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Development and Agronomic Evaluation of *In Vitro* Somaclonal Variation in Sweet Potato Regenerated Plants from Direct Organogenesis of Roots

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ABSTRACT

Plant tissue culture techniques proffer a substitute method of vegetative propagation and regeneration of several crops. Genetic variability occurs frequently in micropropagated and regenerated plants. However, plant tissue culture may generate genetic variability, i.e., somaclonal and gametoclonal variations as a result of gene mutation or changes in epigenetic marks. Somaclonal variation has provided a new and alternative tool to the breeders for obtaining genetic variability relatively rapidly and without sophisticated technology in crops. In this study, the most adventitious buds developed from root explants of sweet potato (Ipomoea batatas) cultured in liquid media under a 16 h photoperiod, and the use of BAP and ZEA promoted the development of more adventitious buds than 2iP. Using an improved protocol, approximately 100 plants were regenerated by direct organogenesis of roots, and a total of 50 morphological somaclonal variants were visually identified in the greenhoue; however, only 13 somaclonal variants (SC-1 to SC-13), with several morphological variants, showed reasonable growth and were selected for further evaluations. The stomatal chloroplasts number, crude protein content and sugar analysis, dry matter and agronomic yield, were evaluated and showed significantly variations.

Keywords: Agronomic evaluation, Direct organogenesis, Ipomoea batatas, Regenerated plants, Somaclonal variation

INTRODUCTION

In the system proposed by the Angiosperm Phylogeny Group, the family Convolvulaceae that comprise nearly 1650 predominantly tropical species is placed with Hydroleaceae, Montiniaceae, Solanaceae and Sphenocleaceae, in the order, Solanales, Core Euasterids [1]. The genus Ipomoea with approximately 500-600 species, comprises the largest number of species within the Convolvulaceae [2], and occurs in the tropics of the world although some species also reach temperate zones [3]. The especies of this genus are mainly distributed throughout the South and Central America countries and Tropical Africa territories [2].

Among 50 genera of the Convolvulaceae family, only sweet potato [*I. batatas* (L.) Lam.], an asexually propagated root crop, is the most important, versatile and underexploited food crops that ranks fourth among the food after rice, potato and wheat and seventh in the world in terms of total production [4]. Nearly half of the *I. batatas* produced in Asia (China with 81.2 m) are used for animal feed whereas most of the crop cultivated in Africa (more than 14.2 m) is used for human consumption referring to its importance as a staple and sustainable crop in that part of the world [5]. Latin America produced 1.97 m, i.e., a little more than 2% of global supply: Brazil holds first place on the continent, followed by Cuba and Argentina [6,7].This highly nutritious crop gives better and faster production under diverse agro-ecological conditions with less input [8] and has immense potential to combat food shortage, malnutrition and poverty [5].

Plant tissue culture technique proffer a substitute method of vegetative propagation of several species, specially horticultural crops as *I. batatas*. Clonal propagation or micropropagation through tissue culture can be realized relatively rapidly within a small space [9,10]. The uniformity of individual plants within a clone population is a major advantage of clonal cultivars in commercial production [11]. However, genetic variations do occur in undifferentiated cells, isolated protoplasts, calli, tissues and morphological traits of *in vitro* raised plants [12]. In 1981, Larkin and Scowcroft coined the term "somaclonal variation" (SV) which describes the genetic, epigenetic or phenotypic variations in stable culture-induced tissue from clonally propagated plant populations [13]; although, in 1958-1959 a novel genetic variability was reported, and the higher plant cells that were cultured *in vitro* showed a genetic instability that also affected the regenerated cell [14,15]. It has also been well documented that somaclonal variants commonly present cytological aberrations such as chromosomal rearrangements, chromosoma damages (deletions, duplications, inversions and translocations), changes in methylation of chromatin and sometimes other more severe alterations caused by changes in the number of chromosomes (aneuploidy or polyploidy) [16-19].

In the other hand, the variation observed in tissue cultured clones are of two types, epigenetic and genetic [20]. Epigenetics can be defined as covalent modifications occurring in the chromatin allowing cells to maintain distinct and different characteristics despite of containing the same genetic material [21]. Changes in DNA methylation often give rise to epigenetic effects, which can cause expression of genes normally suppressed or their expression markedly decreased [22,23]. Epigenetic variation is often unstable and can disappear either after plants are removed from culture or within a few clonal or sexual generations [23], while genetic variation is heritable [24]. Epigenetic process are at the core of several types of phenotypic plasticity, increasing the evolutionary potential of organisms in response to environmental challenges [25], which could potentially by highly relevant in the context of projected global environmental changes [26]. In general, the three epigenetics mechanisms are DNA methylation, histone modifications and micro RNAs during different plant *in vitro* processes from a biological point of view [21].

Somaclonal variation has been observed in many plant species and is an alternative way to create variants and expand the germplasm pool. In St. Augustinegrass (*Stenotaphrum secundatum*), an important turfgrass species for the southern USA, a large scale tissue culture experiment was conducted to induce somaclonal variation to enlarge the germplasm pool for breeding efforts, using immature embryos of cv. 'Raleigh' for callus induction and plant regeneration [27]. Using somaclonal variation, in strawberry (*Fragaria* sp.), several new promising selections were generated and evaluated for their flowering and fruiting ability, adaptability and sustainability [20]. In another study, high genetic and epigenetic stability in *Coffea arabica* plants derived from embryogenic suspensions and secondary embryogenesis as revealed by AFLP, MSAP and the phenotypic variation rate was determined, and this work showed that both embryogenic suspensions and secondary embryogenesis are reliable for tru-to-type propagation of elite material [28]. Likewise, somaclonal variation on *in vitro* callus culture of five potato cultivars, was observed, and the RAPD pattern generated by these primers suggested a high percentage of polymorphic fragments among the five genotypes, indicating a high level of genetic variation among cultivars [29]. A comprehensive review of literature on somaclonal variation has been recently done by Bairu et al. [19], Wang and Wang [30] and Khrisna et al. [10] and epigenetic changes by Marum [31] and Us-Camas et al. [21].

In *I. batatas* the degree of somaclonal variation in plants regenerated by somatic embryogenesis was evaluated by phenotypic analysis under salinity stress condition in media supplemented with 0, 75, 150 and 200 mM of NaCl, and data analysis (number and length of roots, leaf and root condition) suggested a significant variation in salinity tolerance among regenerated and control plants [32]. In another study, preliminary evaluation in field conditions in Gabon revealed that plants regenerated of sweet potato from cultured protoplasts exhibited a great genetic variability in their growth and tuber formation in particular [33]. Likewise, somaclonal variation by *in vitro* mutagenesis in *I. batatas* was induced [34].

Here we report the efforts to regenerate *I. batatas* plants, by direct organogenesis in roots, from cv. UNPRG-358 *in vitro*, to isolate somaclonal variants as an approach for breeding improvement. Visual selection of plants with altered morphological traits, protein markers and field evaluation of these plants were performed to characterize the somaclonal variants.

MATERIALS AND METHODS

Plant materials

The *I. batatas* cultivar, UNPRG-358, was obtained from the Sweet Potato Germplasm Bank of the Universidad Nacional Pedro Ruiz Gallo, Lambayeque (Peru). Stem cuttings (30 to 50 cm length) of three to four months old, in

excellent health conditions and free of lateral buds, were placed in plastic pots containing a mixture of sand and soil at a ratio of 2:1 and grown in a greenhouse with shade screen and permanent irrigation. The stem cuttings were treated with fungicide Orthocide 80 at 0.2% for 5 min and afterwards rinsed away by washing the stem in running water.

Plant material preparation and experimental design

Both apical and axillary buds (1-2 cm) were washed with detergent under running tap water for 30 min to remove the surface contaminants followed by several rinses with sterile distilled water. The buds were posteriorly immersed in 70% etanol for 60 s, followed by 0.5% sodium hypochlorite (w/v) and Tween 20 (one ot two drops per 100 mL) for three minutes, and then washed 4-5 times with sterilize distilled water. Shoots tips of 0.5 to 1 mm in length were cut from the sterile explants under light microscope, placed in 15 × 120 mm test tubes and incubated onto MS [35] semisolid medium supplemented with 2.0% sucrose (w/v) agar and 0.22 mg/L BAP in combination with 0.18 mg/L NAA.

In the micropropagation step (Figure 1a), after 90-120 days of culture, the plantlets were cut into segments of approximately 1.5 cm and placed in 18 × 150 mm test tubes containing 5 mL of semisolid MS medium with 3.0% sucrose, 0.6% agar and without plant growth regulators. In the direct shoot organogenesis step, root segments of 2.0 cm in length, from 4 weeks old cultures, were aseptically excised and placed in MS medium supplemented with 2.0 mg/L BAP and after 2 months regenerated plantlets were transferred onto shoot elongation and root induction medium for generation of microplantlets.

In all experiments, the basal MS medium was supplemented with the vitamins myo-inositol 100 mg/L and thiamine. HCl 1.0 mg/L and 0.6% agar (w/v).

Culture conditions

The pH of all media was adjusted to 5.8 ± 0.1, with KOH and HCl, prior to autoclaving at 121°C at 105 kPa for 20 min. The experiments were evaluated every 30 days. The plant material was kept in a growth room with a controlled temperature of 26 ± 2°C, a relative air humidity of approximately 80%, photoperiod of 16 h light and luminous intensity of 60 $\mu mol.m^{-2}.s^{-1}$ via cool white fluorescent light.

Screening for somaclones from regenerated plants

Weel rooted *in vitro* plants were from culture vessel and washed to remove all traces of agar. Plants were subsequently transferred into pots containing sterilized mixture of sand and soil at a ratio of 2:1 and kept in greenhouse for 2 months. Vigorous plants were subsequently transplanted to the experimental field. The control treatment consisted of plants propagated conventionally by cuttings (Figure 1b).

The experiment was conducted at the Empresa Agroindustrial Pucalá, Lambayeque, Perú, research field. Pucalá is situated at latitude 06º 35' to 06º 48' S and longitude 79º 21' to 79º 41' W and 54 m in altitude, mean montly temperature of about 24°C and high relative humidity of about 80%. In the land preparation, the land was cleared, harrowed and made into 1.0 m row ridges. In plot size, ten plants of each somaclonal variants (SC-1 to SC-13) and control plant were planted at a space of 1 m × 1 m in a randomized complete block design in two replications; plots were kept weed free by regular manual weeding. Inorganic fertilizer was applied at 2 weeks after sowing, and the source of fertilizer was NPK 12:12:12.

Morphological data

The characteristics of ten plants were recorded for 5 months, begenning from the week 4 after transplanting, using the following parameters: Gross morphology, root storage and inflorescence. Gross morphology observations included twining, plant type, ground cover, vine internode (length and diameter), vine pigmentation (predominantly vine colour and secondary vine colour), vine tip pubescence, mature leaf shape (general outline of the leaf, leaf lobes type, leaf lobes number and shape of central leaf lobe), mature leaf size, abaxial leaf vein pigmentation, foliage colour (mature leaf colour and immature leaf colour) and petiole length. Storage root observations included storage root shape, storage root surface defects and storage root skin colour (predominant skin colour, intensity of predominant skin colour and secondary skin colour), storage root flesh colour (predominant flesh colour, secondary flesh colour and distribution of secondary flesh colour). Inflorescence observations included the flowering habit. A calibrated ruler was used to measure the length and diameter of vine internode and the petiole length; while other data, as mature leaf shape, foliage colour and flowering were observed each week. The storage roots were evaluated when the plants were mature and the data collected were based on Descriptors for Sweet Potato [36] evaluation system.

Protein and sugar analysis, dry matter and stomatal chloroplast number

Protein (N x 6.25) was determine by micro-Kjeldahl method. Reducing sugars were analyzed as described by Ross [37]. Briefly, 1 g of powdered dry tuberous roots was vortexed during 45 s with 5 ml of distilled water and sodium

Figure 1: Somaclonal variation in *I. batatas* cv. UNPRG-358 regenerated plants from direct organogenesis of roots (a) *In vitro* propagated plants in MS medium without plant growth regulators and (b) Agronomic evaluation of somaclonal variants

sulfite 0.5 g and centrifuged at 2000x g for 10 min. The samples were suspended in distillid water and filtered through Whatman No 1 filter paper. An aliquot of 2 ml of the supernatant was added to 6 ml of 2,4-dinitrophenol solution (0.038 M). The samples were incubated at 65 to 70°C for 6 min and then cooled under running water. Changes were estimated at 620 nm spectrophotometrically.

The content of partial dry matter was quantified from tuberous roots dried using a forced air circulation oven heated at 60°C, until constant weight of the root was evidenced. To determine the stomatal chloroplast number the first pair of fully expanded leaves were used. A strip of lower epidermis from the middle portion of the leaves was peeled off and mounted in glycerol after staining with lugol. Ten pairs of stomatal guard cell randomly selected from five pairs of leaves were counted per somaclonal variant.

Agronomic evaluation

Plant from *in vitro* cultures were acclimated under greenhouse conditions for one month to reach a height of 15 to 20 cm, 4 to 6 expanded leaves and well developed root system. 25 plants were used for each somaclonal variant, including the control treatment, conformed by plants propagated conventionally by cuttings, in furrows of 15.0 m long and 0.3 m high, separated at 1.0 m and 0.3 m between plants, for a total of 350 plants (13 somaclonal variants and the control) and a density of 40 000 plants per hectare. The yield/ha was extrapoled based on a population density of 40 000 plants per hectare. No fertilizers or pesticides applications were made during the growth and development of the crop. The gravity irrigation was applied twice per month. The experiment was repeated twice. The harvest took place at 5 months after planting, and for every somaclonal variant were harvested 10 plants in a random way, evaluating the weight of fresh roots.

Statistical analysis

In order to compare the treatment means, the results were processed and analyzed by ANOVA and Tukey's HSD

Table 1: Morphological characteristics of 13 somaclonal variants regenerated by direct organogenesis of roots of sweet potato. The evaluation was done in field level

	C	1[n]	2[a]	3[n]	4[a]	5[n]	6[a]	7[a]	8[n]	9[n]	10[a]	11[n]	12[a]	13[n]
Gross morphology														
1. Twining														
0 Non-twining	+	+	+	+	+	+		+	+	+	+	+	+	+
3 Slightly-twining							+							
2. Vine internode														
- Vine internode length														
1 Very short (<3 cm)					+						+			+
3 Short (3-5 cm)	+	+		+		+	+		+	+		+	+	
5 Intermediate (6-9 cm)			+					+						
- Vine internode diameter														
1 Very thin (<4 mm)							+							
3 Thin (4-6 mm)	+	+	+	+	+	+		+	+	+	+	+	+	+
3. Vine pigmentation														
- Predominant vine color														
6 Mostly purple	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7 Mostly dark purple					+									
4. Mature leaf size														
3 Small (<8 cm)							+							
5 Medium (8-15 cm)	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5. Foliage colour														
- Mature leaf colour														
1 Yellow-green	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2 Green							+			+				
6. Petiole length														
1 Very short (<10 cm)		+		+		+	+	+	+					
3 Short (10-20 cm)	+		+		+					+	+	+	+	+

[n]Plants with "normal" morphological characteristics; [a]Plants with "altered" morphological characteristics

multiple-range test ($p \leq 0.05$). All statistical analysis were performed using the software package Statgraphics Plus®, versión 5.0 (StatPoint, Virginia, USA).

RESULTS AND DISCUSSION

Morphological data of somaclonal variants

Approximately 100 plants were regenerated by direct organogenesis of roots, and a total of 50 morphological somaclonal variants were visually identified in the greenhouse; however, only 13 variants showed reasonable growth and were selected for further evaluations, from them 7 were ¨normal" and 6 "altered" with respect to the morphological characteristics; the resting 37 plants showed abnormal phenotypes (Table 1). Phenotype variation at both *in vitro* and field levels were evaluated. The vine internode length was the morphological characteristic of greater variability.

Other features evaluated such as gross morphology, storage root, and inflorescence showed no significant morphological differences. This lack of significant morphological differences were referred to plant type, ground cover, secondary vine colour, vine tip pubescence, mature leaf shape (general outline of the leaf, leaf lobes type, leaf lobe number and shape of central leaf lobe), abaxial leaf vein pigmentation, foliage colour (immature leaf colour) and petiole pigmentation in the case of Gross Morphology; storage root shape, storage root surface defects and storage root skin colour (predominant skin colour, intensity of predominant skin colour and secondary skin colour), storage root flesh colour (predominant flesh colour, secondary flesh colour, distribution of secondary flesh colour) with respect to storage root; and inflorescence for the evaluation of flowering habit.

In our study the objective was to develop an different tissue culture method in *I. batatas*, as direct organogenesis of roots, in the induction of variation leading to the development of new plant genotype. This source of variability is considered as a useful tool for geneticists and plant breeders [38,39]. For instance, in strawberry (*Fragaria* sp.) the regenerated plantlets were evaluated for somaclonal variation under field conditions and qualitative and quantitative different traits observed in the putative somaclones and quantitative data were as follows: canopy size, number of runner, days of flowering, number for flower per plant, number of fruit per plant, average single fruit weight and

percent of summer survival; likewise, was observed several phenotypic differences in the *in vitro* derived plants such as leaf shape, leaf petiole was shorter and thicker and lamina was comparatively bigger than control, and the most of the leaves were lighter green and number of leaves was less than control [20]. In St. Augustinegrass (*Stenotaphrum secundatum*) only 19 variants derived from immature embryos showed reasonable growth and were selected for further evaluations: semi-dwarf growth habit, with shorter leaves and internodes, and grew well; semi-dwarf growth habit and some growth in the greenhouse; longer and wider leaves; variegated leaves with yellow stripes, and thicker internodes [27]. In *Coffea arabica* plants derived from embryogenic suspensions, the frequency of phenotypic variants assessed among more than 600,000 plants in the nursery was very low (approx. 0.1%) and not significantly affected by the proliferation system nor the hybrid variety, and the observation of around 200,000 emblings in the field two years after planting revealed roughly an additional 0.74% of abnormal phenotypes, still without any significant difference between the two proliferation systems (SCE, secondary embryogenesis and ESP, embryogenic suspension) and hybrids (H1 and H3) [28]. In general, our results showed that in most of the cases somaclones were more vigorous than control, despite that morphological characteristics were not significantly variable.

In the other hand, for the induction of somaclonal variation, a high concentration of BAP (2.0 mg/l) or ZEA (2.0 mg/l) was applied in order to regenerate adventitious buds form roots explants, which corresponds with a report for a previous Researchers [40]. The high requeriment of BAP for efficient shoot regeneration and somaclonal variation has also been observed by other researchers. For example, Biswas et al. [20] reported in strawberry that a high concentration of BAP in culture medium successfully resulted in the induction of somaclonal variation, and among the tissue culture techniques adopted, meristem culture was most effective for induction of somaclonal variation, while Li et al. [27] observed that regeneration medium MS supplemented with 1.0 mg/l BA, 0.2 mg/l NAA and 0.5 mg/l GA as suitable for plant regeneration from immature embryos derived calli. Likewise, in potato plant was also regenerated from callus cells with best results on MS basal media supplemented with both 1.0 mg/l BAP and 1.5 mg/l IAA and genetic variability in *in vitro* cultured callus at different hormonal concentrations was assessed by using RAPD technique [41].

Number of chloroplasts

The mean stomatal chloroplast number per stomatal guard cells in somaclonal variants of *I. batatas* were calculated and the data are presented in Table 2. In some somaclonal variants such as SV-6 (12.9 ± 1.3) and SV-10 (13.2 ± 1.3), both presenting "altered" morphological characteristics, the stomatal chloroplast number decreasead in comparison with the control (14.9 ± 2.8) and the somaclonal variant SV-13 (18.9 ± 1.6) with "normal" morphological characteristics". However, no significant difference could be found between the somaclonal variants, and also the mean was no significantly different.

Stomatal chloroplast number have often used as morphological markers for identifying ploidy levels in many plants species as *Dactylis* [42] and wheat [43]. The determination of ploidy level by counting the number of chloroplast in stomatal guard cells is less time consuming, laborious and expensive comparing to chromosome counting in root tip cells or mother pollen cells and flow cytometry methods [44]. For instance, stomatal frequency, epidermal cell frequency, stomatal guard cell length and stomatal index were examined at different ploidy levels in *Coffea arabica* and C. *canephora* [45]; likewise, the chloroplast average number in stomatal guard cells was very similar among the same ploidy genotypes of Chinese cabbage as well as rapeseed, while the variation of chloroplast number in diploid and tetraploid white cabbage plants was significant [44]. It lacks studies about the assessment of chloroplast number of stomatal guard cells in somaclonal variants.

Crude protein and sugar analysis

The crude protein content of *I. batatas* somaclonal variants, SV-5 (1.92 %), SV-1 (1.74 %), SV-4 (1.74 %) y SV-11 (1.71 %), with "normal" and "altered" morphological characteristics, it was statistically higher than the control (1.07 %), but most of the somaclonal variants statistically exceed the control although these values were not significant (Table 3). In similar studies, the crude protein content of four sweet potato varieties cultivated in Rwanda was high in the yellow Kwizekumwe variety with 0.91% while crude protein was least in the yellow 440170 variety with 0.70% [46]; likewise, a comparative analysis of nutritional quality of five different cultivars of *I. batatas* in Sri Lanka showed that the crude protein content is in the range 1.2 to 1.3% on dry basis [47], values that were also reported by researchers [48,49]. In general, the average total protein content of *I. batatas* es low as 1.5% (fwb), however it is superior to other roots and tubers such as cassava, plantains, taro and inferior to potato, yams and cereals even those cooked as porridges [50]; however, the protein quality of *I. batatas* is of acceptable nutritive value and appreciable levels of amino acid lysine [51,52].

The total reducing sugars or total sugars were found to be high in the somaclonal variants of *I. batatas* SV-4 (7.20%), SV-3 (6.87%), SV-7 (6.73%), SV-1 (6.36%) y SV-8 (6.36%), which far exceed the control (4.89 %). In the comparison of the nutrient composition of four *I. batatas* varieties cultivated in Rwanda, the total reducing sugar was high in the yellow Kwizekumwe variety with 2.50% while was least in the yellow 440170 variety with 0.70% [46]. In another

Table 2: Stomatal chloroplast number at different somaclonal variants regenerated by direct organogenesis of roots of sweet potato

Somaclonal variants (cv. UNPRG-358)	Chloroplast number per stomatal cell guard
Control	14.9 ± 2.8 bc
SV-1[n]	15.1 ±1.1 bc
SV-2[a]	15.2 ± 1.9 bc
SV-3[n]	14.5 ± 1.1 bc
SV-4[a]	17.6 ± 1.9 ab
SV-5[n]	15.2 ± 1.5 bc
SV-6[a]	12.9 ± 1.3 c
SV-7[a]	14.9 ± 1.1 bc
SV-8[n]	16.3 ± 1.7 abc
SV-9[n]	14.4 ± 1.3 bc
SV-10[a]	13.2 ± 1.3 c
SV-11[n]	14.3 ± 1.8 bc
SV-12[a]	15.4 ± 1.9 abc
SV-13[n]	18.9 ± 1.6 a
Mean	**15.2 ± 1.5**

[n]Plants with "normal" morphological characteristics; [a]Plants with "altered" morphological characteristics

Table 3: Crude protein and total reducing sugars obtained from different somaclonal variants regenerated by direct organogenesis of roots of sweet potato

Somaclonal variants (cv. UNPRG-358)	Crude protein (%)	Total reducing sugars (%)
(Control)	1.07 bc	4.89 cd
SV-1[n]	1.74 a	6.36 ab
SV-2[a]	1.63 ab	5.76 bc
SV-3[n]	1.60 ab	6.87 a
SV-4[a]	1.74 a	**7.20 a**
SV-5[n]	**1.92 a**	4.89 cd
SV-6[a]	1.04 bc	3.19 f
SV-7[a]	1.02 bc	6.73 a
SV-8[n]	1.63 ab	6.36 ab
SV-9[n]	0.72 c	4.72 d
SV-10[a]	1.60 ab	3.40 f
SV-11[n]	1.71 a	5.39 cd
SV-12[a]	1.48 ab	4.66 de
SV-13[n]	1.36 ab	3.78 ef
	DMS=0.63	**DMS=0.91**

[n]Plants with "normal" morphological characteristics; [a]Plants with "altered" morphological characteristics

studies the sugar composition and starch morphology of backed seven fresh *I. batatas* cultivars in Taiwan ranged between 4.50 and 8.41% (dry weight basis) which were slightly lower than the results reported by Zhangs's [53]; in this study, sucrose was the major sugar composition of fresh *I. batatas* (49.92~92.43% of total sugars), and after the baking tretment, the total sugar content of baked *I. batatas* was dramatically increased due to the formation of maltose. In the Zhangs's results, the total sugars content of fresh *I. batatas* varied from 4.8 to 12.5% (dry weight basis) depending on varieties [54]. Cultivars from South Pacific region were found to have reducing sugar from 0.38 to 5.64% (fwb) and from 2.9 to 5.5% (fwb) in American cultivars and the time of harvest had a significant effect on total sugar content [50].

Dry matter and yield of tuberous roots

Somaclonal variant SV-11 (36.0%), with "normal" morphological characteristics, showed the highest partial dry matter and somaclonal variant SV-1 (26.64%), also with "normal" morphological characteristics, showed the lowest dry matter; while control (34.14%), although it was lower, it was not statistically surpassed. In *Solanum tuberosum*, clones Asterix and SMINIA793101-3 tubers grown in spring had higher dry matter (19.9 and 20.1%, respectively) with the only exception the clone Missaukee that had similar dry matter content (19.2%) in both, spring and autumn, growing seasons [55]. These results were much lower than those obtained in our study.

Table 4 shows that yield of somaclonal variants of *I. batatas* was significantly affected by the *in vitro* culture conditions. Somaclonal variant SV-9, with "normal" morphological characteristics, produced significantly higher tuber fresh

Table 4: Dry matter, tuber yield per plant and yield obtained from different somaclonal variants regenerated by direct organogenesis of roots of sweet potato

Somaclonal variants (cv. UNPRG-358)	Root fresh weight/plant (g)	Yield (Mt/ha)	Dry matter (%)
(Control)	321.3 c	12.84 cd	34.14 ab
SV-1[n]	216.1 d	8.64 ef	26.64 d
SV-2[a]	91.9 e	3.64 g	31.43 abc
SV-3[n]	401.4 bc	16.04 bc	29.95 bcd
SV-4[a]	399.2 bc	15.96 bc	30.10 bcd
SV-5[n]	302.4 c	12.08 de	30.50 bcd
SV-6[a]	186.2 d	7.44 f	26.88 cd
SV-7[a]	201.5 d	8.04 f	30.54 bcd
SV-8[n]	473.4 b	18.92 b	33.77 ab
SV-9[n]	**820.3 a**	**32.80 a**	30.62 bcd
SV-10[a]	370.0 bc	14.80 cd	33.49 ab
SV-11[n]	469.5 b	18.76 b	**36.00 a**
SV-12[a]	326.9 c	13.04 cd	30.80 bcd
SV-13[n]	219.2 d	8.76 ef	30.65 bcd
		DMS=3.52	**DMS=4.75**

[n]Plants with "normal" morphological characteristics; [a]Plants with "altered" morphological characteristics

weight with 820.3 g/plant and 32.8 Mt/ha, broadly superior to the control treatment with 321.3 g/plant and 12.84 Mt/ha. In Nigeria, the evaluation of the agronomic characters of *I. batatas* varieties grown at varying levels of organic and inorganic fertilizer showed production of 767 g/plant of tuber fresh weight [56]. In another study on agronomic and physicochemical evaluation of *I. batatas* collections in Ethiopia the mean storage roots fresh weight of accesions and checks were 484.51 and 486.09 g, respectively [57], and in Costa Rica, after the agronomic evaluation of thirteen genotypes of *I. batatas* researchers concluded that the root yield of all genotypes evaluated was higher than that of the local variety Criollo (6 $t.ha^{-1}$) as well as the national average (5 to 8 $t.ha^{-1}$), ranging from 12 to 48 $t.ha^{-1}$ [58]. In all these cases the storage performance of fresh roots was lower than in the somaclonal variant SV-9 of our study.

CONCLUSION

Somaclonal variation in plant tissue culture is a complex problem and very sensitive regulatory mechanism that needs several approaches to be appreciated correctly. This study have shown that the variability of regenerated buds obtained by *in vitro* direct organogenesis in roots of *Ipomoea batatas* can serve as a valuable tool in improving yield and other properties of *I. batatas* varieties and breeds. For the first time not true-to-the type morphological characteristics of somaclonal variants were detected. The study allows selecting somaclonal variants with higher yields in tuberous roots.

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