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# Isopentenyletransferase Gene Enhances Drought Tolerance in Genetically Engineered Sweetpotato (*Ipomoea batatas* (L.) Lam)

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## Abstract

Sweetpotato is an economically important food crop worldwide. It is a valuable source of carbohydrate in Sub-Sahara Africa and is an industrial raw material in developed countries. Sweetpotato is capable of producing high yield in a short period of time making it suitable for cultivation in regions with limited or erratic rain water supply where other food crops grow with difficulty. However, it is sensitive to water deficit, which adversely affects its growth and yield. The isopentenyltransferase gene (IPT), isolated from Agrobacterium delays drought induced senescence via up-regulation of cytokinin biosynthesis when put under the control of a water-deficit responsive and maturation specific promoter (PSARK). This study therefore aimed at evaluating the effect of IPT on sweetpotato under water deficit conditions. Three sweetpotato genotypes: Jewel, Kemb36 and Ksp36 were transformed using IPT under the control of a water-deficit responsive and maturation specific promoter (PSARK). Transgenic sweetpotato plants were then evaluated for drought tolerance under controlled conditions in the glasshouse. Transgenic plants displayed delayed senescence and greater drought tolerance under water deficit conditions in the glasshouse. These plants exhibited better growth, higher yield, maintained higher water status, chlorophyll content, and thus higher photosynthetic rates under reduced water conditions in comparison to wild-type. These results therefore affirm that inducible expression of IPT gene in sweetpotato significantly improves drought tolerance.

**Keywords:** Drought tolerance; Transgenics; Isopentenyltransferase gene; Sweetpotato; Transformation

## Abbreviations

IPT: Isopentyltransferase; CIP: International Potato Center; MS: Murashige and Skoog; PSARK: Senescence Associated Reporter Protein Kinase promoter; PMI: Phosphomannose Isomerase.

## Introduction

Sweetpotato is an economically important crop in most regions of the world [1]. It is a valuable source of food, animal feed and industrial raw material [2]. Sweetpotato is considered a potentially new source of energy due to its high carbohydrate content in the storage roots. The carbohydrates consist mainly of starch, sugars and a low quantity of pectin, hemicelluloses and cellulose [3]. The high energy levels of sweetpotato therefore, make it an attractive industrial raw material for biodegradable plastics and for bio-fuel production [4]. According to sweetpotato is composed of antioxidant compounds such as anthocyanin, carotenoid and vitamin C [5]. These compounds are of medicinal importance in that they are capable of protecting human body from oxidative stress that may promote aging [6,7]. As such, sweetpotatoes has been welcomed as a better alternative staple food crop as a result of its diverse usage.

In many tropical regions, drought stress is the major abiotic factor that limits sweetpotatoes production. According to Van Heerden and Laurie [8], yield reduction as result of drought stress has been estimated at 60%. Therefore, in the face of escalated drought episodes due to climate change, irrigation could be a perfect solution to overcome drought that greatly affects sweetpotato productivity. However, this may seem an uphill task in the sense that farmers may not be able to access clean water for irrigation due to ever surging human population that impose great pressure on limited available water resources.

Although sweetpotato is considered drought tolerant [4], it is sensitive to water deficit stress especially during establishment and initiation of storage roots. Yields are significantly reduced when water stress occurs within the first six weeks after planting [9]. The crop therefore requires a constant water supply throughout its growing season to produce high yields [10].

In this regard the most sustainable solution is to improve sweetpotatoes production is to develop drought tolerant varieties. Sweetpotato hybridization has been previously used as a breeding strategy to improve drought tolerance. However, the strategy is limited because of sweetpotatoes high male sterility, sexual incompatibility and hexaploid nature of its genome that results in high level of hybrid segregation and loss of valuable traits hence requiring a long time to select suitable plants. Gene technology therefore offers great potential for improving sweetpotato to enhance beneficial traits through expression of foreign genes [11].

Previously there have been considerable efforts to improve sweetpotatoes for drought tolerance. According to Bray [12]. Responses to water stress in plants is controlled by a number of genes with different gene functions and most regulatory responses are initiated immediately c loss water. Therefore, adjustments in cellular metabolism results in changes in gene expression. Changes in expression patterns of genes in plant under drought stress ranges from

genes whose products are involved in early responses such as signal transduction, transcription and translation factors; to late response genes, such as water transport, osmotic balance, oxidative stress and damage repair [13].

For instance, Carotenoids and anthocyanins are important antioxidants in plants. Their accumulation often increases abiotic stress tolerance in plants. Beta-carotene hydroxylase (CHY-beta) and lycopene ε-cyclase (LCY-ε) genes, (εIbCHY-beta and IbLCY-ε) down-regulation enhanced salt tolerance of transgenic sweetpotato cultured cells as a result of increased accumulation of carotenoids [14]. According to findings by Kim et al. [15], Zinc finger proteins are associated with abiotic stress responses in plants. Transgenic sweetpotato (cv. Yulmi) plants expressing the soybean cold-inducible zinc finger protein (SCOF-1) gene under control of SWPA2 promoter showed enhanced tolerance to low-temperature stress. Glycine betaine (GB) accumulation in higher plants enhances tolerance to various abiotic stresses such as drought, salinity and cold [16]. The gene encoding betaine aldehyde dehydrogenase (BADH) functions in GB biosynthesis in plants and therefore its overexpression increases the GB accumulation and subsequently enhancing plant tolerance to abiotic stresses [17-21]. A chloroplastic BADH gene from Spinacia oleracea (SoBADH) was successfully introduced into sweetpotato (cv. Sushu 2) with transgenic plants displaying improved tolerance to multiple abiotic stresses, including salt, oxidative stress, and low temperature [22]. The transgenic sweetpotato exhibited increased BADH activity and GB accumulation resulting in enhanced protection against cell damage via maintenance of cell membrane integrity, stronger photosynthetic activity, and ROS scavenging activation [22,23], introduced sperimidine synthase coding genes in sweetpotato to improve its tolerance to environmental stresses. The transgenic plants displayed tolerance to drought, salt, chilling and heat stresses indicating the ability of the gene in enhancing tolerance to environmental stresses. Previous report by revealed that transgenic potato plants with Cu/Zn superoxide dismutase and ascorbate peroxidase genes showed enhanced tolerance to multiple environmental stresses including a high temperature compared to non-transgenic plants. Additionally, Transgenic sweetpotato (cv. Yulmi) plants expressing CuZn superoxide dismutase (CuZnSOD) and ascorbate peroxidase (APX) with enhanced tolerance to methyl viologen-mediated oxidative stress, chilling, and drought were developed via transformation with CuZn superoxide dismutase (CuZnSOD) and ascorbate peroxidase (APX) genes under the control of an oxidative stress-inducible SWPA2 promoter [24,25]. Leaf senescence, a programmed degeneration process controlled by multiple developmental and environmental signals, occurs at the final stage of leaf development [26]. It is therefore an intrinsic mechanism for mobilization of nutrients from aging leaves to support the development of younger organs [25]. According to Xu et al. [27] modifications of the leaf senescence process directly impact agricultural traits of crop plants such as biomass, seed yield, seed protein composition, and abiotic stress resistance such as drought. Isopentyl transferase gene (IPT) catalyzes the rate limiting step in cytokinin biosynthesis. Overexpression of the gene in plants enhances high levels of endogeneous cytokinin. Therefore, Strategy such as use of isopentenyl transferase gene (IPT) under the control of a water-deficit responsive and maturation specific promoter (PSARK) has been employed to delay drought induced senescence [28]. Cytokinin hormone homeostasis as a result of expression of IPT gene under the control of PSARK promoter has been reported to enhance drought tolerance in a number of crop plants like broccoli [28-34], lettuce, rice, wheat, peanut and rice. In this work, we genetically engineered sweetpotao with IPT gene under the control of a water-deficit responsive and maturation specific promoter, PSARK, to enhance drought tolerance.

## Materials and Methods

### Plant material

Two Kenyan sweetpotato cultivars Kemb36, KSP36, obtained from Kenya Agricultural and Livestock Research Organization, Nairobi and non-African cultivar Jewel, obtained from International Potato Centre, Nairobi were used in this study after virus indexing. The Kenyan cultivars were selected due to their high dry matter content and moderate tolerance to drought. Jewel cultivar was included due to its high transformability and regenerability. The plants were then grown under glasshouse condition at Kenyatta University. The plants were allowed to grow for two months before being used as stock plants for subsequent regeneration experiments.

### Bacterial strains and plasmid

Agrobacterium tumefaciens strain EHA 101 harboring binary plasmid vector pNOV2819-IPT was used in this study. The binary vector contained IPT gene driven by PSARK promoter and a phosphomannose isomerase (PMI) gene as a selectable marker gene that confers resistance to mannose in positively transformed tissues (Figure 1). The IPT gene was graciously provided by Dr. Eduardo Blumwald, Department of Plant Sciences, University of California, Davis, CA, USA.

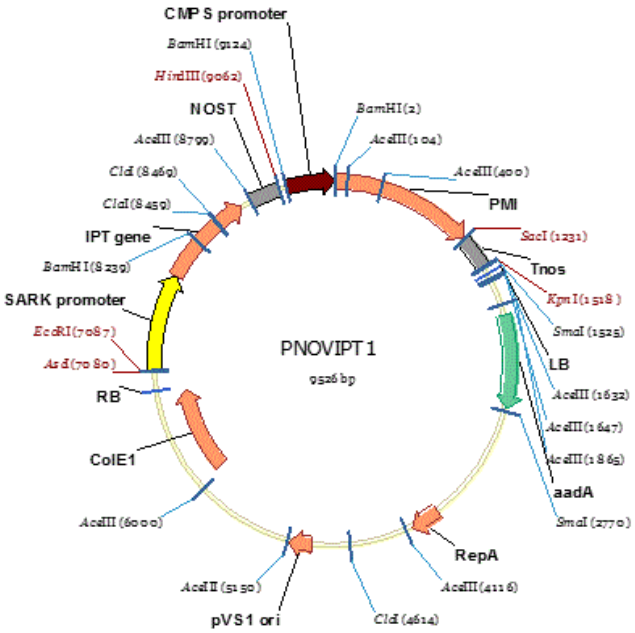


**Figure 1:** Map of the binary vector PNOV-IPT with the T-DNA comprising IPT gene cassette and the selectable marker gene cassette, PMI, driven by CMPS promoter.

The IPT cassette was cloned into pNOV2819 plasmid by and used to transform Agrobacterium tumefaciens strain EHA101 that was then

stored in form of glycerol stocks at the Plant Transformation Laboratory at Kenyatta University where this work was done.

### Transformation and regeneration

Meristematic leaf cuttings (2-6 mm long) from *in vitro* grown sweetpotato plants were used as target tissue for transformation with the gene constructs pNOV-IPT immobilized in Agrobacterium tumefaciens strain EHA 101. The binary vector contained IPT gene driven by PSARK promoter and a phosphomannose isomerase (PMI) gene as a selectable marker gene that confers resistance to mannose in positively transformed tissues (Figure 1).

Transformation of the explants followed the protocol described [35] with some modifications. After 3 days of co-culturing, the explants were transferred to F15 medium (Table 1) supplemented with 30 g/l mannose and 250 mg/l cefotaxime for callus induction. Selection of positive transformants was conducted as described by the induced calli were transferred to F9 media (Table 1) for callus proliferation. The calli were sub cultured every two weeks [36], until embryogenic calli developed. The embryogenic calli surviving selection pressure were subsequently divided and cultured on G24D and F25 media (Table 1) for embryo development and shoot initiation. This followed a preliminary observation of root emergence before shoot regeneration on G24D medium. Fully developed shoots were multiplied on hormone free sweetpotato propagation media (Table 1) and transferred to peat moss for hardening and acclimatization in the glass house.

| Media Type | Media Composition |
|---|---|
| Preinfection (EPM) | LS salts, 30 g/l Sucrose, 0.5 g/l MES, 0.1 g/l NAA, 0.01 g/l GA3, 1 g/l BAP |
| Bacterial Infection (BIM) | LS salts, 30 g/l Sucrose, 20 mg/L acetosyringone, pH 5.5 |
| Co- culture (S CM) | LS salts, 30 g/l sucrose, 20 g/L glucose, 0.5 g/L MES pH 5.5 |
| Callus Induction (F15) | LS salts, 30 g/l sucrose, 0.2 mg/l Zeatin riboside, 0.02 g/l 2,4-D, 30 g/l Mannose, 200 mg/l Cefotaxime pH 5.8 |
| Callus Maturation (F9) | LS salts, 30 g/l sucrose, 0.2 mg/l Zeatin riboside, 30 g/l Mannose, 200 mg/l Cefotaxime pH 5.8 |
| Embryo Formation (G2,4D) | LS salts, 30 g/l sucrose, 30 g/l Mannose, 200 g/l Cefotaxime, 0.05 g/l 2,4-D, 0.01 g/l GA3 pH 5.8 |
| Embryo Maturation (ABA) | LS salts, 30 g/l sucrose, 0.2 mg/l Zeatin riboside, 30 g/l Mannose, 1 g/l Abscisic acid, 200 g/l Cefotaxime pH 5. |
| Shoot Induction (F9) | LS salts, 30 g/l sucrose, 0.2 mg/l Zeatin riboside, 200 g/l Cefotaxime pH 5.8 |

**Table 1:** Composition of different types of media used for transformation and regeneration of sweetpotato.

### Molecular analysis of transgenic plants

The genomic DNA was extracted from the leaf tissues of putative transgenic and non-transgenic (control) plants using cetyltrimethylammonium bromide (CTAB) method as described [37-39]. The presence of IPT gene in the transgenic plants was determined via PCR using gene specific primers: Forward primer; 5'-CCAACTTGCACAGGAAAGAC and Reverse primer; 5'-CTAATACATTCCGAACGGATGAC.

### Drought stress assays

Pot experiments were carried out in the glass house to evaluate the effect of drought stress on both transgenic and non-transgenic sweetpotatoes. Vine cuttings measuring 4 cm in length were obtained and planted in plastic pots containing 15 kg of a homogeneous completely air-dried soil with 1 plant per pot. Plants were watered sufficiently and allowed to grow for 4 weeks before they were subjected to drought stress for 96 days by withholding irrigation. Treatments were a combination of three watering regimes; severe drought stressed (water with 30% of soil holding capacity after every 2 days) moderate drought stress (watered with 50% of soil holding capacity after every 2 days) and control (watered with 80% of soil holding capacity after every 2 days). This was done for both transgenic and non-transgenic plants. After the stress treatment the plants were watered to 100% soil holding capacity every two days until the plants fully recovered. Treatments were conducted in a completely randomized design with 3 replicates of 4 pots per treatment as described [38]. Data on physiological and morphological characteristics (relative water content, chlorophyll content and growth parameters) were collected from mature, healthy and fully expanded third, fourth and fifth leaves before treatment (28 days), during treatment (at day 42, 63 and 96) and after treatment (on the 1st, 5th and 9th day of recovery). This was done on transgenic and non-transgenic plants for comparison. Sampling was done simultaneously on fully watered plants and the different stress regimes for both transgenic and non-transgenic plants.

### Relative water content assay

To monitor the relative water content, the leaves were collected from the mid-section of the vine then weighed to obtain the fresh weight (FW). They were floated in distilled water for 24 h obtaining the turbidity weight (TW). Dry weight (DW) of leaves was determined by oven- drying the leaves at 80°C for 72 h and then the weight determined. Values of FW, TW, and DW were used to calculate the leaf relative water content (RWC) using the formula indicated by equation 1 below.

$$RWC\ (\%) = \left[\frac{(FW - DW)}{(TW - DW)}\right] \times 100$$

Where RWC=Relative water content; FW=Fresh weight; DW=Dry weight; TW=Turbidity weight.

### Determination of leaf chlorophyll content

To determine the leaf chlorophyll content, ten fresh leaf discs from the third fully expanded leaf was sampled, chlorophyll was extracted using 100% v/v acetone by crashing the leaves using a mortar and

pestle. Chlorophyll was obtained from the leaf debris by centrifugation at 5000 rpm for 10 minutes and the supernatant transferred into a clean 2 ml eppendorf tubes. Chlorophyll concentration was measured using a spectrophotometer at 662, 645 and 470 nm wavelengths and the readings at this absorbance levels used to calculate chlorophyll a, chlorophyll b and carotenoid concentration. Measurements and calculations were performed according to Saraswati et al. [40] Following equations below.

Equation 2: Chlorophyll a=$11.75A_{662}$–$2.350A_{645}$

Equation 3: Chlorophyll b=$18.61A_{645}$–$3.960A_{662}$

Equation 4: Total carotenoid (C a+b)=1000 $A_{470}$–2.27 Ca–81.4 Cb/227

### Plant growth parameters determination

Plant growth parameters; vine length, internode length, branch number, leaves per vine, leaf length and leaf width were measured on four randomly sampled plants per treatment a week before stress treatment, at 42, 63 and 96 days after planting (DAP). The increment in each of these parameters was determined at 42 and 96 DAP and expressed as a percentage.

## Data analysis

Differences in transformation frequency, transformation efficiency and regeneration frequency between genotypes were analyzed using ANOVA at 95% confidence interval. Transformation efficiency (TE) was calculated as percentage number of confirmed transformants per the number of transformed explants whereas the transformation frequency (TF) was the percentage number of putative transformed callus per the number transformed explants. Regeneration frequency was calculated as the percentage number of shoots formed per the number of calli put on regeneration on drought stress experiments, data on growth parameters (leaf length, width, internode length, vine length, leaves per vine and number of branches per plant) relative water content and chlorophyll content was analyzed thought ANOVA using statistical analysis software, SAS version 9.2. Treatment means were compared using Turkey's tests at 95% confidence level ($p \geq 0.05$).

## Results

### Transformation and regeneration of transgenic plants

Three sweetpotato varieties under study were evaluated for their response to transformation and regeneration. Jewel had the highest transformation frequency mean of (57.1%) (Table 2) which was significantly different from Kemb 36 (50.0%) and KSP 36 (44.2%) (Table 2). On regeneration from transformed calli, Jewel also had the highest regeneration frequency of (2.1%) followed by Kemb 36 (0.9%). The genotype KSP36 did not give any regenerants despite having calli that tolerated mannose (Table 2).

| Variety | Embryogenic calli formation (%) | Transformation frequency (%) | | Regeneration frequency (%) | Transformation efficiency (%) | Regeneration Efficiency (%) |
|---|---|---|---|---|---|---|
| KSP 36 | 17.66 ± 1.75a | 44.17 ± 4.36a | 0.00 ± 0.00 | | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Kemb 36 | 20.00 ± 2.25ab | 50.00 ± 5.63ab | 0.88 ± 0.83a | | 0.83 ± 0.38a | 5.52 ± 2.16a |
| Jewel | 22.83 ± 0.95b | 57.08 ± 2.36b | 2.08 ± 1.19a | | 2.08 ± 1.19a | 12.81 ± 2.75a |
| LSD | 5.07 | 12.68 | 2.78 | | 2.76 | 7.11 |
| (P ≥ 0.05 | | | | | | |

**Table 2:** Data on Transformation frequency, regeneration frequency and regeneration efficiency.

### Analysis of transgenic sweetpotatoes

Putative transgenic plants were analyzed through PCR on genomic DNA using IPT gene specific primers to confirm the presence of the gene and an amplicon of 500 bp was obtained as visualized on agarose gel after electrophoresis (Figure 2).

Analysis of PCR positive plants through drought stress revealed that under optimal water conditions (before on set of drought stress treatments), both transgenic and wild type sweetpotatoes grew vigorously with a lush green color with no visible differences in growth (Figure 3A).

Fourteen days later after drought induction, the wild type plants began to wilt and leaves droop while transgenic plants still had upright leaves. After 21 days of stress treatment, the wild type plants displayed pronounced senescence of leaves characterized by yellowing as opposed transgenic plants that only exhibited wilting. Pronounced Senescence in transgenic plants began to manifest by day 35 of stress treatment where the leaves wilted, turned yellow then brown before finally falling off the plant. By this time, most of the leaves of the wild type plants had fallen off. Generally, this process of wilting, senescence and falling off of leaves as a result of drought was delayed in transgenic plants (Figure 3B). On recovery after drought stress the transgenic plants recovered much faster compared to the wild type. Transgenic plants fully recovered from effects of drought after 9 days of re-watering while the wild type plants remained chlorotic (Figure 3D).

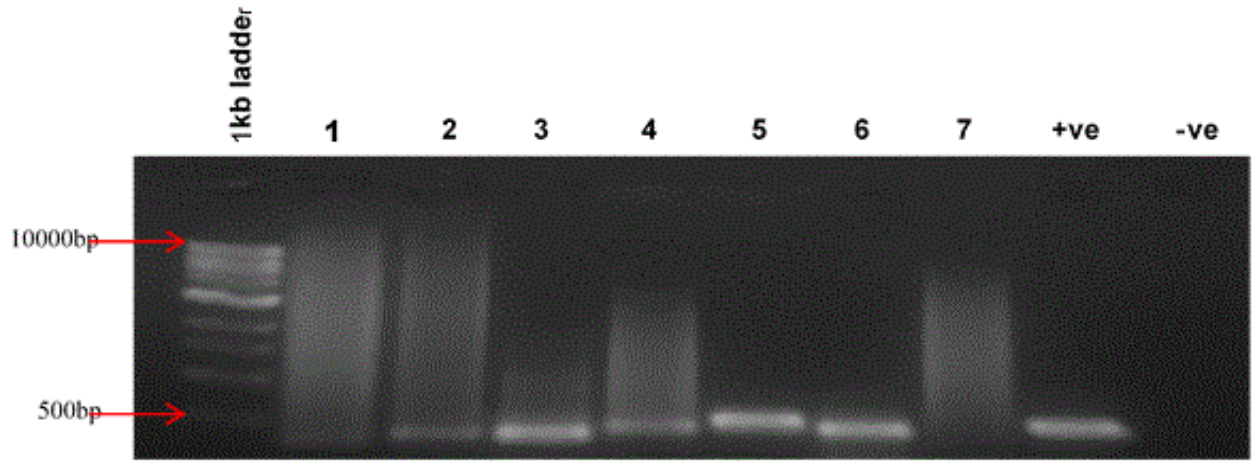


**Figure 2:** PCR analysis of Putative transgenic plants using IPT gene specific primers on sweetpotato genomic DNA; Lanes 1 and 2; putative transgenic Kemb36. Lanes 3-7; putative transgenic Jewel. Lane 8; +ve control (PNOV-IPT plasmid), Lane 9; -ve control.

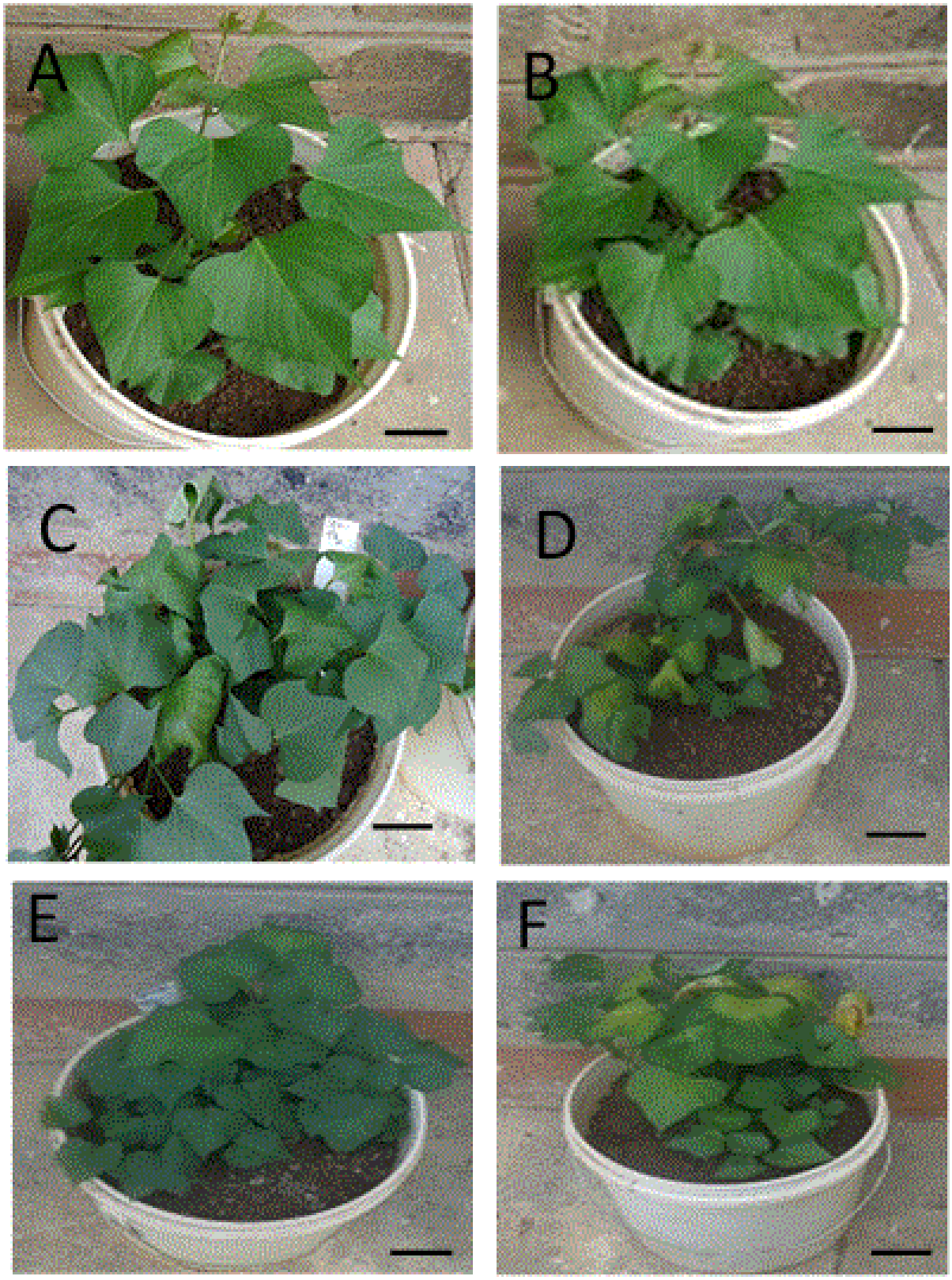


**Figure 3:** Morphological appearance of the sweet potato plants before onset of drought stress treatment; (A) Transgenic jewel and (B) Jewel wild type 28 days after planting.

## Effect of drought stress on morphological characteristics

Both transgenic and wild type plants responded differently to the 3 regimes of stress treatments. Moderate drought stress relatively increased branching in transgenic plants compared to wild types. However, morphology of both transgenic and wild types was significantly affected by severe drought stress as indicated by reduction in leaf area, vine length, internode length and number of leaves per vine. Compared to well-watered plants (controls), moderate stress decreased both vine length and internode length in both transgenic and wild types. The average vine length and internode length in transgenic Jewel variety was 21.57 cm and 0.98 cm respectively and while in transgenic Kemb36 the averages were 26.27 cm and 0.94 cm respectively the corresponding vine length and internode length in wild type plants was significantly lower (12.96 cm and 0.56 cm respectively in Jewel and 23.58 cm and 0.89 cm respectively for Kemb36). Further increase in drought stress treatment (day 35) lead to a further reduction in vine and internode lengths and was more severe in wild type plants (Figure 4).

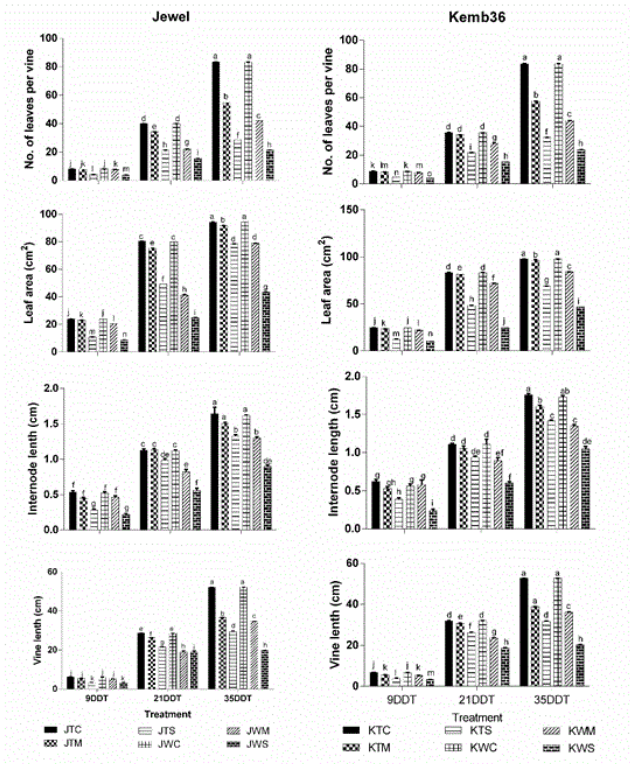


**Figure 4:** Effect of drought stress treatment on growth of transgenic and wild type sweetpotato. (A) Transgenic jewel variety and (B) Jewel wild type 28 days after planting (DAP). (C) Transgenic Jewel variety 35 days (63 DAP) after onset stress treatment. (D) Wild type Jewell variety 35 days (63 DAP) after the start of stress treatment. (E) Transgenic plant at 9 days after re-watering. (F) Wild type plant 9 days after re-watering.

Under well-watered conditions, both transgenic and wild type plants showed no significant difference in leaf area and number of leaves per vine (Figure 4). However, drought stress had a great effect on both leaf area and number of leaves per vine. Compared to the wild type plants, the transgenic plants exhibited a higher leaf area ($P<0.05$) and number of leaves per vine under severe stress. In transgenic Jewel plants the average leaf area and number of leaves per vine were 49.30 $cm^2$ and 21.31 respectively while in transgenic Kemb36 plants they were 48.20 $cm^2$ and 21.80 respectively. Leaf area and number of leaves per vine in wild type plants were significantly lower for both Jewel (24.84 $cm^2$ and 15.36 respectively) and Kemb36 (24.81 $cm^2$ and 15.29 respectively).

## Relative water content in transgenic and wild type plants under drought stress

Under well-watered (control) conditions, plants maintained high relative water content of 96% (Figure 5) throughout the experiment with no significant difference between transgenic and wild type plants for both genotypes. However, for all plants subjected to drought stress treatment, relative water content declined significantly in response to drought stress. Significant change in relative water content was observed in severely stressed plants much earlier (day 14) than in moderately stressed plants (day 21). Two weeks after withholding water, the leaf RWC of Jewel transgenic plants was 85.24% and 93.36% for Kemb36 under moderate stress. Under severe stress, transgenic Jewel and Kemb36 genotypes had 82.05% and 86.94% relative water content respectively. Compared to the transgenic plants, the wild type plants registered significant lower relative water content. Under moderate stress the wild type relative water content for genotypes Jewel and Kemb36 was 81.61% and 90.43% respectively. For severe stress, the relative water content was 73.08% in Jewel and 81.13% in Kemb36. As drought treatment progressed, plants exhibited further reduction in relative water content but IPT transgenic plants maintained a relatively higher RWC of above 80% as which was significantly higher than that of wild type plants (Figure 5).

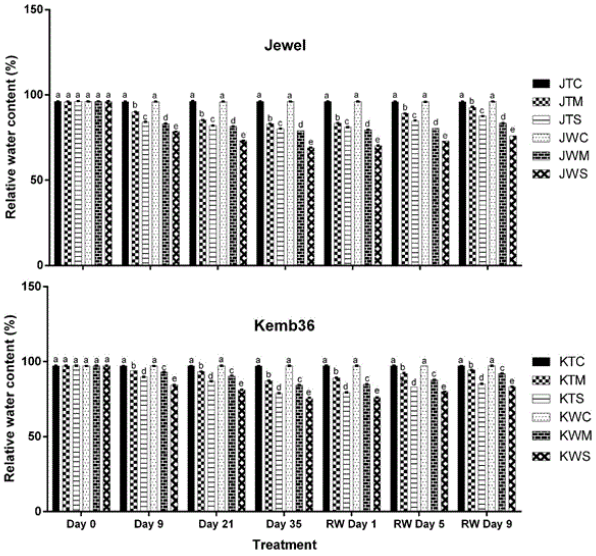


**Figure 5:** Effect of drought stress treatment on relative water content on Jewel and Kemb36 varieties. Bars with same letters are not significantly different.

### Effect of drought stress on leaf chlorophyll content in wild type and transgenic plants

Under optimum watering conditions, transgenic and wild type plants showed no significant variation in total chlorophyll content. However, a significant reduction in total chlorophyll content was observed 21 days after imposing drought stress. The total chlorophyll content in transgenic Jewel plants was 32.09% and 26.01% under moderate and severe stress respectively while corresponding chlorophyll content in wild type plants was 31.44% and 25.5% under similar conditions. For the genotype Kemb36 total chlorophyll content was 35.3% and 29.64% under moderate and severe stress condition respectively while corresponding chlorophyll content in wild type plants was 35.07% and 23.03% under similar conditions. As the drought treatment progressed both transgenic and wild type plants showed a further reduction in the total chlorophyll content, but the reduction was greater in wild types. At 35 days of drought stress, the total chlorophyll content in transgenic Jewel plants was 27.23% and 23.70% under moderate and severe stress respectively while corresponding chlorophyll content in wild type was 26.41% and 23.03% under similar conditions. In Kemb36 genotype, total chlorophyll content for transgenic plants was 32.46% under moderate stress and 27.93% under severe stress and the corresponding values for the wild type plants under moderate and severe stress conditions was 31.26% and 24.74% respectively.

Upon re-watering, the moderately stressed plants recorded a drastic increase in total chlorophyll content within the first 3 days then gradually till they reached the same level as the unstressed control plants by the 7th day (Figure 6). Further, in severe stressed transgenic plants it increased slowly within the first 3 days then rapidly to attain significant recovery in chlorophyll content 9 days after re-watering. However, the severe stressed wild type showed no signs of significant recovery even after 9 days (Figure 6).

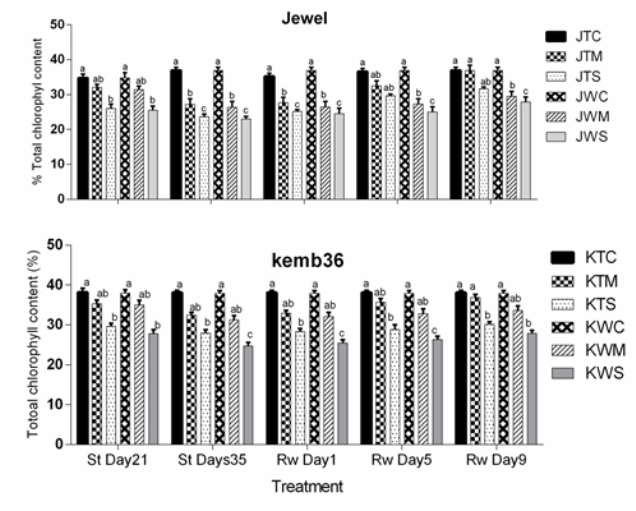


**Figure 6:** Changes in leaf chlorophyll content under well-watered and drought treatment in both transgenic and wild type plants. Bars with the same letters are not significantly different

### Effect of drought stress treatment on tuber and root formation

No obvious phenotypic difference in the tuber formation was observed between the wild type and the PSARK-IPT transgenic plants (Figure 7) under optimum watering condition. However, drought stress resulted in smaller and fewer tubers in wild type plants in comparison to transgenic plants that had higher number of visibly bigger tubers (Figure 7).

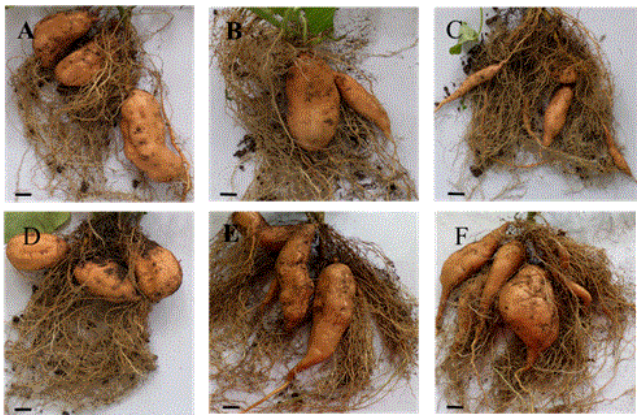


**Figure 7:** The effect of drought stress on visual rating of plants growth and storage root formation in transgenic PSARK-IPT and the wild type sweetpotatoes. (A), Non - transgenic tuber formation under control conditions. (B), wild type plant tuber formed under moderate stress. (C) Wild type plant tuber formed under severe drought stress. (D) Transgenic PSARK- IPT tuber formed under control condition. (E) PSARK- IPT tuber formed under severe drought stress. (F) PSARK- IPT tuber formed under severe drought stress.

## Discussion

This study aimed at developing transgenic sweetpotatoes that can tolerate drought and produce some significant yield under drought. Out of three sweetpotato varieties tested for transformability and regenerability, two transgenic sweetpotato varieties Jewel and Kemb36 were successfully transformed via Agrobacterium mediated gene transfer method. KSP36 variety however, did not regenerate any plants in spite of better performance of the variety during callus formation after transformation. This is consistent with the findings of Saraswati et al. [40] who established that different cultivars show different response to *in vitro* regeneration. Genotype-dependence for regeneration and transformation in sweetpotato has also been previously reported by [41,42]. According to Qin et al. [35] growth regulators are critical in *in vitro* regeneration process since a balance between auxins and cytokinin is important for conversion of somatic embryos into shoots. In this study, the variation in regeneration among genotypes could be attributed to significant variability in genotype response to growth regulator combination and genotype dependency to *in vitro* regeneration.

To evaluate the effect of drought on sweetpotato physiological and morphological parameters as well as yield, the drought treatment was organized to coincide with the critical developmental stage and extended till harvest time. The treatments were designed so because

sweetpotato is relatively drought tolerant [4]. Thus, its sensitivity to water deficit stress is dependent on its growth stage at the time of the stress. More so, sweetpotato is sensitive to drought stress during growth establishment, vine development and storage root initiation [4]. In general, drought stress caused a significant reduction in all growth parameters in wild type plants thus confirming the susceptibility of sweetpotato crop to high intensity of drought stress during the critical stage of plant establishment and root initiation. From the results, this study has demonstrated that expression of PSARKIPT under water deficit conditions improves drought tolerance in transgenic sweetpotatoes. This finding is consistent with the findings of Xu et al. [27,34] who demonstrated that PSARKIPT enhanced drought tolerance in transgenic tobacco and rice respectively.

Under well-watered conditions (control), both transgenic and wild type showed no morphological and physiological differences. This shows that introduction of PSARK IPT gene had no effect on plant growth and development under normal conditions. However, under drought stress conditions, there was a significant effect on all growth parameters. The development of vines was impaired, and the leaves turned yellow and finally fell off the plant. According to leaf yellowing [25] is a convenient and visible indicator of leaf senescence and it reflects chloroplast senescence of mesophyll cells which is the first step in senescence associated programmed cell death. From the study, signs of senescence began much earlier in wild types after induction of severe stress conditions. Wild type plants displayed wilting and drooping leaves that later turned yellow while the transgenic plants remained green and looked healthy. The difference in delayed senescence in transgenic plants can be attributed to expression of IPT gene that up regulated cytokinin biosynthesis thus delaying drought induced leaf senescence and maintaining growth. As drought treatment progressed, both transgenic and wild type plants showed a decline in vine length, internode length, canopy (leaf number and leaf area), tuber size and number as well as root number compared to the well-watered controls. The finding concurs with report Kreuze et al. [38] who observed that biomass and morphological traits decreased in response to drought stress. Relative water content is a measure water status in the plant and reflects the metabolic activity in tissues due to the fact that it is an important index for dehydration tolerance. RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures [43]. Under well-watered conditions, the plants maintained a high level of water content. However, the scenario significantly changed under drought stress. The RWC of plants tested decreased slightly in moderate drought stress and dropped further under severe drought stress. However, the reduction in RWC in transgenic plants was less than that of the wild type. During rewatering, the transgenic plants recovered much faster than the wild type. This result therefore suggests that the improvement might be due to the transgenic lines expressing the IPT gene that delayed drought induced senescence thus maintaining higher water level. Transgenic sweetpotato displayed an increase in all growth parameters compared to wild type signifying the expression of IPT gene which may have enhanced cytokinin availability hence functioning in response to drought stress. According to Walter et al. [44], Phytohormones play an important role in plant growth and development. The levels of cytokinins drop in response to water-deficit. The reduction in the levels of cytokinins is accompanied by the breakdown of proteins and photosynthetic machinery, leading to senescence and programmed cell death. To evaluate the effect of water deficit on photosynthesis in PSARKIPT transgenic sweetpotato, photosynthetic performance was analyzed by determining the chlorophyll degradation under drought stress and normal conditions. Under normal conditions, the transgenic plants displayed a significant difference in chlorophyll a, Chlorophyll b and total chlorophyll. From the study we established that moderate stress did not have a serious effect on the photosynthetic activity of the transgenic plants and wild type plants. The plants under moderate drought stress maintained a high total chlorophyll content and chlorophyll b. This observation is consistent with the finding by Van [8] who reported that chlorophyll content is almost unaffected by moderate stress. After re-watering, the chlorophyll content in both transgenic and wild type increased to a near normal level though the wild type reached this level 2 days later. The most probable reason for this would be that the water potential did not fall below the sustainable level and thus the functional activity of photosynthetic machinery of the plants was not severely affected. Similarly, under moderate soil water deficit, photosynthetic depression could possibly be because of stomatal closure or limitation, but not because of biochemical reaction. Therefore, plants recovered to control level once released from stressful conditions. Under severe and prolonged drought stress, the transgenic PSARKIPT showed a relatively higher level of chlorophyll content than the wild type. This is because the transgenic plants effectively protected the photosynthetic apparatus by efficiently scavenging for ROS hence improving water use efficiency during the water deficit stress. Upon re-watering, transgenic stressed and wild type stressed plants could not get full recovery even after 9 days of re-watering from severe drought conditions. It is possible that they needed more time to recover or the PSII thermal energy dissipation was strongly limited, due to damage to PSII structure and functionality. Further, we demonstrate that the expression of IPT gene under the control of maturation and senescence activated promoter enhances drought tolerance and hence an increase in yield in the transgenic sweetpotatoes. This is consistent with repots Lin et al. [33,34], in rice and peanuts respectively. Compared to the wild type the IPT expressing sweetpotatoes produced larger and more tubers under water stress. This significant difference would be attributed to higher photosynthetic rates in the transgenic sweetpotatoes under water limiting conditions due to conserved photosynthetic apparatus and the higher relative water content. The higher yield therefore is as a result of increased photosynthetic rates, stomatal conductance and transpiration hence supporting the notion of a cytokinins mediated protection of the photosynthesis in the transgenic plants.

## Conclusion

The study successfully developed selected PSARKIPT transgenic sweetpotato plants. However, sweetpotato transformation and regeneration via somatic embryogenesis is still genotype dependent hence affirming that sweetpotato is recalcitrant to transformation and regeneration. Based on drought stress data, PSARKIPT transgenic sweetpotatoes exhibited greater tolerance to drought stress than the wild types. The transgenic sweetpotatoes displayed delayed drought induced senescence, maintained higher relative water content, chlorophyll content, good tuber formation and in overall better growth compared to wild type under drought stress conditions. This shows that IPT gene significantly influenced greater performance in transgenic plants under drought stress conditions. This is a significant breakthrough since the approach can be used to improve other crops under the same prevailing conditions.

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