects potato production due to inadequate soil moisture affecting key physiological and morphological processes, including photosynthesis, chlorophyll content and assimilate translocation, ultimately impairing growth and tuber development. Recent studies confirm that nearly 85% of potato roots are located within the top 0.3 meters of soil, making the crop highly sensitive to moisture fluctuations (Anithakumari et al., 2012; Tadesse et al., 2025). The severity depends on the timing, duration, and intensity of stress, ultimately

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limiting photosynthetic rates and CO_2 assimilation. Stomatal closure and mesophyll layer functionality are critical in maintaining photosynthesis and minimizing photorespiration during stress conditions (Ramirez et al., 2015). Optimal growth typically occurs at daytime temperatures around 21°C with cooler nights, but water stress can severely limit productivity (Obidiegwu et al., 2015; Kooyers, 2015).

Developing new genotypes with drought tolerance traits for warm and low altitude climates of Kenya is crucial for enhancing yields, promoting early maturity, ensuring adaptation to long photoperiods, and conferring resistance to pests and diseases. *In vitro* methods employing osmotic solutions provide a reproducible approach to simulate water stress conditions (Hassan, 2019). Polyethylene glycol (PEG) molecules with a molecular weight ≥ 6000 (PEG 6000) are inert, non-ionic, and virtually impermeable chains that have been extensively used to induce water stress by maintaining a consistent osmotic potential throughout experiments (Sirait and Charloq, 2017; Hassan, 2019). PEG molecules are small enough to influence osmotic potential yet too large to be absorbed by plant tissues, thereby mimicking dry soil conditions more accurately than solutions with low osmotic potential (Githinji et al*.,* 2016), which can infiltrate cell walls and alter intracellular solute concentrations (Mihiela et al., 2016; Sunaryo et al., 2019). Utilizing PEG to induce drought tolerance traits in *in vitro* potato cultures offers a promising avenue for rapid screening and development of varieties capable of thriving under drought conditions prevalent in Kenya's warmer climates. This study focused on determining the optimal PEG level for inducing water stress in six selected *in vitro* potato genotypes**.**

MATERIAL AND METHODS

Potato Clonal Material

Healthy and disease-free stem cuttings from six clonal potato genotypes (*Solanum tuberosum* L.) were sourced from Plant Quarantine Station, Muguga and used in this study. The two clonal potato genotypes tolerant to drought CIP390478.9 and CIP397077.16, two susceptible CIP392797.22 and CIP398190.735 alongside the two local varieties namely *Unica* and *Shangi* which are tolerant and sensitive checks to water stress (CIP, 2018). This experiment was conducted at the Tissue Culture Laboratory, ADC Molo.

Multiplication of Genotypes

Stem cuttings from the plantlets collected were excised from each genotype and inoculated on sterile media containing Murashige and Skoog (MS) media supplemented with sucrose at 20 gL^{-1} maintained at pH 5.8 and solidified with 2.5 gL^{-1} phytagel. Cultures were maintained at 18 °C with white fluorescent light intensity of 700 $\mu mol\ m^{-2}s^{-1}$ provided by light emitting diode (LED), for five weeks, before subculturing.

Optimization of PEG Levels *in-vitro*

To induce water stress, stem cuttings from the six potato genotypes were cultured *in vitro* on MS media supplemented with sucrose 20 gL^{-1} and phytagel 2.5 gL^{-1} maintained at pH 5.8 as per method described by Sihachakar (1997). The study examined the effect of five PEG 6000 treatments (0.0, 0.01, 0.02, 0.03, 0.04 gL^{-1}) on the genotypes. The media was sterilized at 121^{o}C for 15minutes and maintained at 18^{o}C for up to four weeks, following which all cultures were grown under a photoperiod of 16/8 h light/dark of similar LED, light intensity and each treatment replicated three times.

Data Collection

The following growth parameters were measured at predetermined intervals: shoot height, leaf number, apical root number and root length. Measurement began after 21 days of stabilization and continued for up to six weeks. The experimental design was a RCBD (Randomized Complete Block Design) for the six potato genotypes under five PEG levels and replicated three times. Data was analysed using the SAS *PROC REG* and means were separated by least significant difference (LSD) test at 5% level of significance (SAS, 2011).

RESULTS AND DISCUSSION

Effects of PEG on Shoot Elongation

Plant survival: The survival of *in vitro* plantlet varied by potato genotype and PEG level. The controls variety *Unica* and *Shangi* adapted faster to the culture media than the clonal genotypes across all the PEG levels. After 21 days, most genotypes demonstrated a stable level of survival (Figure 1), with highest plant survival being observed when there was no addition of PEG to the media.

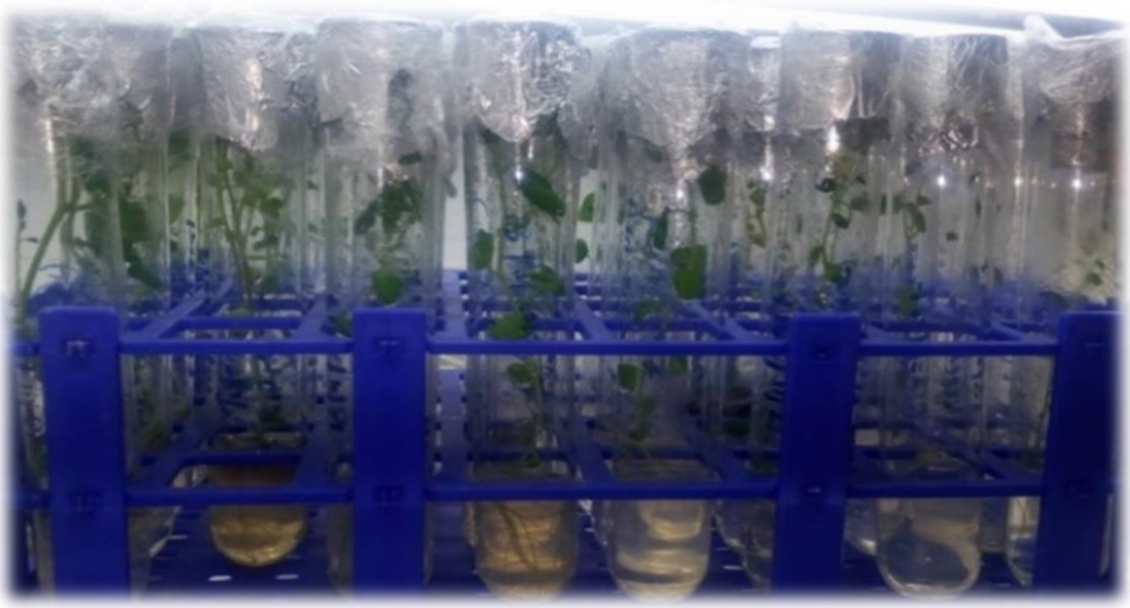

Figure 1: Plant survival in culture grown on PEG media after 21days at the Molo Tissue Culture Laboratory

The clonal genotypes initially exhibited greater sensitivity to the medium with different PEG levels, however by the third week, acclimatization was noted with highest survival being recorded with genotype 392797.22, followed by 397077.16, 398190.735 and lastly by clone 390478.9 (Figure 2). Ebrahim et al. (2006), reported that explant survival was adversely affected by increasing of PEG levels, the intensity of inhibition was clonal genotype dependent, which supports the results in the study.

When the PEG levels were increased, the osmotic stress also increased, leading to water deficit in the plant cells. This could have caused cell dehydration, which inhibited nutrient uptake and reduced metabolic activity ultimately reduce explant survival.

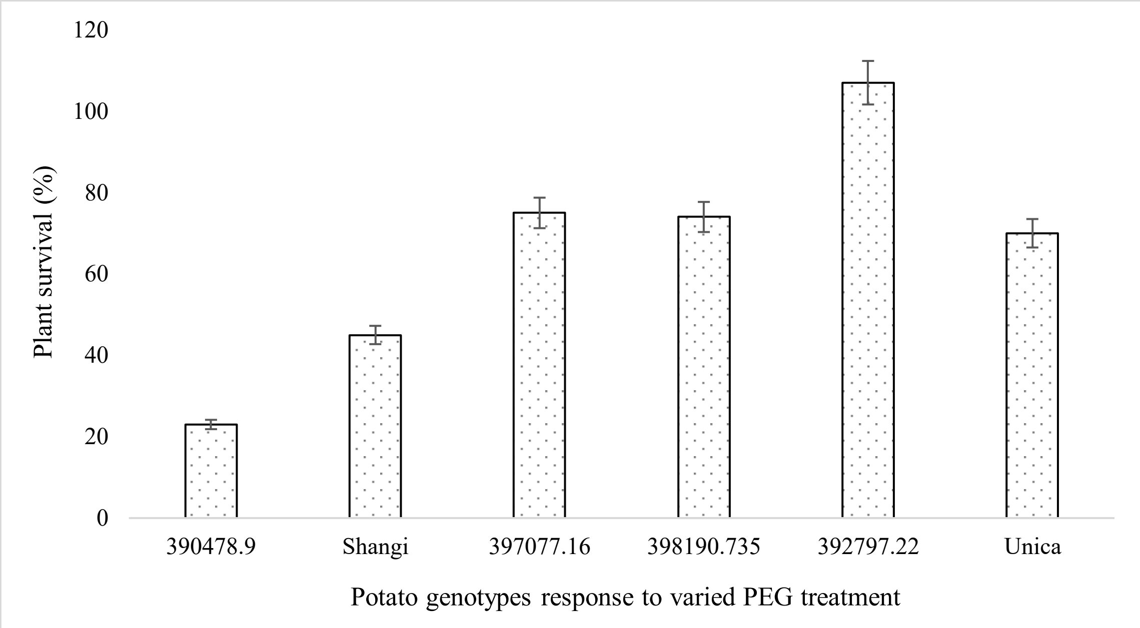

Figure 2: Plant survival (%) observed during the third week of culture across different PEG levels in six potato genotypes

Shoot elongation was significantly affected by varying PEG levels. Potato genotype CIP392797.22 exhibited the highest shoot length of 7.85 cm at 0.03 gL^{-1} PEG, while genotype CIP397077.16 reached 7.35 cm at 0.0 gL^{-1} PEG. Conversely, the lowest shoot length was recorded for variety *Unica* (0.69 cm at 0.04 gL^{-1} PEG) and CIP398190.735 (1.16 cm at 0.01 gL^{-1} PEG). The data indicated that moderate PEG levels (0.02 - 0.03 gL^{-1}) were most conducive for shoot elongation in some genotypes, while higher levels (0.04 gL^{-1}) generally inhibited growth.

The addition of PEG affected shoot elongation, indicating that both high and low levels can inhibit growth in water stress-sensitive varieties. High PEG levels (0.04 gL^{-1}) inhibited shoot elongation in genotypes 390478.9, 397077.16, and 392797.22, which did not produce shoots at lower PEG levels. This addition was suspected to stress and reduce plant cell elongation and expansion, which in turn lowered the growth rate. The decline in water on media induced by PEG, lead to the decreased proliferation of shoot, as PEG had an inhibitory effect of reducing the water potential of the medium, consequently growing shoot tips could not absorb water and nutrients from the medium resulting in deficient performance among lines for shoot vigour (Mohamed et al., 2012).

Different plant genotypes have different genetic makeup that affect how they respond to stress. Some genotypes may possess better water stress tolerance mechanisms such as higher osmotic adjustment capacity, with enhanced expression of stress-responsive genes (Ebrahim et al., 2006).

Number of Leaves Formed

The effect of PEG level and potato genotype showed significant difference on number of leaves that were formed for the six-week period (Figure 3). Leaf formation was highest at 0.02 gL^{-1} PEG in genotype CIP392797.22 at 9 leaves, followed by variety *Shangi* at 8 leaves and CIP398190.735 at 7 leaves. In contrast, the lowest leaf numbers were observed when the PEG level was at 0.04 gL^{-1} with genotype 390478.9 recording one leaf per plant (Figure 3). Some genotypes also exhibited curling of leaves or yellowing under prolonged exposure to PEG-induced stress (Figure 4).

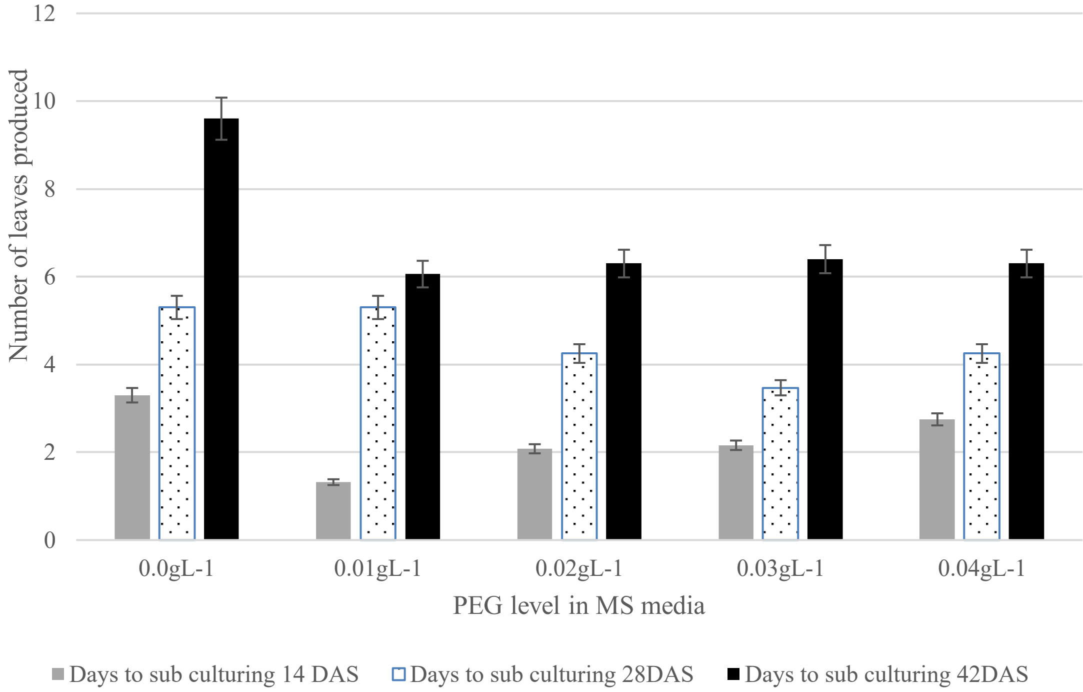

Figure 3: Effect of PEG level and *in vitro* subculture days on the number of leaves formed in six potato genotypes

Figure 4: Leaves from the clonal potato material from the genotype 398190.735 starting to curl and yellow after 28 days of *invitro* culture

Tissue yellowing was observed among some genotypes; as being sensitive and could be the consequence of water loss resulting from exposure of lines to osmotic stress caused by PEG (Hamzar et al., 2018). The observation made with some genotypes exhibited curling of leaves or yellowing under prolonged exposure to PEG-induced stress reflected physiological and biochemical responses to water deficit. Polyethylene glycol (PEG) simulates osmotic stress by creating a water deficit environment *in vitro*. Under prolonged exposure, plants struggle to absorb water, triggering stress responses (Kylyshbayeva et al., 2024). Leaf curling and yellowing are typical stress symptoms in plants under the PEG-induced water stress. The responses were genotype-dependent, reflecting differences in the plants ability to cope with water deficit. Curling was a protective measure to reduce water loss, while yellowing indicated cellular and metabolic stress, particularly involving chlorophyll degradation and impaired photosynthesis.

Effects of PEG Treatment on Root Formation

Root formation was generally stable after fourteen days of culture. Significant differences were observed among the genotypes, with root lengths ranging from 0.71 cm to 7.78 cm. The longest roots were seen in genotype CIP392797.22 (7.78 cm at 0.03 gL^{-1} PEG), while the shortest roots were observed in variety *Unica* (0.71 cm at 0.01 gL^{-1} PEG). Higher levels of PEG (0.03 and 0.04 gL^{-1}) resulted in a marked decrease in root elongation for sensitive genotypes.

Water stress induced by PEG significantly reduced root formation and elongation in certain genotypes indicating limited water uptake and restricted metabolic activity, a phenomenon observed in other crop species exposed to drought stress (Soni et al., 2011; Monneveux et al., 2013). Across all the clonal material there was a decrease in root length with increase of PEG levels. With higher levels of 0.03 gL^{-1} and 0.04 gL^{-1} PEG level (Figure 5) 397077.16, 390478.9 had a significantly decreased root formation and elongation observed over the six-week period.

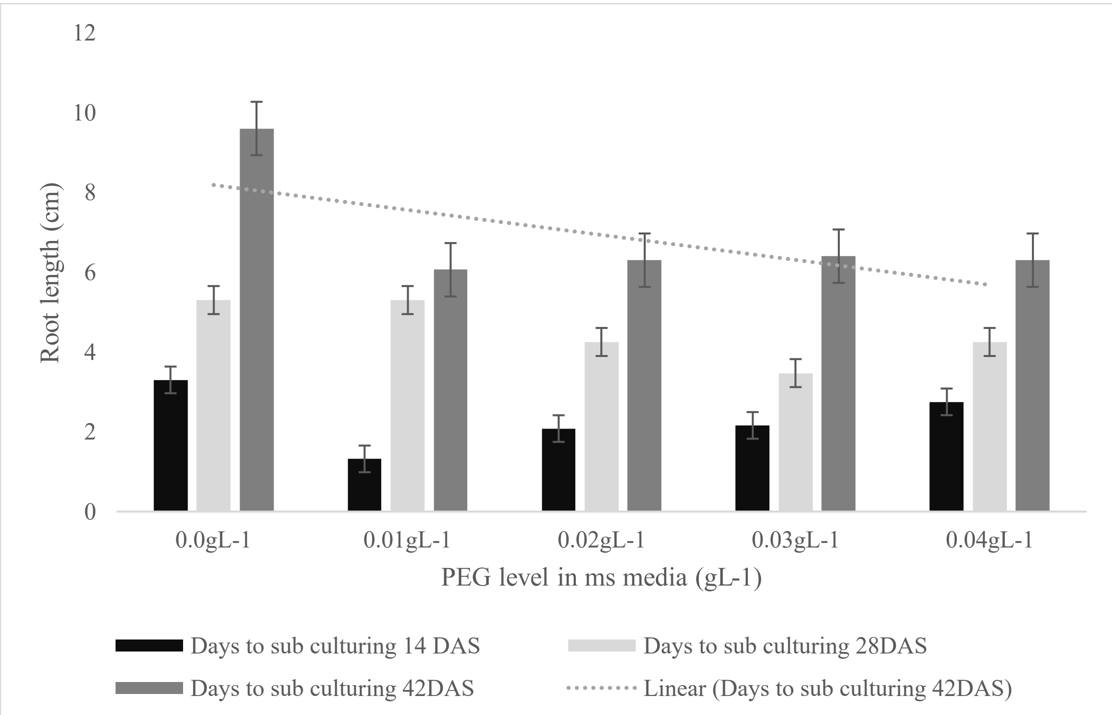

Figure 5: Effect of PEG level on root length formed on s ix potato genotypes induced on water stress media in Tissue culture

Drought stress decreased the root length in various plant species in wheat and maize (Monneveux, et al., 2013). Drought stress caused root formation to decrease, part of survival strategy of the species under stress conditions where availability of sufficient water for further growth only can induce germination. Root length is known to be an important trait in selection of drought resistant variety (Albinski et al., 2012). Research studies confirm that nearly 85% of potato roots are located within the top 0.3 meters of soil, making the crop highly sensitive to moisture fluctuations (Anithakumari et al., 2012). All root characters in these studies were adversely affected by increase in PEG concentration. However genotypic differences were not significant for all these characters

Root Number

The response of the potato genotypes to different PEG level on number of apical roots formed varied and the results indicated the overall highest number of apical roots was clonal material from the potato genotype 397077.16 (5) when the level of PEG added was 0.04 gL-1. This was followed by variety *Unica* and *Shangi* at 4 roots respectively. Water stress induced by polyethylene glycol (PEG) significantly reduced root formation and elongation in certain genotypes, indicating impaired water uptake and restricted metabolic function. PEG creates an osmotic gradient that limits water availability, leading to reduced cell turgor, inhibition of cell division, and suppressed elongation in root tissues. The stress also disrupts hormone signalling and metabolic processes essential for root development. These effects were more pronounced in genotypes with lower stress tolerance, suggesting genotypic variability in osmotic adjustment and drought resilience mechanisms (Anithakumari et al., 2012).

CONCLUSION

This study demonstrated that the level of polyethylene glycol (PEG) significantly influences the response of different potato genotypes *in vitro*. Increasing the level of polyethylene glycol (PEG) in culture media influenced growth performance across the different potato genotypes. The study concluded that MS media supplemented with 0.02 gL^{-1} PEG was optimal for inducing water stress without severely compromising shoot elongation, leaf formation, or root development.

The PEG level promoted early shoot development, increased leaf production, and the formation of more apical roots—factors directly linked to critical parameters for high yield. Notably, at the highest PEG dose tested (0.04 gL^{-1}), the percentage of shoot formation remained stable, although further growth was inhibited and abnormalities appeared

The findings emphasize the potential of *in vitro* screening to rapidly identify drought-tolerant potato genotypes. Furthermore, the development of new potato cultivars with improved tolerance to low moisture conditions is essential in addressing challenges posed by climate change. Future studies may incorporate molecular analyses to elucidate the underlying mechanisms of drought tolerance and coping strategies in these genotypes.

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